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W. VAN DRONGELEN

COMPARATIVE ASPECTS OF TASTE RECEPTORS AND HOST PLANT SELECTION IN LARVAE OF VARIOUS *YPONOMEUTA* SPECIES (LEPIDOPTERA)

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
prof. dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 8 oktober 1980
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

STELLINGEN

I Het verdient aanbeveling bij het gebruik van een "tip recording" techniek geen elektrolyten aan de smaakstof toe te voegen.

Dit proefschrift

II De veel gemeten gevoeligheidscurven van zintuigcellen hebben in vele gevallen nauwelijks betekenis voor het organisme.

Dit proefschrift

III Strikt genomen is geen enkele theorie te verifiëren.

IV Het is wenselijk tijdens de biologiestudie een zwaardere nadruk te leggen op het *toepassen* van hulpwetenschappen.

V De hypothese dat evolutie wordt verklaard met behulp van natuurlijke selectie welke richting geeft aan willekeurige variatie gaat voorbij aan de complexe samenhang binnen het organisme.

VI De functionele betekenis die Iordansky (1964) aan het kaakapparaat van krokodillen toekent is grotendeels onjuist.

Iordansky, N.N. (1964) - The jaw muscles of the crocodiles and some relating structures of the

crocodilian skull. Anat. Anz. 115, 256.

VII De wijze waarop Amoore zijn theorie voor geurperceptie introduceerde maakt deze theorie niet waarschijnlijker.

Amoore, J.E. (1962) - The stereochemical theory of olfaction. Proc. Sci. Sec., Toilet Goods Assn. 37, 1.

VIII Het is de vraag of de huidige tendens tot polarisatie tussen verschillende groepen in de samenleving met post- ofwel met pre-adaptatie aangeduid moet worden.

Proefschrift van W. van Drongelen
Comparative aspects of taste receptors and host plant selection in larvae of various *Yponomeuta* species (Lepidoptera)

Wageningen, 8 oktober 1980

Aan Marije

VOORWOORD

In dit proefschrift zijn verschillende aspecten van de smaakwaarneming van larven van verschillende soorten stippelmotten (Lepidoptera: Yponomeutidae) beschreven. Een overzicht van de taxonomische structuur binnen het genus *Yponomeuta* is in Tabel 1 weergegeven; tevens zijn hierin de waardplant preferenties vermeld.

Het proefschrift bestaat uit een experimenteel gedeelte en een deel waarin nader wordt ingegaan op theoretische aspecten van het beschreven onderzoek. Voor het gehele proefschrift geldt dat het eerste hoofdstuk, over de fysiologie en bouw van smaakzintuigen van de rupsen, als basis van de volgende gedeelten kan worden opgevat. De gemeten gevoeligheid van de zintuigen is in het tweede hoofdstuk nader geanalyseerd. Een onderzoek naar effecten van de gevoeligheid op het gedrag van twee soorten rupsen vindt de lezer in hoofdstuk 3. Genetische aspecten van de smaakwaarneming worden besproken in het vierde hoofdstuk. Een verdere ontwikkeling van de in hoofdstuk 1 beschreven signaal analyse is in hoofdstuk 5 te vinden. Het eerste theoretische hoofdstuk handelt over de vorm van de gemeten signalen. Het volgende onderdeel over convergentie is oorspronkelijk geschreven naar aanleiding van experimenten aan reukzintuigen, maar is onverminderd van belang voor de smaakwaarneming van insekten. Hiervoor verwijs ik tevens naar de discussie in hoofdstuk 1.

Nu de verschillende delen zijn samengevoegd tot dit proefschrift, heb ik behoefte een dankwoord te richten aan allen die aan het tot stand komen hiervan hebben meegewerkt.

Prof. dr. L.M. Schoonhoven ben ik dank verschuldigd voor zijn opbouwende kritiek. Op allerlei wijzen gaf hij aanleiding tot verdere ontwikkeling van het onderzoek, ik ben hem daarvoor bijzonder dankbaar. Prof. dr. J.T. Wiebes en dr. W.M. Herrebout ben ik erkentelijk voor hun aanvullingen met betrekking tot evolutionaire aspecten; onze onderlinge gesprekken hebben mij gestimuleerd bij het beschrijven hiervan. De medewerkers van de vakgroep diersystematiek en evolutiebiologie van de Rijksuniversiteit Leiden dank ik voor de ondersteuning in woord en daad; zonder de door hen verzorgde voorziening van proefdieren was mijn onderzoek niet mogelijk geweest. Prof. dr. R. Hegnauer heeft mijn kennismaking met de chemotaxonomie van de waardplanten op voortreffelijke wijze verzorgd; zijn welhaast onuitputtelijke bron van kennis is mij tot steun geweest bij het opzetten van de experimenten. De inspirerende gedachtenwisselingen met Prof. dr. A. Holley en Prof. dr. K.B. Dφving zijn van groot belang geweest voor het deel over centrale verwerking van zintuigsignalen; ik ben hen dankbaar voor alle aan mij bestede tijd. De automatische verwerking van gegevens is mogelijk gemaakt door de medewerking van Ir. C.J. de Groot en drs. G.D.E. Povel; ik denk met veel plezier aan onze samenwerking. De heer J.J.A. van Loon dank ik voor zijn enthousiaste medewerking en voor zijn bijdrage aan het uitvoeren van experimenten. Ik ben de heer F. Thiel erkentelijk voor zijn hulp bij het vervaardigen van Fig. 2 in hoofdstuk 1. De voor dit type onderzoek zo belangrijke technische hulp werd bereidvaardig verleend door de heren W.R. Bijlsma en A.G.M. Roos. De figuren zijn op een plezierige wijze verzorgd door de heren C. van Eden en J. van Brakel. Mevrouw T. van Bemmel (Vakgroep Fysiologie der dieren) en Mevrouw W.M. Laoh-Gieskes (Afd. Tekstverwerking Landbouwhogeschool) dank ik voor het uitvoeren van het vele typewerk.

Tabel 1. Klassificatie van *Yponomeuta* soorten en hun waardplanten

Waardplanten	*Yponomeuta*					
	Ras	Semispecies	Species	Species-groep	Hogere	Species-groep
(Celastraceae)			verschillende Aziatische spp.	*polystigmellus*-groep		
Prunus padus			1 *evonymellus*			
Euonymus		2 *cagnagellus*				
P. mahaleb		3 *mahalebellus*				
Malus		4 *malinellus*	*padellus*-complex	*cagnagellus*-groep		
Crataegus	1	5 *padellus*				A
P. spinosa	2					
P. domestica	3					
Salix		6 *rorellus*				
Euonymus			7 *irrorellus*			
Euonymus			8 *plumbellus*			B
Sedum tel.			9 *vigintipunctatus*			

Een speciaal dankwoord richt ik tot mijn ouders die mij in de gelegenheid stelden Biologie te studeren. Zonder het geduld en begrip van mijn vrouw was het niet mogelijk geweest zoveel tijd en inspanning te besteden aan mijn studie en dit proefschrift. Aan onze dochter die mijn aandacht waarschijnlijk niet bewust heeft gemist draag ik dit proefschrift op.

CONTENTS

PART I

EXPERIMENTS

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CHAPTER 1 Contact Chemoreception of Host Plant Specific Chemicals in Larvae of Various *Yponomeuta* Species (Lepidoptera)

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Summary. Gustatory sensitivity of larvae belonging to nine different *Yponomeuta* species was studied. A conspicuous behavioural difference between these closely related species is represented in their host plant preferences. Electrophysiological and morphological examination of the anteriorly-located sensilla revealed that a restricted number of contact chemoreceptors are present. A tip recording technique was used to record gustatory responses of neurones in the lateral and medial styloconic sensilla. The following chemicals were applied as stimuli: sucrose, sedoheptulose, sorbitol, dulcitol, coumarin, phloridzin, salicin, prunasin, rutin, (+)-catechin, the trisodium salt of isocitric acid, $ZnCl_2$, NaCl, and KCl. Most of these stimuli were selected on the basis of chemical composition of the array of host plants of the larvae studied (Table 1). Different spike amplitudes displayed in the recordings indicated that most constituents tested are perceived by single cells. Ionized substances sometimes excited more than one cell. In several recordings, a delayed response appeared. This phenomenon seems related to variation in diameter of the distal pore by which inward diffusion of the stimulus takes place. Analysis of the time course of the recordings indicated that the effect of diffusion of stimulus molecules on the initial neural response may be considerable for the non-ionized substances tested.

The functional diversity displayed in the interspecific sensitivity patterns can be partly explained by composition of the host plants on which the larvae feed (Table 2). Compounds of one type act as phagostimulants, and some of these compounds are sufficiently host-specific to act as host-recognition factors. A second type of chemical signal inhibits "wrong" food intake; these substances are classified as deterrents. A few gustatory responses appeared non-adaptive, this type of sensitivity is discussed from an evolutionary point of view.

Introduction

A technique to record neural responses from the tips of insect taste sensilla was first described by Hodgson et al. (1955). Ever since, several aspects of peripheral sensitivity of contact-chemoreceptors have been studied. Most research, focused on the neural mechanisms of gustatory perception and on chemo-electrical transduction, has been carried out on the labellar and tarsal hairs of flies (Wolbarsht and Hanson 1965; Rees 1967; Morita 1969; Stürckow 1971; Maes 1977). A second type of study has been made on the significance of gustatory sensitivity to compounds occurring in the natural or artificial diet (Stürckow 1959; Ishikawa 1963; Schoonhoven en Dethier 1966; Rees 1969; Dethier and Kuch 1971; Schoonhoven 1972; Ma 1972; van der Starre 1972; de Boer et al., 1977; Blom 1978). Lepidopterous larvae offer a good preparation for the latter type of research because they possess a restricted number of gustatory sensilla. In these species three topological types of sensilla are known: the lateral and medial sensilla styloconica situated on the maxilla, a number of sensilla on the maxillary palpi, and in some species a pair of epipharyngeal sensilla located on the ventral side of the labrum (Schoonhoven 1972). The importance of the gustatory neurones was demonstrated in behavioural tests were amputation of

the maxilla or the maxillary palpi leads to acceptance of normally rejected plants (Torii and Morii 1948; Dethier 1953; Waldbauer and Fraenkel 1961). Assuming a relationship between speciation and host plant choice, the latter studies indicate the significance of the gustatory organs from an evolutionary point of view. Various theories on sympatric and allopatric speciation of phytophagous insects have been described by Dethier (1952), Bush (1974), Wiebes (1976) and Labeyrie (1978). In recent comparative studies on neural and behavioural gustatory responses of larvae belonging to the genus *Yponomeuta* it is suggested that there exists a persistence of sensory sensitivities to constituents of the ancestral host plant (Gerrits-Heybroek et al., 1978; van Drongelen 1978). Because many species of the genus *Yponomeuta* seem closely related, but show different food regimes (Friese 1960; Herrebout et al., 1976), they are good candidates for a comparative study on insect-host plant relationship.

This paper aims to describe gustatory activity during application of plant constituents, as recorded in the lateral and medial styloconic sensilla of nine *Yponomeuta* species. Parameters of the neural responses are considered in connection with primary events of the peripheral perception mechanism. To evaluate aspects of the relationship between chemosensitivity and occurrence of plant constituents in the host, an attempt is made to state expectations concerning the sensitivity patterns. The distribution of various compounds over the host plants and assumptions on neural mechanisms represent the input of these expectations. Deviations from expected sensitivity are discussed from an evolutionary point of view. A short description of a part of this work appeared previously (van Drongelen 1978) and preliminary data on the sugar receptors have been described by Schoonhoven et al. (1977).

Methods

Animals. Most larvae of the *Yponomeuta* species (Lepidoptera, Yponomeutidae) were collected in the field. Larvae of *Y. vigintipunctatus* and *Y. irrorellus* were reared in the laboratory on their host plant. The animals were stored in a refrigerator at 6°C for a maximal duration of one month. A list of the *Yponomeuta* species studied and their associated hosts is shown in the first two rows of Table 1. It can be seen that *Y. padellus* is oligophagous and the remaining species are monophagous. In this paper, the host-race of *Y. padellus* feeding on *Crataegus* species will be referred to as host-race 1 and the one feeding on *Prunus spinosa* as host-race 2. It was found practical to use the host plant on which the larvae were collected as the main criterion for species identification. On morphological grounds larvae of *Y. vigintipunctatus* and of *Y. plumbellus* can be clearly distinguished from those of the other species studied; the first two species belong to a different taxonomic group (group B) than the remaining ones (group A) (Gerrits-Heybroek et al., 1978). Larvae of *Y. cagnagellus* were distinguished from those of *Y. irrorellus* by morphological identification of adult individuals of the latter species, which were reared in a laboratory culture.

Morphology. The mouth parts of the insects to be investigated were fixed in Bouin. The fixed preparations were placed in freon liquid, subsequently in liquid nitrogen and were freeze dried. Dried preparations were dissected and coated with gold. The sensory organs were examined in a scanning electron microscope (Jeol, JSM-U_3) in the Service Institute for Technical Physics in Agriculture in Wageningen.

Table 1. Compounds occurring in leaves (together with the ratio dry weight/fresh weight) of various host plant species of *Yponomeuta* species are given in the upper line. Concentrations are indicated in mM and in the first column the concentrations used in the electrophysiological experiments are represented. When no quantified concentration is indicated, a "+" denotes presence of the chemical and a "0" absence or presence in very low concentrations (traces). The contents of this table are based on: *A*. Favre-Bonvin et al. (1968); *B*. Haslam (1978); *C*. and *D*. Hegnauer (1964, 1973); *E*. Maas (1957); *F*. Nordal and Klevstrand (1951); *G*. Raa and Overeem (1968); *H*. Rabaté (1935); *I*. Seybold (1968); *J*. Soderström (1962); and *K*. Tölgyesi (1965). References C and D are not indicated in Table 1.
– A "?" denotes that the figure given is open to doubt. The salicin and (+)-catechin content of *Salix* leaves is very much species-dependent which is indicated by $. The concentration indicated for salicin was calculated from data of salicin-containing willows.

Yponomeuta species	*Evonymellus*	*Cagnagellus Irrorellus Plumbellus*	*Malinellus*	*Padellus*	*Padellus*	*Rorellus*	*Mahalebellus*	*Viginti-punctatus*
Host plant	*Prunus padus*	*Euonymus europaeus*	*Malus* sp.	*Crataegus leav./mono.*	*Prunus spinosa*	*Salix* sp.	*Prunus mahaleb*	*Sedum telephium*
Dry weight/ Fresh weight	0.37	0.35	0.40	0.38	0.36	0.31	0.45	0.08
Sucrose (0.1-100)	+	6-57 (I)	21-33 (I)	+	+	13-64 (H)	+	+ (F)
Sedoheptulose (10-100)	0	0	0	0	0	0	0	principal sugar (F)
Sorbitol (1-100)	38 (E)	0	121-278 (I) 54-104 (E)	96-131 (E)	low concentration	0	+	0
Dulcitol (0.25-25)	0	140-450 (I) 282 (E)	0	0	0	0	0	0
Coumarin (1)	0	0	0	0	0	0	252 (A)	0
Phloridzin (0.02-2)	0	0	167 (G)	0	0	0	0	0
Salicin (10)	0	0	0	0	0	1-36 $	0	0
Prunasin (10)	+	0	0	0	+	0	0	0
Rutin (0.1)	+	+	+	+	+	0	+	0 ?
(+)-Catechin (1)	0 ? (B)	+ ?	0 (A) (B)	0 (B)	0 ? (B)	$	0 ? (B)	+ ?
Isocitric acid (100)	0	0	0	0	0	0	0	7-52 (J)
$ZnCl_2$ (1)	0.1 (K)	+	0.1 (K)	0.1 (K)	0.1 (K)	0.3 (K)	0.1 (K)	+
NaCl and KCl (1-100)	+	+	+	+	+	+	+	+

Recording. Neural responses were recorded using a modification of the tip recording technique described by Hodgson et al. (1955). Two types of preamplifiers have been used in this study, both with high input resistances (10^{10} Ω and 10^{15} Ω), low input capacitances (approximately 0.2 pF) and low input bias currents (10 pA and 0.3 pA). In view of those characteristics responses were obtained without inadvertant electrical stimulation (Maes 1977; van Drongelen and Bijlsma[1]). In most recordings, the blocking artefact accompanying stimulus onset was reduced to approximately 5 ms by the automatic bucking potential source described by van der Molen (1977). In the remaining recordings, blocking artefacts of the same duration were obtained by using the preamplifier to be described by van Drongelen and Bijlsma. Spikes encountered during stimulation with non-ionized substances dissolved in double-distilled water could be recorded, because the preamplifiers had a low input capacitance and the input circuit was guarded. The biopotentials were amplified 1,000 times and the amplified signal was fed to an active filter (Krohn-Hite, model 3750) with a flat band pass from 0.1 to 1.5 kHz. The filtered and DC signals were connected to a dual-beam oscilloscope and were recorded on tape for subsequent analysis.

Recordings were displayed on paper by means of a Siemens Oscillomink inkjet recorder. An example of a recording during stimulation with sucrose is shown in Fig. 1. It can be seen that after stimulus onset (S.O.) a response starts after a short latency (t_1).

Stimulus selection. When comparing chemosensitivity spectra of several species, one can expect to find qualitative as well as quantitative differences in sensitivity. In order to study both aspects, chemicals specific for *one* host plant and chemicals occurring in *more* than one host plant were applied as stimuli. The stimuli and information on their presence in the leaves of various host plants are summarized in Table 1. As far as is known from literature sources, the concentrations of the compounds are given in mM. Otherwise a " + " denotes the presence and a " 0 " the absence, or presence in a very low concentration, of a particular compound. Most of the data concerning *Euonymus europaeus* and *Sedum telephium* are based on Hegnauer (1964). The figures stated for *Crataegus monogyna*, *Crataegus laevigata*, *Malus* sp., *Prunus mahaleb*, *Prunus spinosa* and *Salix* sp. are given by Hegnauer (1973). If data were obtained from other sources, this is indicated in Table 1. The ratio dry weight/fresh weight of the leaves was determined in June 1978 and it is indicated for each host plant in the third row of Table 1. It can be seen that these figures vary considerably from 0.08 up to 0.45. The concentrations given in Table 1 were derived from data in literature in the following way. In Maas (1957) sugar alcohol content is presented in atmospheres, a measure for the osmotic value. It was found that reliable estimates for the amount of sugar alcohols in mg/ml were obtained by multiplying the figures in atmospheres by a factor of 7 (Tables 7 and 8 in Maas 1957). Most authors, referred to in Table 1, either describe the amount of constituents as a percentage of the weight of the dry or fresh leaves, or in (milli) gram or (milli) Moles per unit dried or fresh leaves (Hegnauer 1964, 1973; Raa and Overeem 1968; Rabaté 1935; Seybold 1968; Soderstrom 1962; Tölgyesi 1965). The value for coumarin was calculated from the amount of μCi per unit dried leaves and the specific radio activity of the sample (Table 1 in Favre-Bonvin et al. 1968). The concentrations indicated in Table 1 were obtained by calculating the number of mM in that amount of leaves containing 1 l of water. The figure stated for isocitric acid is based on a number of *Sedum* species, not including *Sedum telephium*. The

1) in preparation

Fig. 1. A response to 10^{-3} mMol/l sucrose recorded from the lateral sensillum styloconicum of *Y. cagnagellus. s.o.*, stimulus onset; t_1, latency time; calibration: 1 mV, 100 ms

concentration of salicin and (+)-catechin in *Salix* is very much species dependent (Hegnauer 1973). The concentration range indicated for sucrose and the sugar alcohols represents short term variation of these constituents in a single plant. This is due to fluctuations either in the water content or in the sucrose and sugar alcohol content of the leaves (Maas 1957; Jeremias 1964; Seybold 1968).

Chemicals used were obtained from commercial sources, i.e., KCl, NaCl, dulcitol, sorbitol, sucrose and (+)-catechin (Baker); salicin (E.G.A.); rutin (Fluka); coumarin (Koch-Light); phloridzin and prunasin (Roth); isocitric acid and sedoheptulose (Sigma); and $ZnCl_2$ (Merck). In the electrophysiological experiments the compounds were tested at concentrations as indicated in the first column of Table 1. In some cases, stimuli were given at lower concentrations than indicated above in order to obtain more complete stimulus-response relationships. Most chemicals were applied at concentrations close to the natural levels in the host plants. Coumarin was tested at a much lower concentration because this compound is released when the plant is dried or damaged, therefore the 252 mM represented in Table 1 is too high. In the cases of dulcitol and phloridzin solutions become saturated at concentrations below the quantities found in *Euonymus europaeus* and *Malus* sp. respectively. Consequently these chemicals were tested at lower concentrations than found in the hosts.

Solutions used were stored at 2°C for a maximal duration of five days. Because some species were not always available, not all species could be tested with all stimuli.

Analysis of the Recordings. Routinely, the number of all spikes encountered in the first 500 ms of stimulation served as an index for the neural activity. A computer program was used to analyse some recordings in greater detail. The X (time) and Y (amplitude) coordinates of the upper and lower peak of each action potential in recordings could be entered manually into core by means of a tablet (TEKTRONIX, 119-0344-01). In the program it is assumed that different cells are represented by different spike amplitudes. For this reason an amplitude histogram was displayed,

offering the means by which one can distinguish activities of different neurones. For each cell recognized in the recording, the program produces three graphs: 1. a plot of action potential's sequence number versus time; 2. a plot of action potential frequency (inverse of interspike interval) versus time; and 3. an interspike interval distribution histogram. After each program run, numerical data were stored on disk.

Procedure. Before the experiments, animals and stimulus solutions were adapted to room temperature. The head and first body segment of the larvae were cut off. The central nervous system was destroyed to avoid electrical artefacts either due to movement or central neural activity. Because of the small size of the heads (0.9 to 1.3 mm) the preparation was placed on a tungsten wire under a stereomicroscope. The tungsten wire served as recording electrode and was, together with the preparation, placed in a guarded box. A capillary placed over the sensillum contained the stimulus solution and served simultaneously as the indifferent electrode. To minimise any increase of stimulus concentration due to evaporation from the capillary tip, the preparation was mounted in a moistened air stream and the fluid in the capillary was sucked through by means of a piece of tissue immediately before each recording. Using the procedure described, stable responses could be obtained for 20 to 70 min.

The contact-chemosensory neurones studied are located in two sensilla styloconica, one situated laterally and one medially on each maxilla. Five responses to each stimulus were recorded in each sensillum of each species. Each such series of five responses was obtained from different individuals of a particular species. Neural recordings were obtained from fifth instar larvae; 10 to 50 individuals of each species. The signal-to-noise ratio of the recordings analysed in the present study varied between 2 and 8.

Results

Shape and Function of the Anteriorly Located Sensilla

A rostral view of the anterior part of the head of *Y. vigintipunctatus* is shown in Fig. 2B. As far as electrical contact with the longdrawn sensilla basiconica (s.b.) could be established, they appear to serve a mechanoreceptive function in all species. The antennae (not shown in Fig. 2) are situated on the ventro-lateral side of the head. Recording from the larger sensilla basiconica on the antennae displayed a high spontaneous spike discharge, which in a number of cases was modified under the influence of deformation. None of the mechanoreceptive sensilla examined electrophysiologically demonstrated any gustatory sensitivity. In a number of caterpillar species, the smaller sensilla located on the antennae appeared to contain olfactory cells (Schoonhoven and Dethier 1966). In *Yponomeuta* species the function of these smaller antennal hairs has not yet been studied. On the maxillary palpiger (m.p.) two protrusions, the palpus (p) and galea (g) are located. As can be seen on the detail of the tip of the palpus of *Y. padellus* (Fig. 2C), eight conically shaped sensilla are located on this structure. In all species studied one of these sensilla is somewhat longer than the remaining ones (sensillum 8, Fig. 2C). Electrophysiological examination indicated that at least one of the palpar sensilla serves a gustatory function. Stimulation with sucrose or salts elicits responses in the palpus of all *Yponomeuta* species. However, because of the small dimensions of the palpar sensilla it cannot be determined which palpar cone(s) is (are) responsible for these

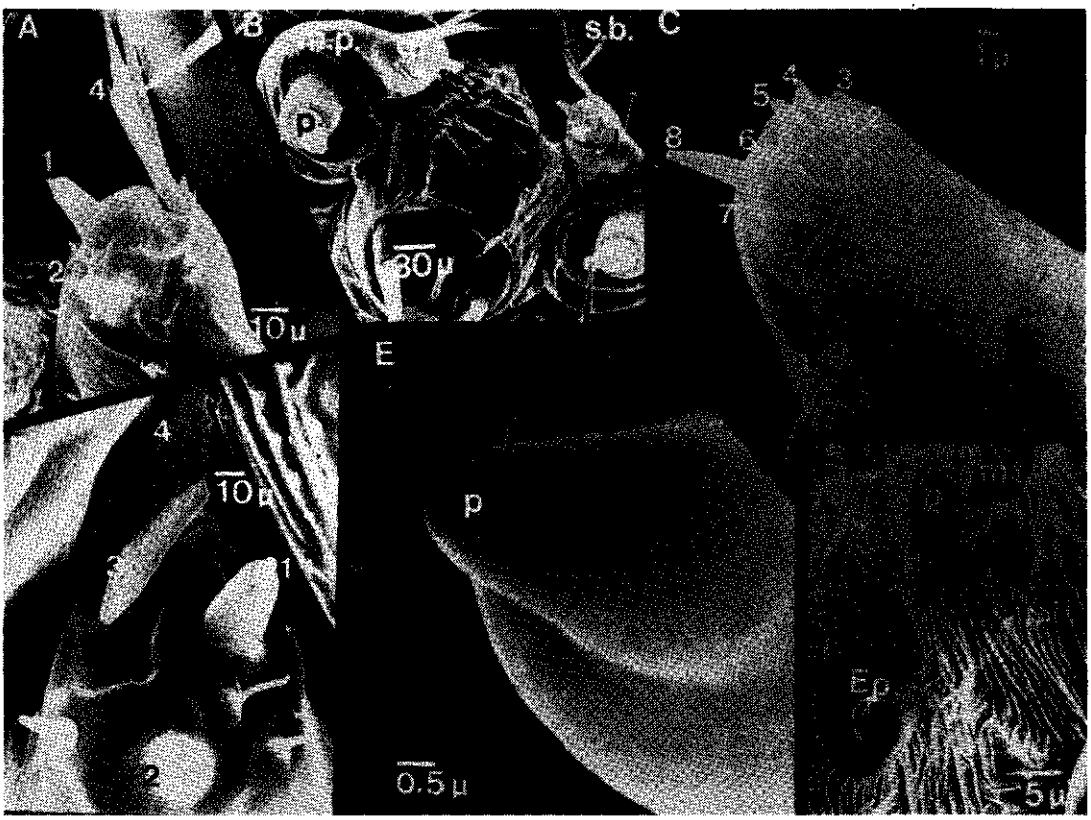

Fig. 2. A Detail of the galea of *Y. vigintipunctatus*. The gustatory sensilla styloconica are indicated by 1 and 2. The shape of sensilla 3 and 4 appears typical for the species groups A and B of the genus *Yponomeuta*. B Rostral view of the head of *Y. vigintipunctatus*. *s.b.*, sensilla basiconica; *m.p.*, maxillar palpiger; *p.*, palpus; *g.*, galea; *l.*, labrum. C Palpus of *Y. padellus*. In all species small conical-shaped sensilla are implanted on the palpus, indicated by numbers 1 to 8. One of these sensilla (8) is larger than the remaining ones. D The galea of *Y. malinellus*. The sensilla are labelled as in A. E Detail of the distal peg of the medial styloconic sensillum of *Y. plumbellus*. The structure indicated by *p* probably represents the pore through which taste substances enter the hair. F Part of the ventral surface of the labrum. The sensillum indicated by Ep. is morphologically similar to epipharyngeal sensilla described in other caterpillar species. Two sensilla campaniforma are indicated by 1 and 2, and m.t. denotes microtrichia.

responses. Furthermore, some of the palpar sensilla contain highly spontaneously active neurones, which obstruct systematic cataloguing of the palpar gustatory response. Two sensilla styloconica which contain gustatory receptors are located on the galea. A determination of the sensitivity spectra of these gustatory neurones is one of the main objectives of this study.

In Figs. 2A and D are shown details of the galea of *Y. malinellus* and *Y. vigintipunctatus*, the first species as a representative of the taxonomical group B and the latter of group A. The difference in shape of sensilla 3 and 4 between both species is a differential character for the taxonomical groups. It can be seen that in both species the gustatory sensilla (1 and 2 in Figs. 2A and D) are divided into two segments. A close-up of the medial sensillum of *Y. plumbellus* (Fig. 2E) demonstrates a structure which may represent the pore (p) through which the neurones may contact outer media. The function of the remaining sensilla on the galea is not yet determined. It is not probable that they serve a gustatory function because application of the chemical stimuli used never elicited any neural activity.

When food is "approved" by means of the external sensory sensilla, food intake behaviour is triggered. At that very moment chemicals contained in the food can contact gustatory receptors located in the buccal cavity. An epipharyngeal organ serving a gustatory function and situated on the ventral side of the labrum has been described in *Pieris brassicae* (Ma 1972; Blom 1978) and in *Manduca sexta* (Ma 1972; de Boer et al. 1977). Examination of the ventral part of the labrum of all species indicated that similar organs are probably present in all *Yponomeuta* larvae.
Sensilla campaniforma as found in a preparation of *Y. malinellus* are shown in Fig. 2F; they have a similar shape and topography as the epipharyngeal organs in *Pieris brassicae* and *Manduca sexta*. One of these sensilla (Ep) is dome-shaped with a central depression containing a small papilla. Based on morphological similarity between this sensillum and the epipharyngeal organ described by Ma (1972), it is likely to be a gustatory organ. The remaining two sensilla, 1 and 2 in Fig. 2F, lack the central depression of the cuticle.

Characteristics of Impulse Trains

A DC recorded action potential displays a negative-to-positive going polarity. It should be noted that reverse polarities are displayed when the recording technique is used as described by Hodgson et al. (1955), i.e., the stimulus pipette as recording electrode and the indifferent electrode in the head of the preparation (van der Starre 1972). Indications exist that the first phase of the spike represents the axonal action potential whereas the second one is caused by an antidromically conducted dendritic depolarisation (van Drongelen 1979).

Neural responses encountered during stimulation with ionized substances display spikes of different amplitudes. The amplitude of action potentials arising in one cell remain approximately constant throughout the recording. A representative response to KCl recorded from the lateral sensillum of *Y. cagnagellus* is shown in Fig. 3A. An amplitude histogram of the spikes during the first second of this recording demonstrates the presence of 4 amplitude classes (Fig. 3B). In both sensilla of all species reactions to salt solutions showed spikes belonging to different amplitude classes. Comparing salt responses of different individuals of one species it appeared that the activity level of the cell firing the largest spikes fluctuated considerably from one lateral sensillum to another. In the recording shown in Fig. 3A, the activity

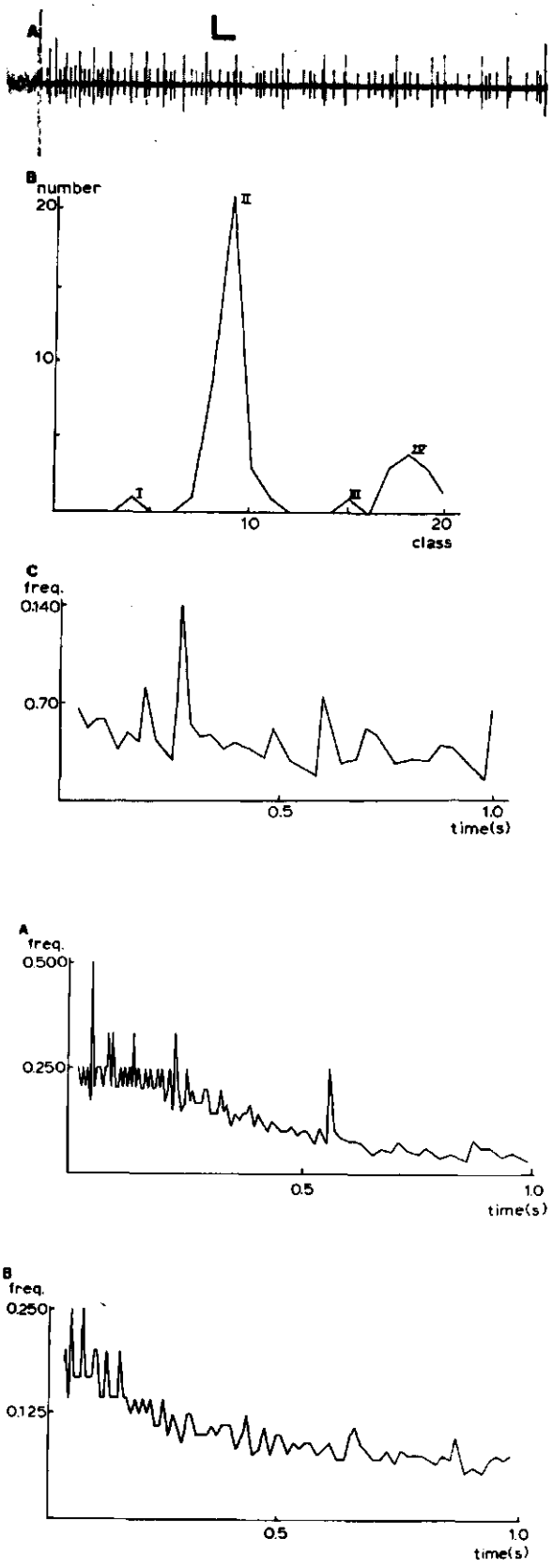

Fig. 3. A Neural activity recorded from the lateral styloconic sensillum of *Y. cagnagellus* during stimulation with 1.000 mMol/l KCl. Calibration: 1 mV, 100 ms. B Amplitude histogram of spikes occurring in the initial second of the recording displayed in A. Four distinct amplitude classes, I to IV, appear present. C Spike frequency (0.8 ms^{-1}) plotted versus time. In this plot, only action potentials from class II of B are considered.

Fig. 4A and B. Action potential frequencies (0.8 ms^{-1}) plotted versus time. A Frequencies measured in a response to sucrose in the lateral sensillum of *Y. padellus*. B Response to prunasin recorded from the medial sensillum of *Y. cagnagellus*.

belonging to spike amplitude class II (Fig. 3B) represents a clear response. The time course of the response to salt stimulation resembles spontaneous discharge observed when applying distilled water, i.e., no clear phasic component is present shortly after stimulus onset and interspike intervals are irregular. This can be seen in Fig. 3C where the frequency (inverse of interspike interval) of the action potentials in class II (during the initial second of stimulation) is plotted versus time. It should be noted that the irregular intervals may be caused by activities of different cells. However, the latter explanation is not probable because non-physiological short intervals were not displayed in responses of this amplitude class.

In all recordings made during stimulation with non-ionized substances only one cell seemed to respond. Considering spike amplitudes in spike trains overtly fired off by a single neurone, responses to non-ionized substances can be classified into three types: 1. the spike amplitude remains constant; 2. the spike amplitude continuously decreases after stimulus onset, or 3. the amplitude increases, subsequently decreases, increases again etc. An example of the third response type can be seen in Fig. 1. The activity shown in Fig. 1 may be generated by different cells, but (in spite of variation in amplitude) most spikes in the initial response overtly belong to a single cell. No correlation is found between stimulus type and any one of these different response types. The different response types might be caused by different physiological states of the gustatory neurones or by variation in diameter of the distal pore in the sensillum, but other causes cannot be excluded. In Figs. 4A and B spike frequencies are plotted versus the time for responses to sucrose (*Y. padellus*, lateral sensillum) and prunasin (*Y. cagnagellus*, medial sensillum). When these time courses are compared to the one for the salt response (Fig. 3C) it appears that responses to non-ionized substances have a pronounced phasic component. Adaptation rates during stimulation are different for each compound. In all species studied the responses to salts display little adaptation, whereas receptors stimulated by phloridzin, prunasin, salicin or (+)-catechin show a moderate adaptation rate and cells responding to sucrose or sugar alcohols exhibit high adaptation rates.

Qualitative Sensitivity

The results of a qualitative screening of the sensilla styloconica of all *Yponomeuta* species are summarised in Table 2. The compounds tested and the corresponding concentrations are indicated in the first column, the *Yponomeuta* species in the first row. A " + " denotes that an excitatory response was encountered and a " 0 " indicates the absence of such a response. In this case, recordings that displayed a total activity of more than 20 spikes in 500 ms after stimulus onset were considered to represent true responses. Inhibition of action potential discharge frequency as compared to neural activity during application of double-distilled water (the stimulus solvent) was never observed. When a particular compound occurs in the associated host plant of the species a (p) is assigned in the appropriate columns of Table 2. The presence or absence of prunasin in the host of *Y. padellus* is related to the fact that two *Y. padellus* races studied occur on different hosts (£ in table 2). The (+)-catechin and salicin content in *Salix* varies considerably due to large interspecific variations. A sensitivity to sucrose is found in the lateral hair of all species, but the medial hair is insensitive to this compound in all species tested. In contradiction to a previous report by Schoonhoven et al. (1977), *Y. vigintipunctatus* appears insensitive to sedoheptulose, the difference probably being due to the use of a different

Table 2. Qualitative sensitivity patterns as determined for the lateral sensillum and the medial sensillum of *Yponomeuta* species indicated in the upper rows; evo., *Y. evonymellus*; cag., *Y. cagnagellus*; mal., *Y. malinellus*; pad., *Y. padellus* (both host-races); ror., *Y. rorellus*; mah., *Y. mahalebellus*; irr., *Y. irrorellus*; vig., *Y. vigintipunctatus*; plu., *Y. plumbellus*. Stimuli tested with their concentrations in mM are given in the left columns. A "+" denotes presence, "0" absence of a response. When a particular compound occurs in the associated host plant this is indicated by (p), or (p?) when the occurrence is not certain. The symbol (£) indicates that prunasin content of the host depends on the *Y. padellus* host-race (Table 1). Presence of salicin and (+)-catechin in the leaves of *Salix* sp. is *Salix* species dependent, indicated by ($). The concentration of $ZnCl_2$ is higher in *Salix* species than in the remaining plants which is denoted by (h).

A. Lateral sensillum

Compound		Species								
	C.	evo.	cag.	mal.	pad.	ror.	mah.	irr.	vig.	plu.
Sucrose	100	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)
Sedoheptulose	100								0 (p)	
Sorbitol	100	+(p)	0	+(p)	+(p)	0	+(p)	0	0	0
Dulcitol	25	+	+(p)	0	+	0	+	+(p)	0	+(p)
Coumarin	1				0	0	+(p)			
Phloridzin	2	0	0	0 (p)	0	0	0	0	0	0
Salicin	10	+		+	+	0 ($)	+			
Prunasin	10	0 (p)	0	0	0 (£)	0	0	0	0	0
Rutin	0.1	0 (p)	0 (p)	0 (p)	0 (p)	0	(p)	(p)	(p?)	(p)
(+)-Catechin	1	0	0 (p?)	0	0	0 ($)		(p?)	(p?)	(p?)
Isocitric Acid	100	+	+	+	+	+	+		+(p)	
$ZnCl_2$	10		0	0	0	+(h)				
NaCl and KCl	1,000	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)

B. Medial sensillum

Compound		Species								
	C.	evo.	cag.	mal.	pad.	ror.	mah.	irr.	vig.	plu.
Sucrose	100	0 (p)	0 (p)	0 (p)	0 (p)	0 (p)	(p)	0 (p)	0 (p)	0 (p)
Sedoheptulose	100								0 (p)	
Sorbitol	100	0 (p)	0	0 (p)	0 (p)	0	0 (p)	0	0	0
Dulcitol	25	0	+(p)	0	0	0		+(p)	0	0 (p)
Coumarin	1				0	0	0 (p)			
Phloridzin	2	+	+	0 (p)	+	+	+	+	+	+
Salicin	10	+	+	+	+	+($)				
Prunasin	10	+(p)	+	+	+(£)	+	+	+	+	+
Rutin	0.1	0 (p)	0 (p)	0 (p)	0 (p)	0	(p)	(p)	(p?)	(p)
(+)-Catechin	1	0	+(p?)	+	0	+($)		(p?)	(p?)	(p?)
Isocitric Acid	100	0		0	0	0			+(p)	
$ZnCl_2$	10			+	+	+(h)				
NaCl and KCl	1,000	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)	+(p)

stimulus solvent. Sorbitol is perceived exclusively by a cell in the lateral hairs of the four species feeding on rosaceous plants. Members of this plant family specifically contain high amounts of this sugar alcohol (Table 1). Sensitivity to dulcitol, a stereoisomer of sorbitol, is found in the lateral sensillum in the three species feeding on *Euonymus*. This plant is typified by the presence of dulcitol. In *Y. cagnagellus* and *Y. irrorellus* dulcitol elicits responses not only in the lateral, but also in the medial sensillum. However, dulcitol sensitivity is not restricted to *Euonymus* feeders. It is also observed in the lateral sensilla of *Y. evonymellus*, *Y. padellus* and *Y. mahalebellus*. A common characteristic of the latter three species is that, with the exception of host race 1 of *Y. padellus*, they feed on *Prunus* species. A solution of coumarin when applied to the sensilla of *Y. mahalebellus* encounters a weak response in its lateral hairs. Larvae of *Y. padellus* (both host races) and *Y. rorellus* do not show any reaction to coumarin. Phloridzin can be perceived by a cell in the medial sensillum of all species except *Y. malinellus*. The insensitivity of the latter species to phloridzin is remarkable in view of the fact that it is a highly specific compound for its host plant. Salicin shows a similar sensitivity pattern as found for phloridzin, albeit in this case in the lateral hair of the species tested; i.e. a sensitivity in those species, which do not feed on *Salix* and an insensitivity in *Y. rorellus* feeding on *Salix*. However, salicin elicits also responses in the medial hair of those species tested. Prunasin excited a cell in the medial hair of all species. Quantitative aspects of the salicin and prunasin responses in the medial sensilla are described in one of the following sections. Rutin appears totally inert for both sensilla in the five species tested. Stimulating effects of (+)-catechin are found for the medial hairs of *Y. cagnagellus*, *Y. malinellus* and *Y. rorellus*, but the same sensillum of *Y. padellus* and *Y. evonymellus* appears to be insensitive to this compound. Sensitivity to NaCl and KCl is present in all species. Therefore it is not surprising that most species also respond to $ZnCl_2$ and the trisodium salt of isocitric acid. Neurones in the lateral sensillum of *Y. rorellus* are slightly more sensitive to $ZnCl_2$ than those in the other *Yponomeuta* species (Table 2A). In the medial sensillum of *Y. vigintipunctatus* a slightly higher activity is encountered during stimulation with $(Na)_3$-isocitric acid as compared to the other species (Table 2B). However, it should be noted that interspecific differences in the reactions to $ZnCl_2$ and $(Na)_3$-isocitric acid are small. In my opinion it cannot yet be decided whether the sensitivity patterns to these compounds play a role in host recognition or not.

From the results presented in Table 2 it may be concluded that most *Yponomeuta* species can be identified on the basis of their chemosensory patterns. Both host-races of *Y. padellus*, interestingly, show identical responses and consequently cannot be discriminated by means of the functional criteria presented so far. Also, no distinction between *Y.cagnagellus* and *Y. irrorellus* can be made on the basis of a qualitative comparison of their sensitivity spectra.

Stimulus-Response Relationship

In order to determine the role of concentration fluctuations of the substances under natural conditions on host plant recognition, it seems important to know the stimulus-response characteristics of the receptors involved. The results of responses to sucrose, sorbitol, dulcitol, phloridzin, NaCl, and KCl recorded on both lateral and medial sensilla are represented in Fig. 5. As far as responsiveness is present, most curves are more or less sigmoid: a low subthreshold activity, a steep rise of neural

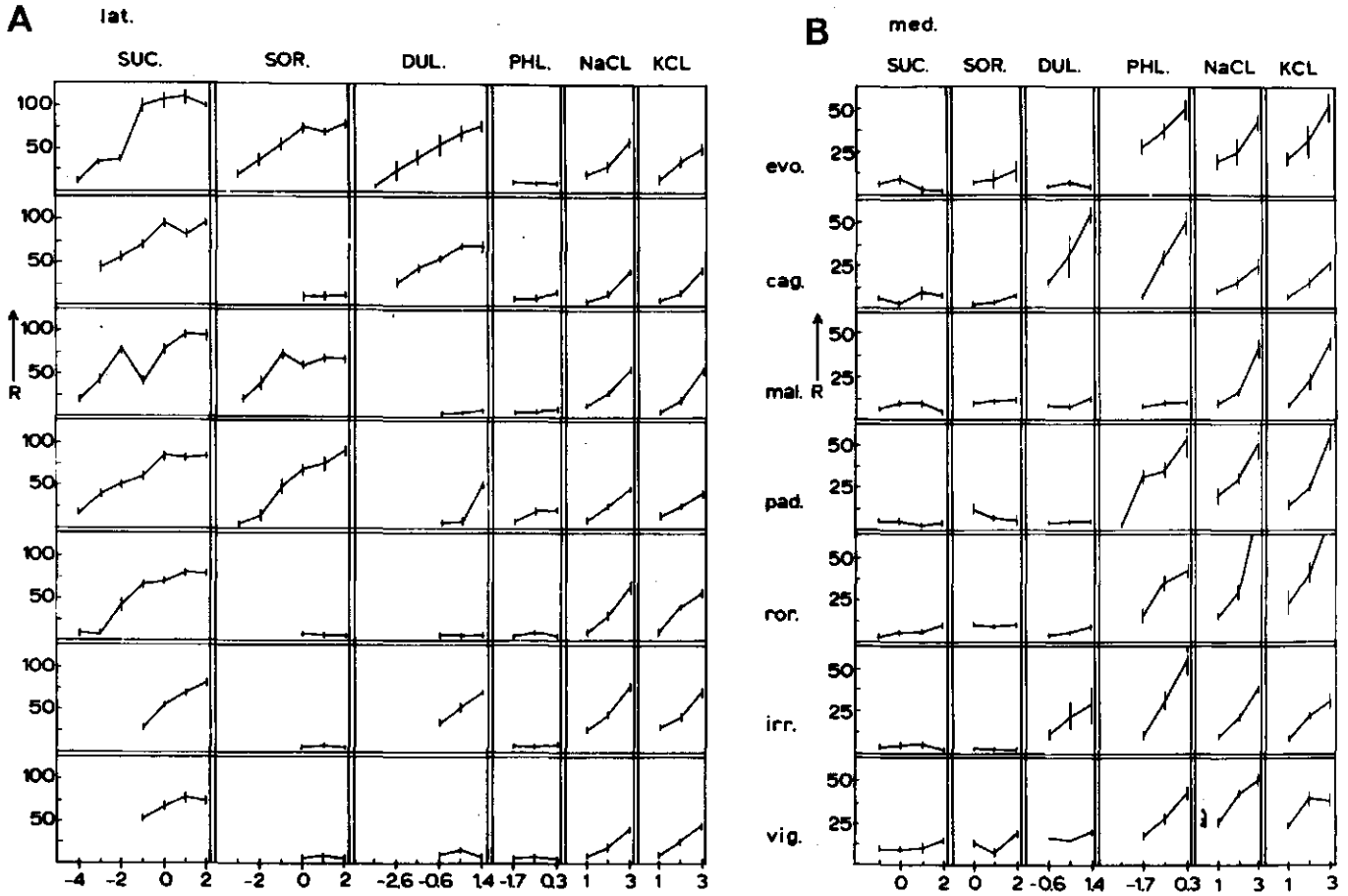

Fig. 5A and B. Stimulus-response relationships as recorded in the lateral (A) and medial sensilla (B). Abscissa: stimulus concentrations in log mM. Ordinates: average of the total number of action potentials encountered during the initial 500 ms of stimulation. For abbreviations of the species names see Table 2. Stimuli used are: *SUC.*, sucrose; *SOR.*, sorbitol; *DUL.*, dulcitol; *PHL.*, phloridzin; NaCl, and KCl. Most of the points represent an average value of five responses; vertical bars: standard error of the mean.

activity upon stimulation with increasing concentrations, in some cases followed by an approximately constant level of response at highest concentrations. When interpreting the graphs of Fig. 5 it should be realised that activities up to approximately 20 spikes/0.5 s reflect spontaneous activity. Below this level of activity, spike patterns are irregular and seldom demonstrate adaptation. The curves of dulcitol and phloridzin cannot be completed with data for higher concentrations, due to the limited solubility of these compounds. The stimulus-response curve recorded for sucrose in the lateral sensillum of *Y. malinellus*, interestingly, shows two peaks of sensitivity. The slope of this curve between 0.01 and 1.00 mM is too clear-cut to explain the bimodal shape of the curve by statistical fluctuation of neural activity. On the basis of spike amplitudes seen in the recordings it cannot be concluded that this phenomenon is due to the presence of more than one sucrose-sensitive cell, each cell possessing a different sensitivity range.

Although distinction between *Y. cagnagellus* and *Y. irrorellus*, both feeding on *Euonymus*, appears impossible on the basis of qualitative comparison (Table 2), it may readily be done with their conspicuous quantitative differences with respect to their sucrose, dulcitol and salt responses. The cells in the lateral hair of *Y. irrorellus* are less sensitive to sucrose and dulcitol than those of *Y. cagnagellus*. The same phenomenon is found for dulcitol sensitivity in the medial hair of these species. However, dulcitol failed to elicit responses in the medial sensillum of several specimens, which is manifested by the relatively large standard errors in the stimulus-response curves. Responses in both hairs to NaCl and KCl at the higher concentrations are low in *Y. cagnagellus* as compared to *Y. irrorellus* and the other species. The sensitivities of the medial hair of *Y. cagnagellus* and *Y. irrorellus* to phloridzin are remarkably similar. Somewhat unexpectedly in view of the fact that this compound is lacking in their host plants, *Y. evonymellus* and *Y. padellus* show a sensory reaction to dulcitol. Larvae of both *Y. padellus* host races are only sensitive to dulcitol solutions at near-saturated concentrations. On the contrary, larvae of *Y. evonymellus* are very sensitive to dulcitol, close to the sensitivity levels met in *Y. cagnagellus*.

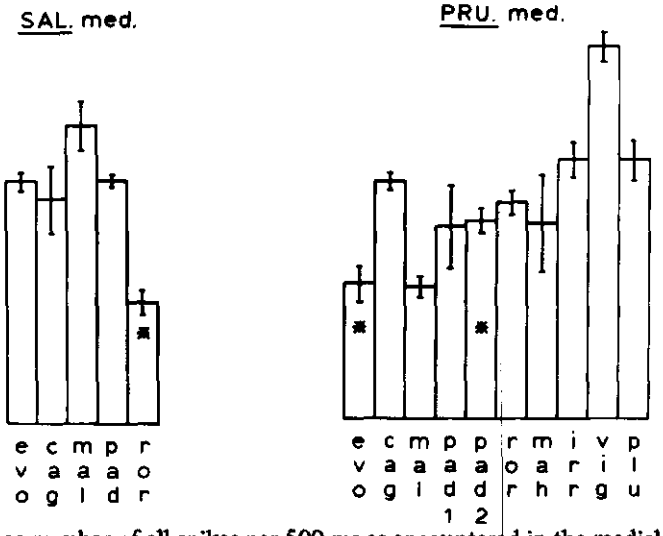

Fig. 6. Average number of all spikes per 500 ms as encountered in the medial sensillum in response to salicin (SAL.) and prunasin (PRU.). Vertical bars: standard error of the mean. For abbreviations of species names see Table 2. Symbol indicates that the compound occurs in the associated host. Highest response 85 spikes.

As represented in the response parameter chosen, no clear differences are found between the responses to NaCl and KCl. Only the cells in the medial hair of *Y. vigintipunctatus* seem more sensitive to higher concentrations of NaCl than of KCl. In *Y. rorellus* high responses to salt stimulation were recorded from the medial hair. The relationship between the sensitivities shown in Fig. 5 and the constituent concentrations represented in Table 1 will be discussed.

Salicin and Prunasin Responses

The responses to salicin in the lateral sensillum and to phloridzin in the medial sensillum generate interspecific patterns as may be expected for deterrents, i.e., the insects perceive compounds specific for non-host plants (van Drongelen 1978). Because prunasin and salicin responses in the medial hair occur in species which do not feed on plants containing these compounds, one might expect a similar but less pronounced sensitivity distribution for these compounds. The average number of spikes per initial 500 ms of salicin responses recorded from the medial sensilla are shown in Fig. 6. It can be seen that in the case of salicin, a sensitivity distribution as described above appears present for the species tested, i.e., species that do not feed on *Salix* are significantly more sensitive to salicin than *Y. rorellus*.

A histogram of impulse frequencies elicited by prunasin in the medial hair is shown in Fig. 6. Indeed *Y. evonymellus* (host: *P. padus*) displays a low activity, but host-race 2 of *Y. padellus* (host: *P. spinosa*) does not. Peculiar responses to prunasin are recorded in medial sensilla of *Y. malinellus* and *Y. vigintipunctatus* which show a weak and a strong reaction, respectively. In the remaining species the responses to prunasin appear to fluctuate within the same range.

Discussion

Recordings

In the species studied sucrose and sugar alcohols are mainly perceived by the lateral sensilla. One may ask whether these compounds elicit responses in one or different cells. On the basis of spike amplitudes, it is concluded that sucrose, dulcitol and sorbitol (as far as perceived by a particular species) stimulate different cells in each species. The spike amplitudes in the responses of the medial sensillum do not suggest that (+)-catechin, phloridzin, prunasin and salicin are perceived by different neurones in the species studied.

Examination of the fine structure of gustatory sensilla revealed the presence of a distal pore via which the sensory neurones contact outer media (Fig. 2E). In several cases it has been reported that such pores may vary in diameter (Blaney and Chapman 1969; Bernays et al. 1972; van der Wolk 1978). During some of the recordings it seemed as if the pore diameter varied from small to large. After the stimulus solution contacted the hair, neural activity encountered was very low and the noise level was sometimes high. These facts indicate that inward diffusion is obstructed and that electrical contact is established via a high resistance. When contact between stimulus electrode and the hair was continued, occasionally a blocking artefact accompanied by a sudden drop in resistance and response onset was displayed. Recordings of this type were met from time to time during stimulation with all com-

pounds used in this study.

The peripheral gustatory perception mechanism can be subdivided into the following sequential processes: 1. absorption of the water dissolved stimulus molecules from the stimulus pipette into the solution in the dendritic lumen, 2. diffusion of absorbed molecules in the tip chamber and 3. molecules affecting the neurone's membrane, and triggering chemo-electrical transduction. Each of these processes is probably performed at different rates for each stimulus. Because the three processes take place sequentially, the slowest one will mainly determine the time course of the end product: the neural response. Considering absorption as a reversible exchange of molecules between the stimulus and the internal medium of the hair, the time course of the number of molecules in the tip chamber at time t will satisfy the equation: $A\ (1\text{-}e^{-\lambda t})$, with A and λ constants. Qualitatively, when the absorption process determines the time course of the neural response R, then in a plot of R versus t, R will increase proportionally with t without an inflection point. The solution of the diffusion equation yields the expression: $C(d, t)=B+\varphi$ erfc(d, t) (Rees 1968). In this equation $C(d, t)$ is the concentration of stimulus molecules as a function of d and t, B and φ are constants, d is the distance between the sensitive membrane and the terminal pore in the gustatory sensillum, and erfc is Gauss' complementary error function. The function erfc(d, t) implies that the graph of the stimulus concentration at distance d plotted versus time t displays a slightly S-shaped slope. Thus if diffusion is the slowest process of the three described above, the time course of the neural response may be expected to be similar to erfc(d, t) and a graph of R as a function of t will be characterised by an inflection point. Any experimental data on the third process, chemo-electrical transduction, are lacking. However, one can try to determine whether there are response parameters that vary in time, similar to one of the first two processes. Graphs in which the sequence number of the action potential is plotted versus time demonstrate weakly S-shaped curves for the responses to non-ionized substances. An example of such a plot of a sucrose response encountered in the lateral sensillum of *Y. padellus* is shown in Fig. 7. This plot indicates that the time course

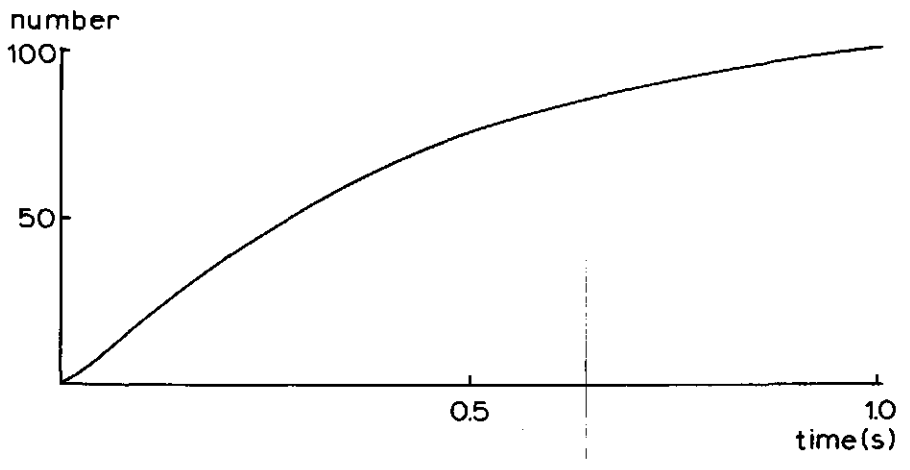

Fig. 7. Action potential sequence number plotted versus time. This plot is based on a response to sucrose in the lateral sensillum of *Y. padellus*.

of the initial response may be determined by the diffusion process. If this is true it means that the initial action potential frequencies in the responses to non-ionized substances are proportional to the rate of change of the stimulus concentration around the receptor cell. The salt responses show a cumulative plot with a very irregular slope. Absorption and diffusion processes can explain such an irregular slope when few molecules generate a single action potential. Thus one can conclude that the time course of the salt response is mainly determined by either stochastic fluctuations in the absorption and diffusion processes or by the transduction mechanism.

In order to investigate whether one may describe action potential occurrence by a stochastic process, interspike interval histograms of the responses were made. These interval histograms appeared to differ considerably from one response to another; even between responses to the same compound. One general aspect in the interval histograms is the presence of few short and very long intervals as compared to the moderate ones. An example of a histogram showing this property is shown in Fig. 8. In a number of recordings, the special Erlang and Γ-distributions (Cox 1962) appear reasonable candidates to describe action potential generation in terms of statistical events.

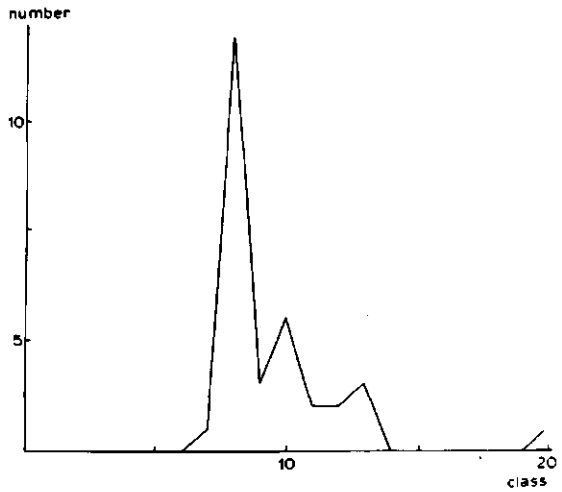

Fig. 8. An interspike interval distribution histogram. Interval ranges are indicated on the abscissa, the occurrence of intervals in each range on the ordinate.

Receptor Sensitivity and Host Selection

Considering the stimulus-response relationships in Fig. 5, it can be concluded that no *subtle* quantitative differences between the curves in relation to host plant composition appear present. Furthermore one may note that, with the exception of the curves representing spontaneous spike discharge, the sensitivities to the same substances are approximately all in the same concentration range for different species. Of course statistical analysis may demonstrate significant differences between the sensitivities recorded. But in order to have a biological criterion to judge similarity or dissimilarity of the curves presented in Fig. 5, it would be necessary to compare them with corresponding curves recorded from other caterpillar species. A comparison between the sucrose sensitivity in the lateral styloconic sensillum of *Pieris brassicae* and *Mamestra brassicae* (Ma 1972; Blom 1978) and the sucrose sensitivity

found in the small ermine moth species, leads to the conclusion that the former species are somewhat less sensitive (concentrations above 10^{-1} mM) than the latter (concentration above 10^{-3} mM). However, these authors used a salt solution as a stimulus solvent and it is known that the sucrose response is lowered under influence of salt ions (Schoonhoven 1967). An important aspect of the system studied here is that probably few contact-chemoreceptors are involved in host selection. This is an essential difference with the invertebrate and vertebrate olfactory system and the gustatory senses in, e.g., adult flies and locusts, which are characterised by huge convergence ratios. Due to convergence of receptor cell axons, weak sensory responses are "amplified" in the central nervous system (van Drongelen et al. 1978). Thus in systems with high convergence ratio the natural stimulus intensities are, as may be expected, in the range of receptor cell sub-threshold levels (Kaissling and Priesner 1970; van der Starre 1972). In caterpillar gustatory system a few receptor cell responses determine food selection behaviour. Accordingly, it has been shown in *Pieris brassicae* that (for a number of relevant compounds) gustatory receptor cell sensitivity is approximately one-to-one related to behavioural sensitivity (Ma 1972; Blom 1978). In connection with this it is interesting to find that natural constituent concentrations are situated in that part of the stimulus-response relationship where the response is highest and approximately constant for varying concentrations (Table 1 and Fig. 6). The classical idea of receptor function is that the organism would use the slope of the stimulus-response curve for quantitative discrimination. In spite of possible synergistic effects and adaptation at the chemo-electrical transduction level, which can (under natural conditions) reduce sensitivity for the compounds tested, this may not be the case here. As can be seen in Table 1, plant constituent concentrations fluctuate during the day and one day to another (Seybold 1968; Maas 1957; Jeremias 1964). When these fluctuations take place in that part of the stimulus-response relationship where a change in the stimulus intensity effects a minimal change in response, the perception mechanism assures a relatively stable neural response. This in its turn may be important in maintaining a stable across-fiber pattern of the gustatory organ, probably involved in food recognition (Schoonhoven 1977).

Comparing sensitivity spectra, and classifying their functions in relation to host selection is difficult because of lack of knowledge of the central neural pathways. However, by using general assumptions it may be possible to understand the functional diversity described. One of the simplest assumptions is that host constituents will directly or indirectly stimulate food intake; accordingly one expects the insects to be sensitive to some of the compounds that occur in their associated hosts. The sensitivity patterns for sucrose, sedoheptulose, sorbitol, coumarin, phloridzin, prunasin, rutin, (+)-catechin, $ZnCl_2$, NaCl, and KCl in the lateral hair (Table 2A) and for sucrose, sedoheptulose, sorbitol, dulcitol, coumarin, rutin, isocitric acid, $ZnCl_2$, NaCl, and KCl in the medial hair (Table 2B) are not in contradiction with the expectation stated above. If it is assumed that non-host constituents (directly or indirectly) inhibit food intake one can expect to record sensitivity as found for phloridzin in the medial sensillum and for salicin in the lateral one. In addition, it may also be partially true for salicin and prunasin perception in the medial sensillum (Fig. 6). An assumption in the model used here is that the central nervous systems of all species considered act in a similar way. In addition, a few observations on feeding behaviour advocate the assumption of similar central nervous systems: food intake of *Y. evonymellus* is stimulated by dulcitol and the same behaviour in *Y. cagnagellus* is inhibited by phloridzin (van Drongelen, unpublished results).

Some of the results cannot be understood by the simple assumptions presented. This is the case for the sensitivities to prunasin, salicin and (+)-catechin in the medial sensillum and to dulcitol and isocitric acid in the lateral one. The peculiar sensitivity profiles for dulcitol and prunasin will be discussed from an evolutionary point of view in the next section.

A comparison between qualitative sensitivity patterns in other lepidopterous species, as cited in the literature, must be limited to a few examples. Sucrose and salt sensitive neurones are described in a large number of species (Schoonhoven 1972). Sorbitol receptors are known from the lateral styloconic sensilla of *Malacosoma americana* and *Episema caeruleocephala* both feeding on sorbitol-containing Rosaceae (Dethier and Kuch 1971; Schoonhoven 1972). Salicin receptors have been found in *Manduca sexta*, *Bombyx mori*, *Estigmene acrea*, *Danaus plexippus*, *Ceratomia catalpae*, *Papilio poluxenes*, *Pieris rapae* and *Isia isabella* (Ishikawa 1966; Dethier and Kuch 1971; Schoonhoven 1972); none of these species normally feeds on salicin-containing hosts. Amygdalin, a compound chemically similar to prunasin, can be perceived by *Estigmene acrea*, *Ceratomia catalpae* and *Isia isabella* feeding on plants which do not contain this compound but it cannot be perceived by *Malacosoma americana* collected on *Prunus virginiana* (Dethier and Kuch 1971). Phloridzin, highly specific for apple, can be sensed by the tobacco hornworm *(Manduca sexta)* and *Adoxophyes reticulana* (Schoonhoven 1972), the latter species feeding on apple. This finding indicates that interspecific differences in central mechanisms of gustatory data processing are probably present, i.e., food intake of *M. sexta* larvae is inhibited by phloridzin, but in the case of *A. reticulana* it is unlikely that this compound inhibits feeding activity. This difference may be caused either by different central pathways or by identical peripheral sensitivity located in non-homologous and differently connected receptor neurones. This may be related to the fact that *M. sexta* and *A. reticulana* belong to different superfamilies.

Evolutionary aspects

Comparative investigation of sensitivity to compounds occurring in plants of the natural environment seems a useful approach to analyse plant-insect interactions from an evolutionary point of view. Screening of chemosensitivity spectra of the lateral and medial styloconic sensilla demonstrated some illogical differences in sensitivity to specific compounds. For instance, dulcitol sensitivity is found in species that feed on plants which positively do *not* contain this compound. Furthermore, food intake of larvae of *Y. evonymellus* is stimulated by dulcitol (van Drongelen, unpublished results). If prunasin inhibits food intake it is logical that *Y. evonymellus* (feeding on *P. padus*) responds comparatively weakly to this compound. But in this context it is not *a priori* to be expected that *Y. malinellus* (feeding on *Malus*) responds equally weakly and that host-race 2 of *Y. padellus* (feeding on *P. spinosa*) (Fig. 6B) responds relatively strongly. To assign a biological role to the sensitivities described above seems impossible. Of course one can suggest that for instance the dulcitol receptor site in *Y. evonymellus* may be able to sense other *host* plant constituents but that still does not assign a biological role to dulcitol sensitivity in these insects! From an evolutionary point of view one may assume that these sensitivities are pre- or post-adaptative. In the following, the latter assumption will be examined.

The eggs from which the *Yponomeuta* larvae hatch, are laid on the appropriate host by the ovipositing female. Larvae of the small ermine moth species have

limited mobility and only use gustatory specificity when different plants grow interwovenly. Suppose there exists some direct coupling between the larval and adult host preference, then a preference alteration in one of these stages would cause an alteration in the other stage too. When no such coupling is present, which seems in view of the present knowledge more probable, an alteration of host preference in an adult insect leaves its offspring with a choice between eating and dying. It is conceivable that some of the constituents of the "new" host stimulate the larval chemoreceptors. Then survival of the altered individuals is likely when two conditions are satisfied: (1) not too many receptor responses must inhibit food intake; (2) the "new" host should contain sufficient nutrients and should not contain toxic substances. Such a process may initiate speciation and could explain persisitence of sensory sensitivity to compounds of ancestral hosts.

Based on the concept of sensory persistence and the sensitivity patterns for dulcitol and prunasin, three phylogenetic relationships between the species studied will be outlined. Behavioural aspects will be incorporated in the following consideration because conspicuous deviations from natural food-choice behaviour corroborate the conclusions to be drawn. First it is concluded that *Euonymus* (Celastraceae) probably served as a host plant of a common ancestor. This is based upon the fact that three species, all non-*Euonymus* feeders have receptors sensitive to dulcitol. Furthermore, the host plant distribution amongst the ermine moth species indicates an ancestral relationship with either Rosaceae or Celastraceae (Wiebes 1976); dulcitol is a typical compound for Celastraceae (Hegnauer 1964). In connection with this it is interesting to find that *Prunus*-feeding *Yponomeuta* species accept *Euonymus europaeus* as food-plant under experimental conditions (Gerrits-Heybroek et al. 1978). In accordance with the dulcitol sensitivity patterns found (Table 2), *Y. malinellus* does not accept *Euonymus* leaves (Gerrits-Heybroek et al. 1978). However, the observation that *Y. rorellus* accepts the whole array of *Yponomeuta* host plant, including *Euonymus europaeus*, is difficult to reconcile with dulcitol sensitivity of this species. Second conclusion: the three *Yponomeuta* species living on *Prunus* "recently" evolved from an ancestor(s) feeding on Celastraceae. This suggestion is based on two facts: (1) with the exception of host-race 1 of *Y. padellus*, examples of sensitivity to dulcitol are displayed by small ermine moth species feeding on *Prunus* sp., and (2) all species living on *Prunus* can perceive prunasin, a compound which commonly acts as deterrent. Third conclusion: some *Prunus* species may have been the host plant of the ancestor of *Y. malinellus*. This conclusion is drawn from the fact that *Y. malinellus* displays a low sensitivity to prunasin as is the case in larvae of *Y. evonymellus*. This seems in accordance with the finding that *Y. malinellus* larvae show a preference for *P. spinosa* and *P. domestica* over their normal natural host (Gerrits-Heybroek et al. 1978).

Neither sensory aspects nor behavioural data permit any hypothesis on the phylogenetic relationship between *Y. rorellus*, *Y. vigintipunctatus* and the remaining species.

Significance of Signal Compounds

In one of the previous sections it is suggested that different chemicals may act as phagostimulants or may inhibit food intake. The latter type of compounds are indicated as deterrents (Schoonhoven and Jermy 1977). On a neurophysiological basis, chemical signals may be obtained by various perception mechanisms. At the receptor level, for example, chemicals may elevate or inhibit discharge of receptor neurones.

Combinations of chemicals may show positive or negative synergism. At the central level compounds may stimulate or inhibit food intake either directly or indirectly. The question may arise whether there is a relationship between the neurophysiological action of the signal and its significance to the animal. In this context, significance can have various meanings such as: (1) the signal compound is nutritionally important or its occurrence (within the plant metabolism) is coupled to synthesis of nutritionally important compounds, (2) the signal compound is poisonous to the animal itself, its predators, its parasites or its competitors and (3) the occurrence of the signal compound in different plant species is specific enough to permit host recognition. The sugars and sugar alcohols tested in this study probably have a nutritional value to the larvae. In addition, the characteristic distribution of sorbitol and dulcitol render them suitable as host recognition factors. But, as pointed out by Dethier (1952), signal compounds are certainly not restricted to nutritionally important constituents. For instance coumarin is toxic to larvae of *Y. padellus* (both host-races) and *Y. rorellus* potential competitors of *Y. mahalebellus* (van Drongelen and van Loon, unpublished results) and its distribution over other plant species is sufficiently restricted to make it at the same time a host recognition factor. Phloridzin is known to inhibit general enzymatic reactions and thus lowers the metabolism in species that cannot deal with this compound. Although the occurrence of phloridzin is specific enough for a potential host-specific phagostimulant, this is not the case in *Y. malinellus*. In conclusion it may be stated that the present results indicate that the relationship between the effects of plant constituents on sensory activity and their biological significance is not *a priori* predictable. The relationship between host-plant composition and sensory sensitivity is intelligible in many of the cases studied, but the discussion in the previous sections indicated that natural selection seems to permit the presence of some non-adaptive sensitivity.

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CHAPTER 2 Gustatory Sensitivity and Taxonomic Relationships in Larvae of some *Yponomeuta* species (Lepidoptera)

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Caterpillars perceive their food's taste by a small number of contact chemoreceptors (Ma, 1972; Schoonhoven, 1973). Most receptors are situated in lateral and medial styloconic sensilla, which are located on the mouth parts (Ma, 1972).

In the present study, diversity in electrical responses of gustatory neurones of seven *Yponomeuta* species are analysed using statistical techniques. The species studied and their host plants are represented in the left columns of Table 1. In this table, it can be seen that the larvae display restricted and different host plant preferences. Though most of the insects are closely related from a taxonomist's point of view, the different hosts on which they feed may suggest interspecific diversity in the gustatory perception mechanism (Herrebout et al., 1976). The question to be answered is whether the contact-chemosensitivity patterns of the species will merely reflect their taxonomic relationship, or will display a functional diversity correlated with their natural food.
Electrophysiological and evolutionary aspects are described in a separate paper (van Drongelen, 1979).

Gustatory responses encountered during stimulation of lateral and medial styloconic sensilla were lead off using a tip recording technique (Hodgson et al., 1955). Taste substances were tested at concentrations close to those occurring naturally (van Drongelen, 1979).

Qualitative sensitivity as recorded in the styloconic sensilla of the various species is indicated in Table 1; "+" denotes presence, "o" absence of a gustatory response. When a particular compound occurs in the associated host plant, this is indicated by (p). Sucrose and salts (NaCl and KCl) occur in all hosts. Sorbitol and dulcitol are specific sugar alcohols for Rosaceae and Celastraceae respectively. Phloridzin is highly specific for apple and prunasin for *Prunus* species. A response to sucrose, prunasin and salts is encountered in all species. Sorbitol sensitivity is restricted to species feeding on Rosaceae. Dulcitol responses are recorded in species feeding on *Euonymus europaeus* (Celastraceae), but sensitivity to this constituent is not restricted to them. A typical response pattern is found for phloridzin: this compound elicits responses in the medial sensillum of all species with the exception of *Y. malinellus* (feeding on apple). On the basis of the qualitative sensitivity patterns presented, distinction can be made between the following four species groups: 1. *Y. evonymellus* and *Y. padellus*, 2. *Y. cagnagellus* and *Y. irrorellus*, 3. *Y. malinellus* and 4. *Y. rorellus* and *Y. vigintipunctatus* (Table 1).

In order to investigate sensitivity patterns in more detail, most stimuli were applied at different concentrations (legend, Fig. 1). Responses were quantified by the total number of action potentials displayed during the initial second of stimulation. A measure for intra-specific variation was obtained by applying a single stimulus to five individuals of each species. In this way, each species is characterized by five arrays containing indices of responses to different compounds as recorded in

Table 1. *Yponomeuta* species and associated host-plants. Qualitative gustatory sensitivity for different compounds is indicated by the symbols "+" (positive) and "o" (negative response). The occurrence of the compounds in the host-plant is indicated by (p). On the basis of the qualitative response patterns four species groups can be distinguished, which are indicated in the last column.

Yponomeuta species	Host-plant	sucrose	NaCl and KCl	sorbitol	dulcitol	phloridzin	prunasin	species group
Y. evonymellus	*Prunus padus*	+(p)	+(p)	+(p)	+	+	+(p)	1
Y. cagnagellus	*Euonymus europaeus*	+(p)	+(p)	o	+(p)	+	+	2
Y. malinellus	*Malus sp.*	+(p)	+(p)	+(p)	o	o (p)	+	3
Y. padellus	*Crataegus laevigata*	+(p)	+(p)	+(p)	+	+	+	1
Y. rorellus	*Salix sp.*	+(p)	+(p)	o	o	+	+	4
Y. irrorellus	*Euonymus europaeus*	+(p)	+(p)	o	+(p)	+	+	2
Y. vigintipunctatus	*Sedum telephium*	+(p)	+(p)	o	o	+	+	4

both sensilla. These arrays can be used as descriptions of the "physiological specimens" in a multidimensional vector space, taking the stimuli as vectors and the scores as coordinates. After standardisation of the vectors, in order to obtain a vector space with a uniform scale, the Mean Square Distance between the specimens was computed and grouping of the arrays with the smallest fenetic resemblances was accomplished by means of Ward's Average as cluster analysis (Sneath & Sokal, 1973). This analysis was carried out by the IBM 370/158 computer of the University at Leiden, using the computer program BIOPAT, developed by P. Hogeweg and B. Hesper[1].

The results of the cluster analysis is represented as a dendrogram in Fig. 1. The electrophysiological responses of the individuals used, appear to cluster in distinct species-clusters, with the exception of one specimen of *Y. padellus* (arrow, Fig. 1). In relation to this aberrancy, it is interesting to note that another host-race

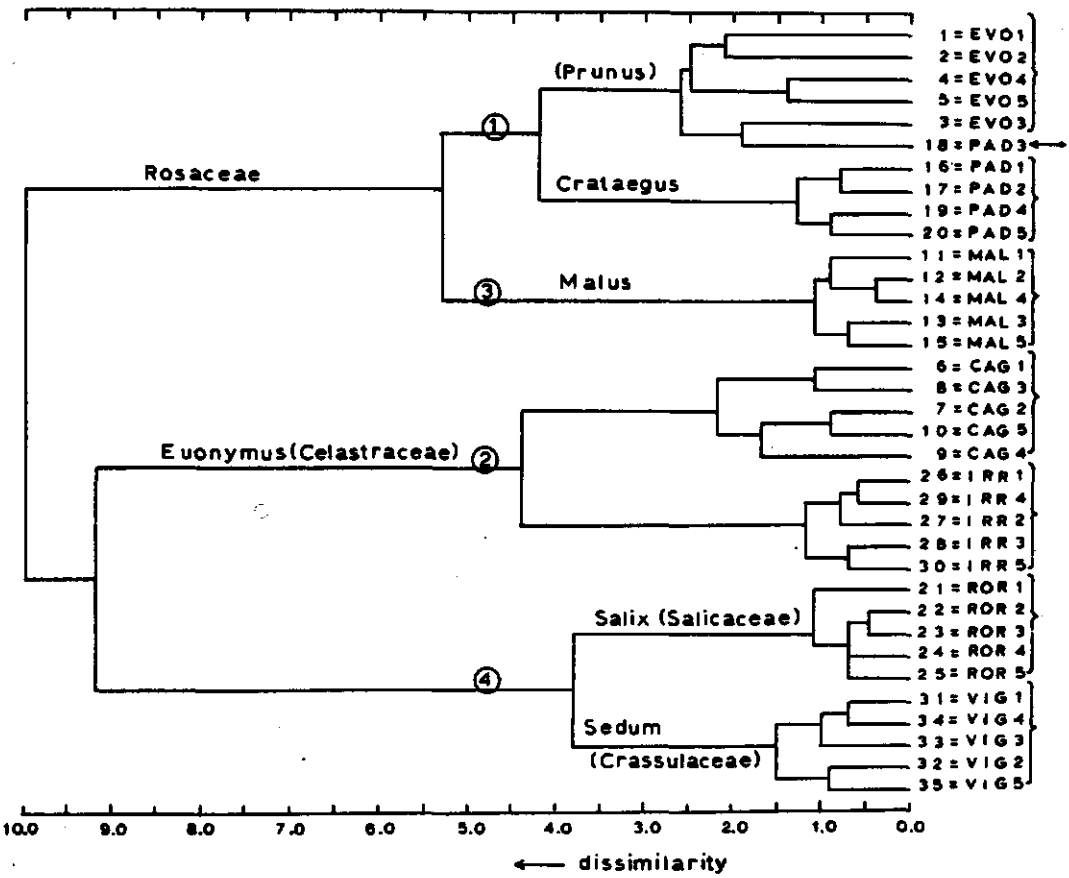

Figure 1. A dendrogram of clusters as based upon quantitative chemosensitivity patterns of seven *Yponomeuta* species. The compounds (as indicated in Table 1) were tested at the following concentrations (Mol): sucrose (10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}), NaCl and KCl (10^{-2}, 10^{-1}, 1), sorbitol (10^{-3}, 10^{-2}, 10^{-1}), dulcitol ($2.5\ 10^{-4}$, $2.5\ 10^{-3}$, $2.5\ 10^{-2}$), phloridzin ($2\ 10^{-5}$, $2\ 10^{-4}$, $2\ 10^{-3}$), prunasin (10^{-2}). The group numbers assigned in Table 1 appear in the four largest clusters. With the exception of *Y. padellus* (arrow), all species can be classified in distinct groups. The species are clustered according to taxonomic relationships of the host-plants.

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of *Y. padellus* does not feed on *Crataegus*, but on *Prunus spinosa*. As far as studied, both host-races of *Y. padellus* seem to have similar gustatory sensitivities (van Drongelen, 1979). This might explain a close functional relationship between *Y. evonymellus* (feeding on *Prunus padus*) and *Y. padellus*. In Fig. 1 it can be seen that the *Yponomeuta* species are mainly grouped according to taxonomic relationships of their host plants. A remarkable accordance appears to exist between the pattern displayed in Fig. 1 and hypothetical phylogenetic relationships as described by van Drongelen (1979).

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CHAPTER 3 Behavioural responses of two small ermine moth species (Lepidoptera: Yponomeutidae) to plant constituents

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Abstract

On the basis of electrophysiological screening of lateral and medial styloconic sensilla of caterpillars of *Yponomeuta* species, several hypotheses concerning behavioural effects of plant constituents can be posed (van Drongelen, 1979). Feeding experiments have been carried out with intact larvae of *Y. cagnagellus* and *Y. evonymellus*. Both species display neural responses to dulcitol, phloridzin and prunasin (van Drongelen, 1979). Leaf discs of the host plants of these species were modified using these three constituents and effects were studied in choice and non-choice situations. Dulcitol appears to stimulate food intake of both species, whereas phloridzin acts as a deterrent exclusively in a choice situation. Prunasin inhibits feeding of *Y. cagnagellus* in a choice situation, but *Y. evonymellus* does not show a reaction to this compound at the concentration applied. Neural mechanisms probably underlying these behaviours are discussed.

Introduction

Larvae of different *Yponomeuta* species display different host plant preferences (Herrebout et al., 1976; Gerrits-Heybroek et al., 1978). Some aspects of host choice can be related to sensitivity patterns of the gustatory neurones of the caterpillars; e.g. gustatory neurones of larvae which feed on *Rosaceae* respond to sorbitol, a constituent typically occurring in this plant family (van Drongelen, 1978, 1979). However, in several cases peripheral sensitivity to non-host plant chemicals was also found and may either play a rôle in recognition of non-host plants, or be post- or pre-adaptive (van Drongelen, 1978, 1979; Gerrits-Heybroek et al., 1978). In the case of host plant recognition, chemicals characteristic of non-hosts probably inhibit food intake, whereas in a post- or pre-adaptive situation non-host chemicals which can be tasted may stimulate food intake or be neutral.

The purpose of the present study is to compare electrophysiological and behavioural reactions of *Y. cagnagellus* and *Y. evonymellus* to constituents specific for three *Yponomeuta* host plants. In a number of cases, the chemicals tested (dulcitol, phloridzin and prunasin) are known to elicit neural responses in larvae of a number of species which do not feed on plants containing these substances (Hegnauer, 1964, 1973; van Drongelen, 1978, 1979). Taxonomical affiliations of the species used in the experiments are described in Gerrits-Heybroek et al. (1978).

Materials and Methods

Fifth instar larvae of *Y. cagnagellus* and *Y. evonymellus* were collected in the field and stored in a refrigerator at 6° for a maximal duration of one month. The larvae were fed on leaves of their associated hosts (Table 1).

When testing whether phloridzin acted as a deterrent, it was added to host plant leaf discs (1.3 cm diam.). These discs were impregnated with solutions to be

tested under vacuum over 24 hr at 6°. When dulcitol was to be tested for phagostimulatory effects, this impregnation technique did not seem suitable as the host plant itself may represent the optimal food and it seemed doubtful whether adding stimulant to host leaves would raise food intake. For this reason, host plant leaves were freeze dried and plant substances were extracted by placing the dried leaves in ethanol (*pro analyse* quality) for a duration of two weeks. In this manner, a relatively neutral medium for adding hypothetical stimulants and preparing "leaf" discs was obtained. Leaves of *Prunus padus* (host of *Y. evonymellus*) contain prunasin and leaves of *Euonymus europaeus* (host of *Y. cagnagellus*) do not. Therefore freeze dried and leached host plant discs impregnated with dulcitol (known to stimulate feeding of both species) were chosen as the control medium to investigate effects of prunasin. Immediately before each experiment leaf discs were taken out of the test solutions and dried on filter paper. Chemicals (*pro analyse* quality) were obtained from commercial sources; dulcitol (Baker), phloridzin and prunasin (Roth).

Two types of experiments were carried out; in one type ten larvae of a single species were placed in a petri-dish (4 cm diam.) with two differently treated leaf discs (choice experiment), in the other type one larva and one disc were placed in a petri-dish (non-choice experiment). In the latter type of experiment, food intake was quantified by measuring the surface area of the leaf disc before and after the experiment. In the choice experiment all insects contributed to the feeding behaviour described below, as was noted visually. During a period of 24 hr preceding the experiments, the larvae were kept in the petri-dishes with their natural food. No period of starvation was applied. In order to reduce effects of water loss from the leaf discs, all experiments lasted maximally 3 hr at mean temperature of 21°.

Results

Data concerning sensory sensitivity to compounds used, the occurrence of the constituents in *Yponomeuta* hosts, the species used and their associated hosts are represented in Table 1. The symbols "l" and "m" denote that a neural response to a particular compound is encountered in the lateral or medial maxillary styloconic sensillum.

Table 1. *Yponomeuta* species, their hosts, chemicals tested and their occurrence. Symbols "l" and "m": presence of a neural response in lateral and medial styloconic sensilla, respectively. Brackets: effects of compounds at behavioural level.

Chemicals and Occurrence	Dulcitol (*Euonymus europaeus*)	Phloridzin (*Malus sp.*)	Prunasin (*Prunus padus*)
Yponomeuta species and associated hosts			
Y. cagnagellus (*Euonymus europaeus*)	l, m (stimulant)	m (deterrent)	m (deterrent)
Y. evonymellus (*Prunus padus*)	l (stimulant)	m (deterrent)	m (neutral)

Dulcitol can be perceived by larvae of both species (van Drongelen, 1979). In the case of *Y. cagnagellus* this sensitivity appears logical because dulcitol is specific to Celastraceae upon which it feeds. The presence of dulcitol sensitivity in *Y. evonymellus*, on the contrary, seems redundant because sugar alcohols commonly activate caterpillar food intake and dulcitol is not contained in its natural food. On the basis of several arguments it has been assumed that dulcitol sensitivity in *Y. evonymellus* is related to its association with ancestral host plant(s) (van Drongelen, 1978, 1979; Gerrits-Heybroek et al., 1978). To examine the behavioural response to dulcitol, two choice experiments (as described above) with freeze dried and extracted host plant leaf discs, one impregnated with distilled water (none of the species displayed a neural response to water) and the other with a solution of 25 mM dulcitol, were carried out on larvae of both moth species. In all four experiments dulcitol impregnated discs were eaten within two hours, only small bites were taken from the water impregnated discs. In addition, the behavioural effect of dulcitol was tested in a non-choice experiment on 35 larvae of *Y. evonymellus*; 19 insects received a freeze dried and extracted host plant disc impregnated with a 25 mM dulcitol solution and 16 insects received an identical prepared disc impregnated with distilled water only. In three hours 30% of the dulcitol impregnated discs were eaten and only a few bites were taken from the water impregnated discs.

With the exception of *Y. malinellus*, sensitivity to phloridzin is encountered in gustatory neurones of larvae of all nine european small ermine moth species (van Drongelen, 1979). This is a peculiar sensitivity pattern because phloridzin is highly specific to apple, the host of *Y. malinellus*. Therefore, it was assumed that phloridzin inhibits food intake in larvae that can perceive this compound. To test this assumption two choice experiments were carried out on both species; one host plant leaf disc was vacuum impregnated with distilled water and one with a phloridzin solution of 2 mM. In these experiments, larvae of both species first ate the water impregnated disc. But after this disc was eaten, the disc impregnated with phloridzin was fed upon. Because of this observation it was concluded that phloridzin probably does not block food intake in a direct manner. This hypothesis was examined in a non-choice experiment; 29 larvae of *Y. cagnagellus* received a water impregnated host plant leaf disc and 28 larvae received a phloridzin (2 mM) impregnated one. In relative units, the group that received water impregnated discs ate 13.90 $\pm$ 3.65 (representing the mean amount $\pm$ standard deviation), the other group 14.14 $\pm$ 5.67. As judged by a Mann-Whitney U-test the mean amounts eaten did not differ significantly at a 0.05 level. However, the variance of the amounts eaten in the group that received the phloridzin impregnated disc was larger than in the control group. By visual examination it appeared that insects that started a meal on a phloridzin impregnated disc displayed retracting movements with the anterior part of the body, which was occasionally followed by locomotion. This type of behaviour was infrequently observed in larvae feeding on water impregnated leaf discs.

Prunasin elicits a neural response in the medial styloconic sensillum of nine *Yponomeuta* species (van Drongelen, 1979). From this fact one might question which behavioural effects this constituent may have in species that live on prunasin containing hosts as compared to effects in the other species. In addition, it has been found that neural sensitivity to prunasin of *Y. evonymellus* (living on *Prunus padus* which contain high levels of prunasin) is significantly lower than the sensitivity in larvae of *Y. cagnagellus* (van Drongelen, 1979). The action of prunasin at the behavioural level was studied in two choice experiments on larvae of the two species. In

these experiments one freeze dried and leached host plant disc was impregnated with dulcitol (25 mM) and the other with a mixture of dulcitol (25 mM) and prunasin (10 mM). In the case of *Y. cagnagellus*, larvae preferred discs impregnated with dulcitol only but they continued feeding on the prunasin containing disc after the most attractive one was swallowed. Larvae of *Y. evonymellus* did not display any preference, they fed on both discs in equal amounts. The behavioural effects of the chemicals tested is summarized in Table 1.

Discussion

Small ermine moth caterpillars possess externally located gustatory sensilla, i.e. lateral and medial styloconic sensilla, several small hairs on the palpi and a pair of epipharyngeal organs (van Drongelen, 1979). The lateral and medial styloconic sensilla have been screened electrophysiologically and are known to respond to the stimuli used in this study. Recordings of palpal hairs never displayed any reaction to the chemicals applied here, but the impact of the epipharyngeal organs on the behaviour of intact insects cannot be taken into account. In spite of this fact, some neural mechanism which may underly the behaviour observed are described below.

The control experiments in which freeze dried and leached discs impregnated with distilled water were applied, demonstrate that continuous feeding demands some gustatory stimulus.

The experiments with phloridzin demonstrated that this compound acts as a deterrent in a choice situation only. However, this action of phloridzin may not be effected by direct inhibition of the neural pathway which stimulates the motor system governing food intake. In this case, the inhibition of food intake can be achieved indirectly; phloridzin probably stimulates central motor systems of functions which are "contradictory" with feeding, such as locomotion. Behavioural responses to prunasin demonstrate that differences in central processing of sensory signals may exist between the species studied. Alternatively, it might be hypothesized that the relatively low responses to prunasin as encountered in larvae of *Y. evonymellus* (as compared to most of the other species) are not recognized as "true" responses by the central nervous system of this insect.

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Ent. exp. & appl., (in press)

CHAPTER 4 Inheritance of gustatory sensitivity in F1 progeny of crosses between *Yponomeuta cagnagellus* and *Yponomeuta malinellus* (Lepidoptera)

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Abstract

Larvae of *Yponomeuta cagnagellus* and *Y. malinellus* display clearly different neural responses to dulcitol, phloridzin and sorbitol (van Drongelen, 1979). Reciprocal crosses of these species have been examined for gustatory sensitivity to these plant constituents. Evidence is obtained that sensitivity to a particular compound is dominant or semi-dominant over non-sensitivity. No significant differences could be found in neural responses between F1 progeny reared on different host plants. Quantitative results concerning inheritance of sorbitol sensitivity cannot be presented. This is due to a small signal-to-noise ratio in the sorbitol responses of the crosses.

Introduction

In lepidopterous larvae, contact chemoreception plays a crucial rôle in host plant preferences (Waldbauer & Fraenkel, 1961; Ishikawa, 1963; Schoonhoven, 1972; Dethier & Kuch, 1971; van Drongelen, 1979). In *Pieris brassicae*, the amount of food intake of artificial diets appears closely related to peripheral gustatory sensitivity to diet constituents (Ma, 1972; Blom, 1978). Examination of genetic mechanisms controlling inheritance of gustatory sensitivity is one step in the analysis of insect-host plant relationships from an evolutionary point of view. At the behavioural level, a few studies on this subject have been made (Hovanitz & Chang, 1963; Falk & Atidia, 1975).

The present study is a first attempt to reveal genetic coding of gustatory sensitivity in two closely related *Yponomeuta* species. The F1 progeny of reciprocal crosses between small ermine moth species has been examined on contact chemoreceptor cell sensitivity to some plant constituents.

Materials and Methods

Parent specimens of *Y. cagnagellus* (host plant: *Euonymus europaeus*) and *Y. malinellus* (host plant: *Malus sp.*) were collected in the field. Under natural conditions, these insects behave as different species, however they can be crossed under artificial conditions (Herrebout et al., 1976). In our experiments, some insects were crossed in the laboratory, other crosses were initiated in cages in the field. In the laboratory, parent individuals of each species were kept in cages with dried branches of both their associated hosts. Approximately three weeks after oviposition, first instar larvae emerge. Under natural conditions these larvae enter diapause and remain under the scale formed by the outer part of the egg cluster. To prevent larvae going into diapause, the scale was lifted and larvae were placed on host plant leaves under long day illumination. The same procedure was carried out with larvae of both parental species. In this manner, six experimental groups were obtained (abbrevations used in the following are indicated in between brackets). 1. *Y. cagnagellus* reared on *Euonymus europaeus* ($\frac{c}{E}$), 2. *Y. malinellus* on *Malus sp.* ($\frac{m}{M}$), 3. and 4. F1

of ♀♀ *Y. cagnagellus* x ♂♂ *Y. malinellus* reared on *Euonymus europaeus* and *Malus sp.* ($^{cxm}_{E}$ and $^{cxm}_{M}$ respectively), 5. and 6. F1 of ♀♀ *Y. malinellus* x ♂♂ *Y. cagnagellus* on *Euonymus europaeus* and *Malus sp.* ($^{mxc}_{E}$ and $^{mxc}_{M}$ respectively). Up to the fifth instar, mean mortality was 85% ± 3.4%. In one of the groups mortality was 100%, none of the $^{cxm}_{M}$ individuals reached fifth instar and apple leaves were not eaten by this group. In the field, crosses of $^{cxm}_{E}$ and $^{mxc}_{M}$ were made, in this case intact host plants were used. No offspring of the latter crossing was obtained. A fraction of 98% of the $^{cxm}_{E}$ individuals collected as first and second instar larvae reached the fifth instar. Approximately 10% of all F1 offspring that reached fifth instar possessed abnormally shaped sensilla.

Responses of lateral and medial styloconic sensilla were obtained using a tip recording technique (van Drongelen & Bijlsma, unpubl.). The following chemicals were applied as a stimulus at concentrations indicated in between brackets: dulcitol (25 mM), phloridzin (2 mM), prunasin (10 mM), salts (100 mM), sorbitol (100 mM) and sucrose (10 mM). Sucrose and salts were applied to test the condition of the hairs. The remaining chemicals were selected because the parental species are known to react differently to them and because they occur in *Yponomeuta* host plants (van Drongelen, 1979). All stimuli were applied in double-distilled water. During stimulation with water a relatively low neural discharge was encountered, so the responses were easily recognized. Recordings that displayed a signal-to-noise ratio lower than 2 were not considered. An index for neural activity was obtained by counting all spikes in the second following stimulus onset. A t-test was used to judge significant differences between the groups of data. All figures to be presented are based on five individuals unless differently indicated.

Chemicals (*pro analyse* quality) were obtained from commercial sources: i.e. Baker (dulcitol, salts, sorbitol and sucrose) and Roth (phloridzin and prunasin).

Results

Larvae $^{cxm}_{E}$ reared in the laboratory did not display significantly different responses as compared to those partly reared in the field. Therefore, results of both groups are lumped.

In Fig. 1, dulcitol sensitivity as recorded in the lateral sensillum of crosses and parental species is represented. Larvae of *Y. cagnagellus* respond to dulcitol which is specific for their host plant, larvae of *Y. malinellus* do not react to this constituent. A dulcitol response is normally encountered in the lateral styloconic sensillum of *Y. cagnagellus*. As can be seen in Fig. 1, the three groups of F1 progeny tested appear to be sensitive to dulcitol in the lateral sensillum; as is the case in *Y. cagnagellus*.

Sorbitol, a stereo-isomere of dulcitol occurring in apple, elicits responses in the lateral sensillum of *Y. malinellus*. Larvae of *Y. cagnagellus* are insensitive to this compound. In most crosses a poor signal-to-noise ratio of sorbitol recordings prevented analysis. These poor recordings displayed the bad-contact phenomenon, i.e. a high noise level probably related to the diameter of the distal pore of the sensillum (Bernays et al., 1972).

Phloridzin, occurring in apple leaves, can be perceived by a cell in the medial sensillum of *Y. cagnagellus* and does not elicit a neural reaction in *Y. malinellus* (Fig. 1). In larvae of *Y. cagnagellus* this constituent acts as deterrent (van Drongelen, unpubl.). The F1 offspring of $^{cxm}_{E}$ and $^{mxc}_{M}$ clearly display an intermediate sensitivity to phloridzin as compared to the parental species (Fig. 1). The responses of

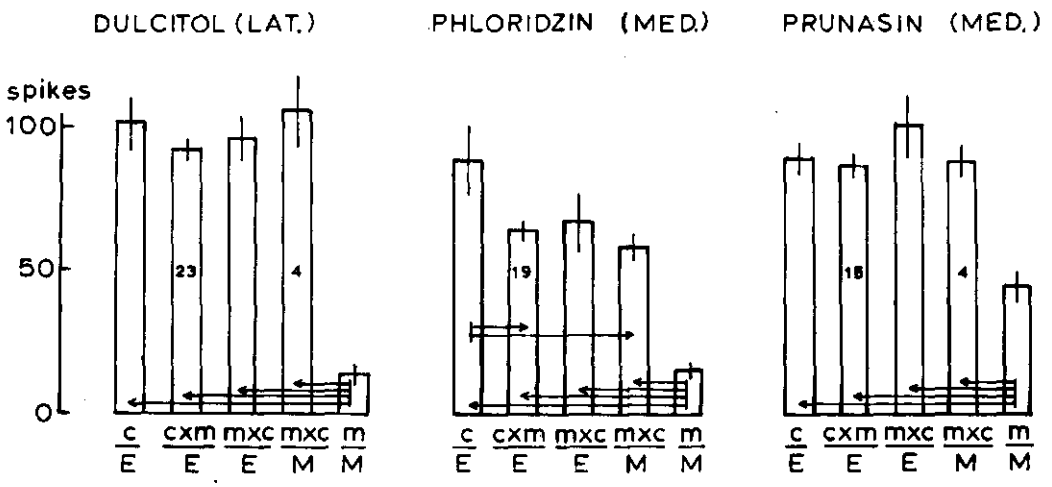

Fig. 1. Responses to dulcitol (25 mM), phloridzin (2 mM) and prunasin (10 mM) quantified by the mean number of action potentials during the first second of stimulation. Vertical lines indicate standard error of the mean. Significant differences are indicated by arrows. Columns are based upon five individuals unless differently indicated.
Abbreviations $\frac{c}{E}$, $\frac{cxm}{E}$, $\frac{mxc}{E}$, $\frac{mxc}{M}$, and $\frac{m}{M}$ are indicated in the text; LAT.-response in lateral sensillum; MED.-response in medial sensillum.

$\frac{mxc}{E}$ larvae are significantly different from those of $\frac{m}{M}$ but not from the phloridzin responses in $\frac{c}{E}$ specimen.

Prunasin, specific for some Prunoidea, elicits responses in the medial sensillum of both parental species. However, in *Y. malinellus* this compound elicits a significantly lower response than in *Y. cagnagellus*. Offspring of the $\frac{cxm}{E}$, $\frac{mxc}{E}$ and $\frac{mxc}{M}$ crossings displayed responses identical to that of $\frac{c}{E}$ caterpillars (Fig. 1).

Discussion

Larvae fed with *Euonymus* leaves displayed a slightly lower mortality than those fed with apple.[1)] However, no clear differences between mortality of the crosses and of the parental species were scored. Thus no indication is obtained that specific genotypes were selected.

In the experiments, sensitivity for dulcitol and phloridzin appears dominant and semi-dominant over non-sensitivity. In case of prunasin, higher sensitivity is found dominant over lower sensitivity. If one assumes receptor proteins to be responsible for the specificity of the gustatory response, the findings can be explained by postulating existence of autosomal genes coding for these proteins. In the F1 progeny, sensitivity of both parents comes to expression by synthesis of both parental receptor protein types. Under this assumption, the amount of proteins synthesized

1) Mortality of the larval stage of the crossing $\frac{cxm}{E}$ which was initiated in the field could not be determined as the insects were collected in the first or second instar.

may be related to quantitative gustatory sensitivity. The number of genes coding a gustatory sensitivity can be further investigated by F2 segregation. In spite of the low signal-to-noise ratio encountered with sorbitol stimulations in $\frac{cxm}{E}$ individuals, it cannot be excluded that differences in sensitivity to this sugar alcohol between reciprocal crossings exist. If this is true, the simple hypothesis described does not explain inheritance of sorbitol sensitivity.

Reciprocal crossings were reared on both parental hosts to investigate a possible realtionship between peripheral sensitivity and food composition. Though induction plays a rôle in some caterpillar species (Schoonhoven, 1969), in the mxc individuals no such effects appear present (Fig. 1).

Most $\frac{cxm}{E}$ individuals displayed bad contact recordings upon sorbitol stimulation, this may be related to the fact that none of the $\frac{cxm}{M}$ individuals reached the fifth instar. For *Yponomeuta* species, sorbitol is probably one of the important stimulants of apple leaves. Absence of stimulating input of receptor cells, as may be the case during "bad contact", can lead to food rejection (van Drongelen, unpubl.). The mxc individuals clearly accept both parental host plants. The findings on acceptability of host plants indicate that crosses between small ermine moths of apple and spindle tree can survive under natural conditions. Probably, the isolation of these species is mainly achieved by different sex-pheromone systems (van der Pers & den Otter, 1978; Hendrikse, 1979).

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CHAPTER 5 Computerized Analysis of Multi-Unit Spike Activity

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Abstract

A method for analysing multi-unit recordings is presented. Programs for spike recognition, for separation of neural activities belonging to different cells and for parametrization of these activities were written to run on a PDP 8 mini-computer. Recordings with a signal-to-noise-ratio of 2 and higher can be analysed by the procedure described. The programs were tested by analysing different types of chemosensory responses recorded on caterpillars of *Yponomeuta rorellus* and *Y. cagnagellus*.

Introduction

In many preparations, analysis of multi-unit spike trains can be avoided by isolating activities of single cells. When studying responses in insect sensilla, isolation is technically impossible. In these cases, neural activities belonging to different cells can theoretically be separated on the basis of amplitude and shape of the action potentials. When using a computer to perform this type of analysis, digitising the neural signal is the first and most time consuming problem. Several solutions for this problem have been described (Kent, 1971; O'Connel, Koesis and Schoenfeld, 1973; Wiemer, Kaack, Kesdi and Klatt, 1975; Schneider, Samanen and Bernard, 1978; and van der Molen, de Kramer and Pasveer, 1978).

The purpose of this study is to investigate different possibilities of automatic analysis of multi-unit spike trains as recorded on gustatory sensilla of larvae of *Yponomeuta rorellus* and *Y. cagnagellus* (Lepidoptera). Several parameters characterising single spikes are examined. To describe neural activity, a comparison is made between the time course of gustatory responses and predictions of two different models described in literature.

Procedure

Responses (Re, Fig. 1) are read from tape at a sample interval of 65 μs for a duration of 1.3 s. In recordings of insect gustatory sensilla, an electrical artefact (a, Fig. 1) accompanies stimulus onset. This artefact is used to initiate sampling; it is transformed into a TTL startpulse (Fig. 1) by a rectifier, a comparator and a monostable. The digitised signal, produced by software module SPIKSM, consists of 20.000 samples and is stored on disk (SAMPnn.SM, Fig. 1). Since the spikes are recorded as bipolar waves, the sampled file can be interpreted by peak detecting software (PEAK). The amplitude of peaks in the signal as a function of time (t) is displayed (1, Fig. 1). Then, peak detection can be checked by plotting parts of the sampled signal by the program PNTPLT (2, Fig. 1). Parameters characterising the action potentials (TAS.DA) are displayed in histograms (3, Fig. 1); this is done to allow separation of activities belonging to different cells. These histograms are generated by HIST and corresponding numerical values are printed by a type terminal (TTY:). Parameters characterising the time course of each neural activity, separated

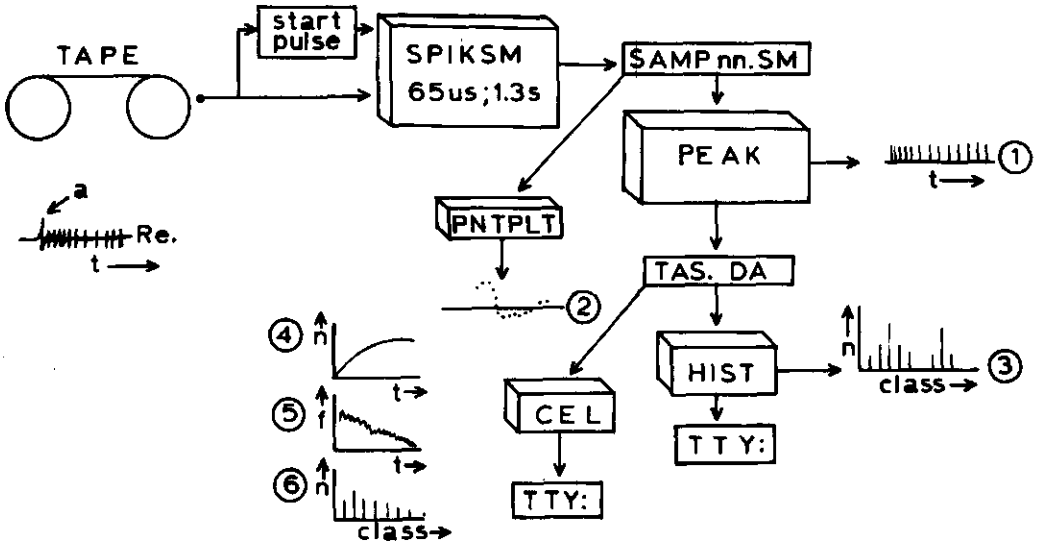

Fig. 1. The procedure used to analyse spike trains. Recordings (Re) are read from tape and digitised by the program SPIKSM. The artefact at stimulus onset (a) is used to initiate sampling. Action potentials in the digitised file (SAMPnn.SM) are recognized by software module PEAK; the operation of PEAK can be controlled by comparing plots 1 and 2. Parameters of the spike can be plotted by HIST (3). The program CEL can separate activities and each activity is analysed (4, 5, 6). Numerical values are printed by a type terminal (TTY:). Description of the software and plots is given in the text.

according results in the histograms, is displayed (4 and 5, Fig. 1) and models from literature sources are tested. In addition, a spike interval histogram is displayed (6, Fig. 1). The latter operations are performed by the program CEL and numerical values (corresponding to the plots and for the fit of the models tested) are printed. Details of several parts of the procedure are described below.

Sampling

Sampling is done using a PDP 8 mini-computer. For this purpose a program called SPIKSM is written. The maximum sample rate in our configuration is 15,385 samples per second. At this speed there is not enough time to move the data in real-time to backup-story (flexible disk). Because of the short duration of each spike, it is necessary to sample as fast as possible. Therefore, the following strategy is used: 1. sample at maximum speed and store data in memory, this will fill up all available memory in 1.3 s; 2. store this data on flexible disk in a suitable format; 3. use separate FORTRAN-programs to process this data-file.

In order to be as independent as possible of main memory organization (core-size, RAM-ROM-memory etc.), the available memory is divided in sections. The start address and length of each memory-section is stored into a table. In this way all the memory which is not occupied by the program itself can be used. The file

containing the sampled data is declared in the FORTRAN program as a random-access unformated file. A machine-coded FORTRAN-callable routine had to be written to convert the 10-bit samples into a floating point number. This routine is imbedded in a FORTRAN-coded subroutine which gives the user the idea of a one dimensional array containing 20,000 samples.

Spike recognition

Software module PEAK checks the sampled file (SAMPnnSM) for maxima and minima (with the exception of the first 80 samples, which represent an electrical artefact at stimulus onset). An example of an action potential is shown in Fig. 2.

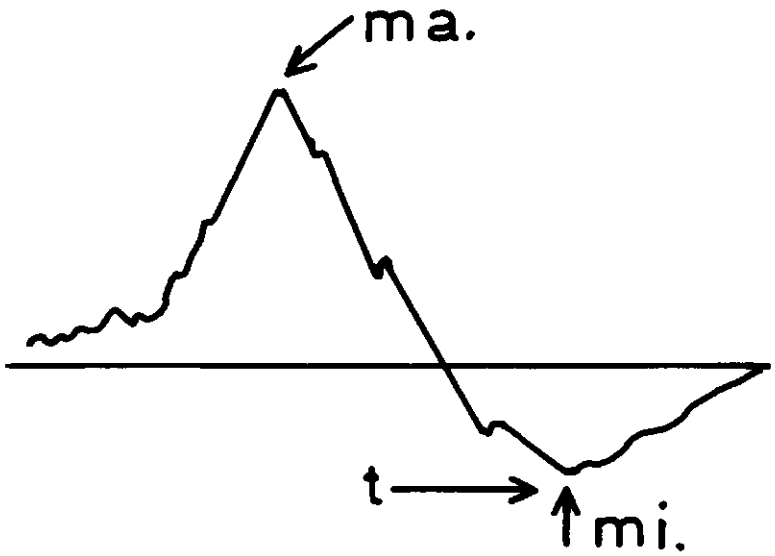

Fig. 2. A single action potential. The maximum (ma.) and minimum (mi.) of the bipolar wave is used for spike recognition. See also Fig. 3.

The maximum (ma.) and minimum (mi.) are detected; when the time interval between ma. and mi. is within certain limits, a spike is recognized. This method of spike recognition is essentially the same as procedures described by Wiemer et al. (1975) and Schneider et al. (1978). To limit computer time, only maxima and minima above and below a noise level criterium are considered. A convenient noise level criterium (CRIT) was obtained by calculating 1.4 x SD, with SD - standard deviation of the last 1000 samples. The last 1000 samples were chosen because the last part of the signal (1235-1300 ms) contains relatively few spikes. The flow chart of the maximum, minimum and spike detecting module is shown in Fig. 3. As is shown in Fig. 2, noise peaks are superimposed on the spike signal. To discriminate between noise peaks and peaks of the spike, samples with the largest extreme values (within each spike signal) are retained as maximum and minimum. This refinement is not incorporated in the flow chart in Fig. 3.

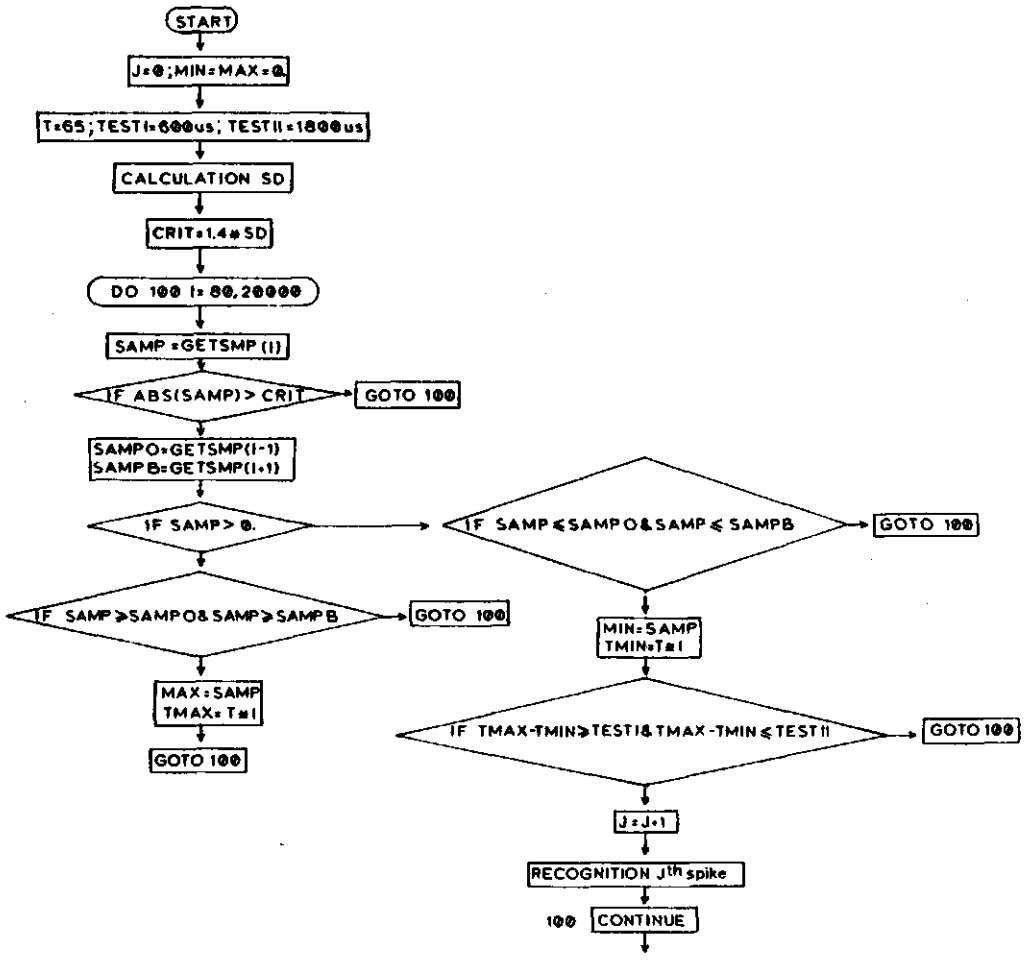

Fig. 3. Flowchart of maximum-, minimum- and spike detecting software, which is a subroutine of the program PEAK. After initialisation of several parameters, the standard deviation SD of the last 1000 samples is calculated. A noise level criterium (CRIT) is set at 1.4 x SD. In the DO-loop, the samples 80 up to 20,000 (SAMP) are checked for being a maximum or minimum if they satisfy the condition ABS(SAMP)>CRIT. The samples are read by the function GETSMP(I). If a maximum is recognized, its amplitude (MAX) and time (TMAX, which equals sample sequence number (I) multiplied by the sample interval (T)) are retained. When a minimum is recognized, the amplitude (MIN) and time (TMIN) are retained and if a correct corresponding maximum exists (Fig. 2), the J^{th}-spike is recognized. In our program, TMAX-TMIN has to lie in between the limits TESTI and TESTII.

Separation of activities

For each spike the following parameters are calculated: 1. the time interval between spike (ma. Fig. 2) and stimulus onset; 2. the amplitude of the spike, defined as the sum of the absolute values of ma. and mi.; 3. the standard deviation of 40 sample points of the digitised spike signal; 4. an index for the shape. The two latter parameters are used to take into account the wave form of the spike. This was done because the amplitude of spikes, overtly fired by a single neuron, appears to vary in some of the gustatory responses (van Drongelen, 1979). The standard deviation of the sample points reflects shape as well as amplitude of the action potential. To parametrize shape, each spike is standardized, i.e. 40 samples of the spike signal are corrected for a standard spike amplitude from zero to one volt. A convenient shape index is obtained by calculating the square of the sum of these corrected samples.

Software module HIST reads amplitudes, standard deviations and shape indices in the output file of PEAK (TAS.DA, Fig. 1). The maximum value of each of the parameters is searched. The class-width of the histograms is obtained by dividing maximum parameter values by the number of classes; in most recordings 20 classes is reasonable. Then the parameters are classified and, for each parameter group, a graph of class-number versus frequency of occurrence is plotted (3, Fig. 1). When disjunct class-groups appear in one of the histograms, activity of more than one cell is presumably present.

Spike train analyses

Data file TAS.DA can be split into parts belonging to different cells by the program CEL (Fig. 1). The separation parameter (amplitude, standard deviation or shape index) and associated boundaries, as determined in the histograms, are entered interactively.

For each activity, spike's sequence numbers and spike frequencies (inverse of spike intervals) are plotted versus time (4 and 5, Fig. 1). Spike frequency is one of the best indices for instantaneous activity of a neurone. Responses studied by us displayed high initial firing rate adapting to a constant activity level of approximately 20 spikes/s. During adaptation, correlation between spike frequency (f) as a function of time (t) and two models is calculated: 1. an empirically established hyperbolic model (van der Molen, van der Meulen, de Kramer and Pasveer, 1978) and 2. a chemical model (Heck and Erickson, 1973). In order to fit both the models with linear regression, they are transformed in straight line relationships. The hyperbolic model; $f = A/t + B$ (A and B - constants) can be transformed in a straight line relationship by multiplying with t. The chemical model; $f = A + B\ e^{-\lambda t}$ (A, B and λ - constants) cannot be transformed into a linear relationship. For this reason, the minimal spike frequency was considered a good estimate of A. In this manner, $\ln (f - A) = \ln B - \lambda t$ can be fitted to observed values by linear regression. In order to test whether spike generation during the response follows some statistical process, spike interval distributions are made (6, Fig. 1).

Recordings

The software was tested by analysing 60 gustatory responses recorded on lateral and medial styloconic sensilla of caterpillars of two *Yponomeuta* species.

Activities were recorded using a tip recording technique, which implies that the electrode which contains the stimulus is also part of the recording circuit. This explains the variable amount of noise produced by electrodes containing different stimuli; the signal-to-noise ratio of the recordings successfully analysed varied between 2 and 8. Details of the recording conditions are described by van Drongelen (1979). Special attention was paid to the parameter which gave best separation of activities, as judged in the histograms displayed by HIST. The shape index gave maximal separation of spikes in 43 cases, the amplitude in 15 cases and the standard deviation in only 2 cases. When the parameter giving maximal separation was determined the result of separation according this criterion was judged in the plot of frequency versus time (5, Fig. 1). In 23 recordings, the time course of at least one of the activities was irregular indicating imperfect separation.
A plot showing this phenomenon is represented in Fig. 4A. If non-physiological intervals (below 2 ms) were displayed, they were removed by deleting one of the spikes causing this interval. Only in 16 cases a regular activity appeared directly after

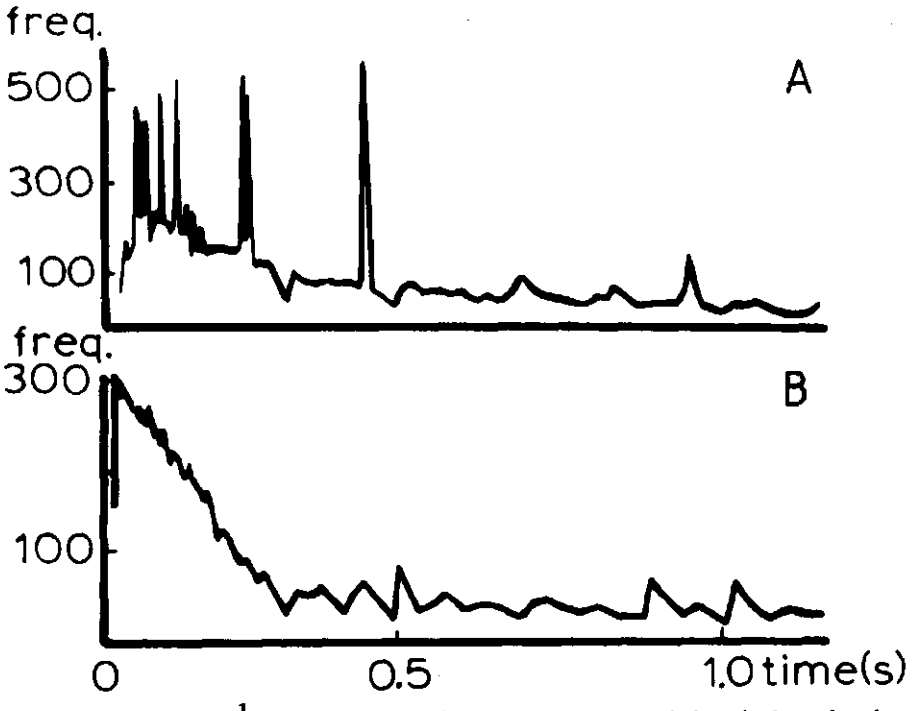

Fig. 4. Plots of frequency (s^{-1}) versus time based upon activities recorded in the lateral styloconic sensilla of different individuals of *Y. cagnagellus*. In both cases the hairs were stimulated with 10 mM sucrose.
A. Imperfect separation; B. good separation.

separation by means of the histograms; an example is shown in Fig. 4B. The models describing the time course of discharge were fitted to 55 activities showing a high initial firing rate followed by adaptation. The mean correlation found for the hyperbolic model is 0.46 and for the chemical model 0.53.

The distributions of spike intervals (6, Fig. 1) displayed few very short and very long intervals as compared to the moderate ones. A representative is shown in Fig. 5.

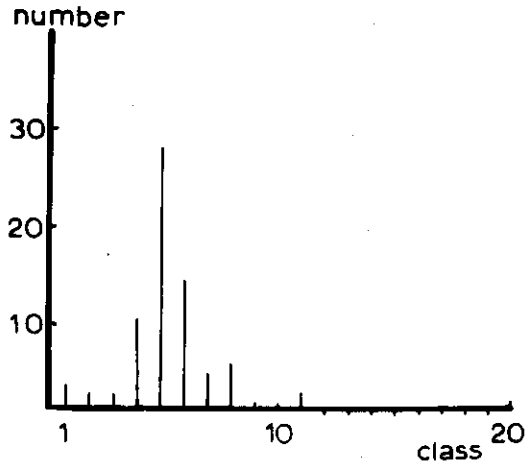

Fig. 5. A spike interval distribution. This plot is based upon activity during stimulation of the medial sensillum of *Y. rorellus*. Stimulus: catechin 1 mM.

Conclusions

By using the procedure described, many recordings can be analysed within a short period. The program PEAK asks a lot of computer time (several minutes), but with a floating point processor it would be 10 times faster. With our processor, it is convenient to use BATCH to interprete a number of sampled files at a time without operator intervention.

The comparator for the startpulse has to be adjusted once and no further adjustments are necessary. Occasionally, noise peaks are interpreted as action potentials, but by using histograms of spike parameters these are easily eliminated.

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PART II

THEORY

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CHAPTER 6

Pores in the insect contact-chemosensory hair; a theoretical study

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Abstract. In most insects the contact chemosensory sensilla are separated into two compartments, one containing the dendrites of the receptor neurones and one dendritic free lumen. Two types of pores occurring in these sensilla were reported in literature, one primary pore, situated distally, that permits gustatory neurones to contact outside media and a secondary type that either connects both compartments or connects the dendritic free lumen with the outside. On the basis of anatomy of the gustatory sensilla, two electrical models have been constructed; 1. a unipore model assuming the exclusive existence of a primary pore and 2. a multipore model assuming both types of pore being present. Comparing theoretical predictions of the models it is concluded that multipore theory fits well to experimental evidence, whilst unipore theory must be rejected. Furthermore, evidence is obtained that the secondary pore connecting both compartments is present. The significance of the secondary type of pore is discussed.

1. Introduction

In insect species three to seven contact-chemosensory cells are located in each sensillum. Most types of contact-chemosensory sensilla contain two lumina, the smallest lumen contains the dendrites of the sensory neurones and its cuticular wall is termed scolopoid body (Larsen, 1962; Stürckow, 1962). Basally, the scolopoid body forms a tight constriction around the dendrites. A primary pore at the distal tip of the hair connects the small lumen with the outside. The perikarya of the sensory cells are enveloped in a tormogen, a trichogen and/or a sheat building cell. The larger lumen is, via the trichogen cell in electrical contact with the basal part of the receptor cells.

Electrical responses of the gustatory cells have been recorded using tip recording techniques (Hodgson, Lettvin and Roeder, 1955) and side-wall recording techniques (Morita and Yamashita, 1959). In the latter technique separate recording and stimulus pipettes are used, the first technique combines the recording and stimulation operations in a single pipette. Based on recorded slow potentials of receptor cells and/or impedance measurements, several authors assume a secondary type of pore to be present either in the scolopoid body or in the outer wall of the sensillum (Rees, 1967; Morita, 1969; Stürckow, 1971; Maes, 1977). Such a secondary pore would establish an electrical contact between the larger and smaller lumina at the distal side of the sensillum. However, the recorded polarity of the receptor cell action potential and the effects of electrical stimulations gave rise to a theory that assumes the absence of a secondary pore (Wolbarsht, 1958; Wolbarsht and Hanson, 1965; Stürckow, 1964). In the following, both theories described shall be referred to as multi and unipore theories respectively. In order to understand electrical generation of receptor cell action potentials, Rees (1967, 1968) has presented an electrical model of the insect contact-chemosensory hair and estimated the magnitude of

several resistances. A theory of non-electrical generation of action potentials was advocated by Maes (1977).

The present study aims to analyse the polarity of recorded biopotentials and general electrical behaviour of insect sensilla in multi versus unipore theories. Properties of a simple electrical model, based on anatomy of the sensillum, are considered in relation to experimental evidence.

2. Electrical models

A schematic representation of a longitudinal section of an insect taste sensillum is shown in Figure 1. In this Figure, a simplified electrical circuit corresponding to different anatomical structures is indicated by the stippled lines. The elements R_e, R_i and r_m denote the resistance of the larger lumen (including hypothetical pores), the receptor cell's inner medium and its membrane. Resistance r_e represents a fraction of the resistance R_e. Capacitor C_s indicates the capacitance between both lumina over the scolopoid body. The resistance of the cuticular separation between both lumina is considered infinite. Recording electrode positions are indicated by I, A_t and A_s representing the indifferent electrode and the active electrodes when using tip recording or sidewall recording techniques. It is assumed that a generator potential (V_g) causes initiation of action potentials. The action potential (V_a) arises near the cell body, in the basal part of the sensillum (Morita and Takeda, 1959; Wolbarsht and Hanson, 1965). From a teleological point of view, it seems reasonable to assume

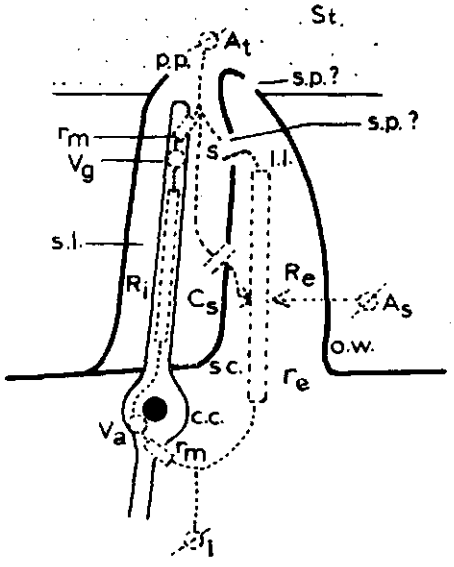

Fig.1 A schematical representation of a longitudinal section of an insect taste sensillum. An electrical circuit corresponding to various structures is indicated by stippled lines. St. – stimulus solution; p.p. – primary pore; s.p.? – hypothetical secondary pores; sc – scolopoid body; o.w. – outer wall; s.l. – smaller lumen; l.l. – larger lumen; c.c. – perykaryon of chemosensory cell; I, A_s and A_t indicate electrode positions; V_g and V_a indicate the potential sources of the generator – and spike potentials; R_e, R_i and r_m – the resistance of the larger lumen, the receptor cell's inner medium and its membrane; s – switch; re is part of Re between A_s and I; and C_s – capacitance over the scolopoid body.

that stimulus perception and transduction occurs at the distal tip of the dendrites. This assumption seems to be justified by recorded slow potentials in the distal parts of the sensillum (Morita and Yamashita, 1959). Because the general electrical behaviour of the insect sensillum is studied, the following simplifications were introduced: (1) capacitance of the cell membranes was not considered; (2) changes in conductivity of the receptor membranes were simulated by voltage steps in batteries V_a and V_g (Figure 1); (3) only potential changes were considered, i.e. analysis did not include any resting potential over the membranes. It should be noted that a transepithelial standing potential can play a role in the multipore model as was pointed out by Thurm (1974). However, in the model presented here such a resting potential would only affect the magnitude of the biopotentials considered. The switch s in Figure 1 is on if one considers multipore theories. In the model presented it is not essential whether the secondary pore is in the scolopoid body and/or in the outer wall. That is to say, during stimulation with a conducting solution, in both cases mentioned, the outside medium contacts the larger lumen via a pore. The resistor in series with C_s shall be neglected in calculations in multipore theory, because it renders calculations on the circuit complicated, but does not affect the electrical behaviour of the model in an essential manner. The unipore theory implies that s is switched off. In this theory r_e shall be considered because then it is essential to the circuit. The simplified electrical circuits, based on the circuit shown in Figure 1, for both multi and unipore theories are shown in Figures 2A and B respectively. For both theories and recording techniques, the voltages as a function of time at A_t (V_{A_t}) and A_s (V_{A_s}) shall be calculated by defining I at zero potential and simulating the generator and action potentials by voltage steps as shown in Figure 3A. The generator potential is represented by a step of 30 mV in V_g at t_o. In the time interval between t_1 and t_2 a pulse with an amplitude of 100 mV simulates a single action

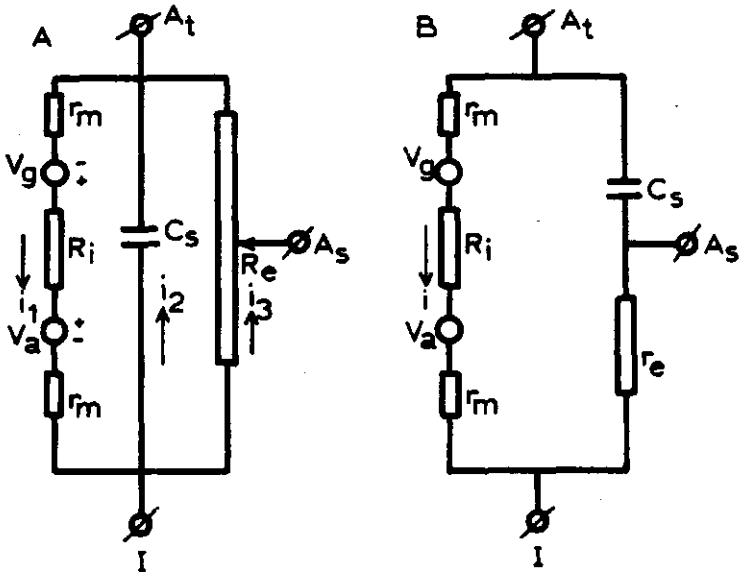

Fig.2 Simplified electrical circuits based on the circuit of Figure 1. A: multipore theory; B: unipore theory.

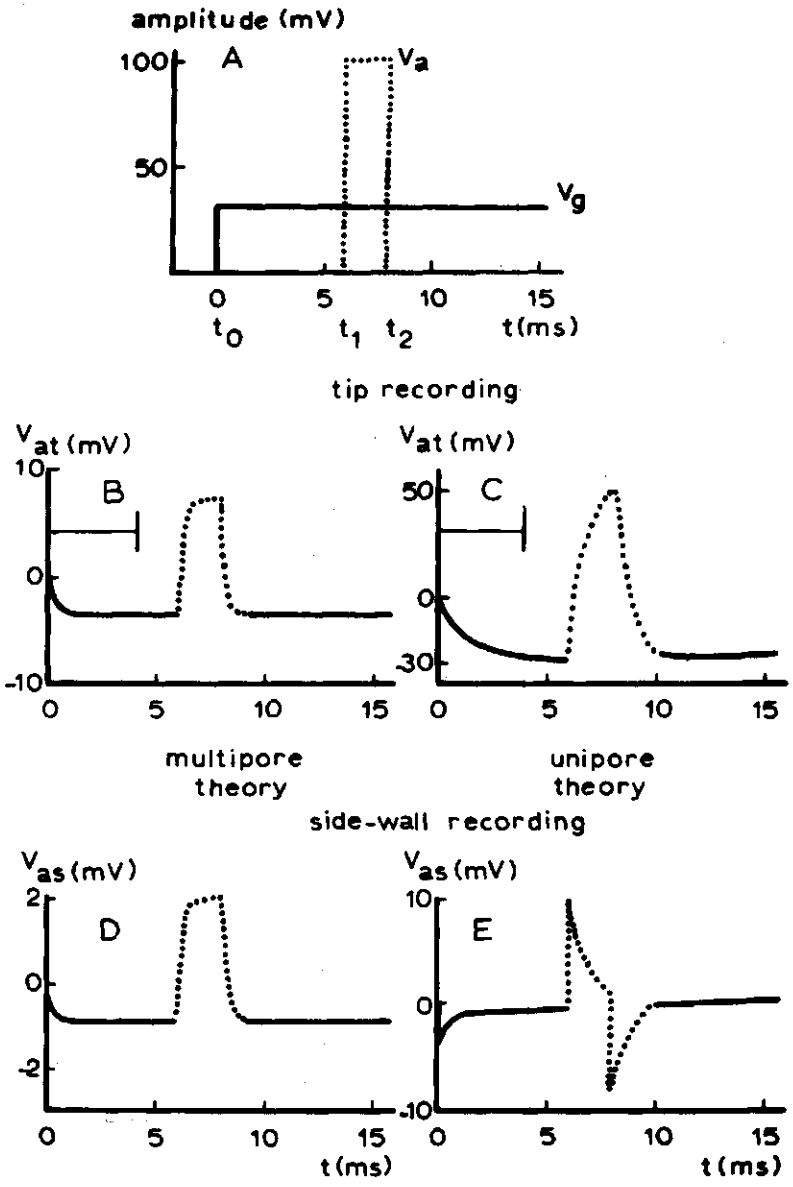

Fig.3 A: The generator potential (V_g, line) and action potential (V_a, dotted line) plotted versus time.
B and D: Multipore theory. Voltage as a function of time at A_t and A_s respectively. Based on formulas (5a), (5b) and (6).
C and E: Unipore theory. Voltage plotted against time, at A_t and A_s respectively. Based on formulas (9a), (9b) and (11). Horizontal bars in B and C indicate blocking of the amplifier under experimental conditions.

potential. The values of the electrical components used in the calculations are: $R_i \approx R_e \approx 10^8\ \Omega$; $r_e \approx 0.3.10^8\ \Omega$; and $r_m \approx 10^9\ \Omega$. The value of C_s was estimated to be approximately 1 pF using the formula: $C_s = \varepsilon_o\ \varepsilon_r \frac{S_{sc}}{t_{sc}}$, with ε_o and ε_r (the absolute and relative dielectric constants) $8.85\ 10^{-12}\ N^{-1}\ m^{-2}\ C^2$ and 20 respectively, S_{sc} (the surface of the scolopoid body) estimated as 500 μm^2, and t_{sc} (the thickness of the scolopoid body) estimated as 0.1 μm.

It is assumed that a negligible current flows through the recording circuit, because of the use of high input impedance amplifiers, i.e. no current leaves or enters the circuit in Figure 2 at any of the points I, A_t or A_s.

Multipore theory

2.1. Calculation of V_{A_t}

In Figure 2A, the current flowing through the different leads are defined as i_1, i_2 and i_3. Applying Kirchhoff's first and second laws, we may write:

$$i_1 = i_2 + i_3 \tag{1}$$

and

$$V_{A_t} = i_1(2r_m + R_i) - V_g + V_a \tag{2}$$

Furthermore we may state:

$$V_{A_t} = \frac{-\int i_2 dt}{C_s} = -i_3 R_e \tag{3}$$

Combining formulas (1), (2) and (3) we obtain:

$$-V_g + V_a = AV_{A_t} + B\frac{dV_{A_t}}{dt} \tag{4}$$

with:

$$A = 1 + \frac{2r_m + R_i}{R_e} \text{ and } B = (2r_m + R_i)\,C_s$$

To facilitate calculation, the solution of V_{A_t} shall be indicated separately for application of V_a and V_g. Furthermore, in calculation of V_{A_t} when V_a is applied, time is set at zero at t_1. Equation (4) shall be solved using Laplace transform (c.f. Appendix).

The Laplace transforms of V_{A_t}, $\frac{dV_{At}}{dt}$, V_a and V_g are respectively defined as S(p), pS(p), $V_a \frac{(1-e^{-(t_2-t_1)p^1})}{p^1}$ and $\frac{V_g}{p}$.

In the Laplace transform of V_a, p^1 is used instead of p to indicate that time at t_1 is reset at zero. When the step at V_g is applied, the Laplace transform of equation (4) becomes:

$$\frac{V_g}{p} = AS(p) + BpS(p) \qquad (4^1a)$$

From this it follows:

$$S(p) = -\frac{V_g}{B} \cdot \frac{1}{p\left(p + \frac{A}{B}\right)} \qquad (5^1a)$$

The inverse transform (Appendix) gives:

$$V_{A_t} = -\frac{V_g}{A}\left(1 - e^{-\frac{A}{B}t}\right) \qquad (5a)$$

An identical procedure when step V_a is applied at $t_1 = 0$ leads to the following transform of equation (4):

$$\frac{V_a(1 - e^{-(t_2 - t_1)p^1})}{p^1} = AS(p) + BpS(p) \qquad (4^1b)$$

The expression for S(p) yields:

$$S(p) = \frac{V_a\,(1 - e^{-(t_2 - t_1)p^1})}{p^1(A + Bp^1)} \qquad (5^1b)$$

and the inverse transform considering the original time scale (e.g. $t_o = o$) becomes:

$$V_{A_t} = \frac{V_a}{A}\left\{\left(1 - e^{-\frac{A}{B}(t - t_1)}\right) \cdot \mu(t - t_1) - \left(1 - e^{-\frac{A}{B}(t - t_2)}\right) \cdot \mu(t - t_2)\right\} \qquad (5b)$$

with:

$\mu(t - a) = 0$ if $t < a$ and

$\mu(t - a) = 1$ if $t < a$, a represents the variable t_1 and t_2 in formula (5b). The solution of V_{A_t} when both biopotentials V_a and V_g are applied, that is to say the combined solution of (5a) and (5b), is plotted versus time in Figure 3B.

2.2 Calculation of V_{A_S}

Because the voltage of A_s is measured over a part of R_e instead of over the whole resistance, one obtains:

$$V_{A_S} = f.V_{A_t} \qquad (6)$$

where f indicates a fraction, the value of f depends on the location of A_s. Using formula (6), formulas (5a) and (5b) may be rewritten to describe V_{A_S}. A graphical representation of V_{A_S} for $f = 0.3$ can be seen in Figure 3D. In practice, application of side-wall recording techniques sometimes results in a better signal-to-noise ratio of the recordings as compared to those obtained by the tip recording techniques. However, this seems to be predominantly due to a lower noise level in recordings

obtained by the side-wall technique, where the amplifier is coupled to the inside medium of the hair via a low impedance as compared with a tip recording situation.

Unipore theory

2.3. Calculation of V_{A_S}

When Kirchhoff's second law is applied to the circuit represented in Figure 2B, one has:

$$V_{A_S} = i(2r_m + R_i) + \frac{\int i dt}{C_s} - V_g + V_a \tag{7}$$

and

$$V_{A_s} = -ir_e \tag{8}$$

Formula (8) indicates the importance of the presence of r_e in this circuit. Using the identical procedure for solution of equations (7) and (8) as indicated in the previous sections one obtains:

$$V_{A_s} = -\frac{V_g}{\psi} e^{-\frac{D}{\psi} t} \tag{9a}$$

when the generator potential is applied, and:

$$V_{A_s} = \frac{V_a}{\psi} \{ e^{-\frac{D}{\psi}(t-t_1)} \cdot \mu(t-t_1) - e^{-\frac{D}{\psi}(t-t_2)} \cdot \mu(t-t_2) \} \tag{9b}$$

for application of an action potential. The values ψ and D have the following definitions

$\psi = \frac{2r_m + Ri}{r_e}$; $D = \frac{1}{r_e C_s}$. Formulas (9a) and (9b) are represented graphically in Figure 3E.

2.4 Calculation of V_{A_t}

From Fig. 2B can be seen that:

$$V_{A_t} = -V_g - V_a - i(2r_m + R_i) \tag{10}$$

Combining of formulas (8) and (10) gives:

$$V_{A_t} = V_g + V_a - \frac{V_{A_s}}{r_e} \cdot (2r_m + R_i) \tag{11}$$

The values for V_{A_s} are given by equations (9a) and (9b), so V_{A_t} can be calculated from (11). A graphical representation is shown in Figure 3C.

3. Discussion

Both the action potential and generator potential reduce the membrane potential. A

peculiar feature is that these biopotentials, when recorded with the side-wall technique, display opposite polarities (Morita and Yamashita, 1959); Rees, 1968). Using a tip recording technique, a generator potential onset cannot be recorded properly because recording of neural activity upon stimulus application is possible after some delay; this is indicated by the horizontal bars in Figures 3B and 3C.

According to the models presented, such an opposite polarity can be explained by the electrode positions, the presence of the scolopoid body, and the locations of the biopotential sources. That is to say,the active electrodes become negative by the dendritic events, whereas indifferent electrode becomes negative by the potential changes in the cell body and axon. This causes that a depolarization of the dendrite's membrane is recorded negative and such a potential shift in the basal part of the neurons displays a positive polarity. The models demonstrate that the presence or absence of a secondary pore does not affect the polarities of both types of biopotentials (Figure 3). However another criterion to distinguish between both theories is present in the time course and magnitude of the biopotentials recorded in the sensillum. In unipore theory the model predicts that: 1. It should be possible to lead off spikes with amplitudes of approximately 80 mV (Figure 3C); 2. When using the side-wall technique, a measurement of the biopotentials (D.C.-recorded) should contain high frequencies (Figure 3E). The latter property shall be indicated by the term high-pass effect, the opposite, as displayed in Figures 3B, 3C and 3D, by the term low-pass effect. Both properties assigned to unipore theory have not been displayed in any record of neural activity of insect contact chemoreceptors. As such, the prediction of the multipore theory model is not in contradiction with experimental evidence. Recorded amplitudes of the biopotentials appear to be approximately a factor 5 less than the predicted ones, but this may be explained by misestimation of the electrical components in the model. The recorded time course of the generator potential always shows low-pass effects as predicted in the multipore theory.

The time course of an averaged, D.C.-recorded spike, measured with a tip recording technique is shown in Figure 4. Assuming a monophasic spike to occur at the axon hillock it is peculiar to note that a positive-negative going wave is displayed. This is by no means predicted by the model as presented in Fig. 2A; i.e. because of the low-pass characteristics of V_{A_t} (Figure 3B) it is unlikely that the negative phase is caused by capacitance effects. One possibility is that the spike generated at the hillock is conducted antidromicly towards the distal part of the dendrite (Wolbarsht and Hanson, 1965). Under this assumption the first positive peak in Figure 4 would represent the spike in the cell's axon, whilst the second negative one reflects a dendritic spike. This is in accordance with the multipore theory model that predicts that a depolarisation in the dendrite is recorded negatively. Maes (1977) suggested that the antidromicly conducted spike in the insect sensillum should be recorded positively. His suggestion is based on the time course of an action potential recorded in a volume conductor. In this situation a spike recorded from a receptor cell axon, near the hillock, is recorded as a negative-positive going wave. However, the positive going wave seems not to be an antidromicly conducted spike but seems to reflect the membrane current after the so called active region of the spike potential passed the recording electrode.

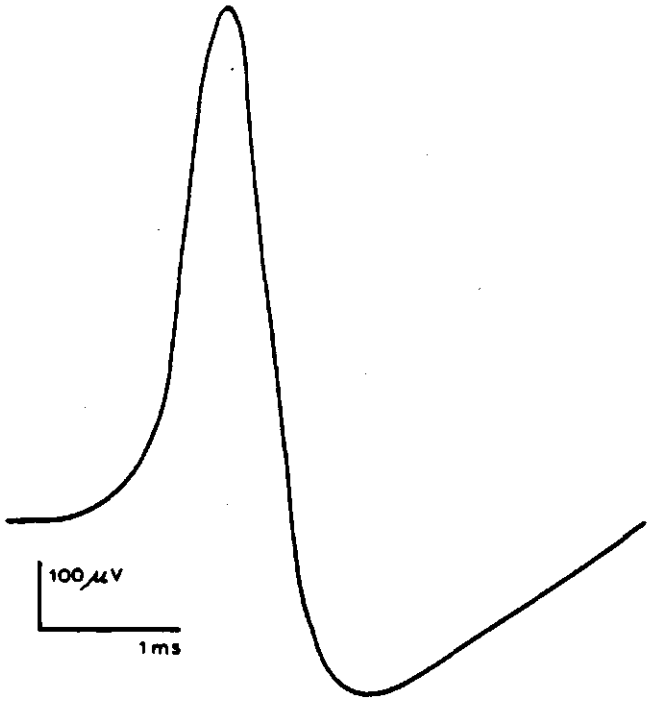

Fig.4 An averaged D.C. recorded action potential. Positive deflection upwards.

Comparing experimental evidence with the analysis of the electrical behaviour of the sensillum it is concluded that at least one of the secondary pores is present. From a teleological point of view, it is attractive to postulate the existence of a connection between the smaller and larger lumina. That is to say when a hair contacts an outside medium, the chemical composition of the smaller lumen is relatively quickly affected, the larger lumen could counteract this process, i.e. the larger lumen (besides being a mechanical protection for the dendrites) could act as a buffer compartment for the fluid surrounding the dendrites.

An experimental argument to assume the presence of a secondary pore in the scolopoid body is furnished by side-wall recordings. That means if a distal connection between the larger lumen and smaller lumen (the pore) would be absent, one would have a situation equivalent to the unipore theory when spikes are lead off from an unstimulated sensillum. That is because in this case the larger and smaller luminae would not be connected electrically via the stimulus solution. Thus in the absence of a pore in the scolopoid body one may expect a difference between the time courses and amplitudes of the action potentials in the absence of stimulation and those during stimulation. Such differences in time courses or amplitudes do not seem present in side-wall recordings shown by Rees (1968).

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Appendix

Equations developed in this study have been solved using the Laplace transform technique. This implies that some function f(t) in the concrete demain is transformed into F(p) in a symbolic domain following the definition:

$$F(p) = \int_0^\infty e^{-pt} \cdot f(t)\, dt$$

Using this technique, an equation can be transformed and the solution of the symbolic equation can be transformed inversely to the concrete domain. The inverse transform of a function F(p) is defined as:

$$f(t) = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} e^{tp} \cdot F(p)\, dp$$

All transforms and inverse transforms can be found in Roberts, G.E. and Kaufman, H. (1966), Table of Laplace transforms, W.B.Saunders Co., Philadelphia, London.

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CHAPTER 7

Convergence in the Olfactory System: Quantitative Aspects of Odour Sensitivity

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The olfactory organ offers a model of a neural system where a large number of receptor cells converge onto a small number of secondary neurones. A mathematical analysis of the effects of neural convergence in terms of response probability of secondary cells has been carried out. The parameters studied have been the ratio of neural convergence, the individual response probability of primary neurones, and the acceptor distribution over the receptor cells. The results indicate that a neural system with a high convergence ratio can detect stimuli at intensities below the one which is commonly used to demonstrate a conspicuous response in the primary neurones. An analysis of the response probabilities of secondary neurones in a system where the olfactory receptor cells have a multimodal sensitivity *v.* a unimodal one, shows that the response probabilities remain the same as long as the total number of "acceptors" is the same in the two modalities.

1. Introduction

In the olfactory system of vertebrates the sensory receptors are numerous and converge onto a much smaller number of secondary neurones. This convergence seems to manifest in all vertebrate species studied and indicates a functional adaptation which has been retained throughout evolution. The ratio between the number of receptor cells and secondary neurones has been estimated in some species. Allison & Warwick (1949) found in the rabbit

5×10^7 olfactory receptors, $4{\cdot}5 \times 10^4$ mitral cells and $1{\cdot}3 \times 10^5$ tufted cells. In some species of bats Bhatnager & Kallen (1975) have estimated the ratio of receptors to mitral cells to be 900:1. In the burbot the ratio between olfactory receptors and myelinated fibers in the olfactory tract was found to be close to 1000:1 (Gemne & Døving, 1969).

The degree of convergence presumably has consequences for odour sensitivity and qualitative discrimination of odours. Knowledge about the properties of olfactory receptors is a prerequisite for a theoretical formulation of these consequences. Studies of the activity of single receptors in a number of vertebrate species have shown that the receptors respond to a variety of different chemicals (Gesteland, Lettvin & Pitts, 1965; Gesteland, Lettvin, Pitts & Rojas, 1963; Altner & Boeckh, 1967; Mathews, 1972; O'Connel & Mozell, 1969; Duchamp, Revial, Holley & MacLeod, 1974). Recent studies of frog olfactory receptors have shown that odours can be grouped into several classes (Holley, Duchamp, Revial, Juge & MacLeod, 1974; Revial, Duchamp & Holley, 1977; Revial, Duchamp, Holley & MacLeod, 1977). These studies demonstrate that olfactory receptors respond to one or more representatives of the odour groups, and most receptors cells have a multimodal sensitivity. Studies of the events in frog olfactory receptors stimulated at low concentrations show that the probability of an increased firing rate in a receptor is dependent upon the stimulus concentration in a fashion that is predicted by a Poisson distribution (van Drongelen, 1978*a*, *b*).

In the following we have used information concerning receptor function in formulating some quantitative properties of convergence in the olfactory system. We give a theoretical demonstration of the amplification or increased sensitivity to stimuli that can be obtained in a neural system with a high convergence ratio. We also treat some consequences which multimodal sensitivity of the olfactory receptors may have for the olfactory system.

2. Convergence Ratio

In the absence of overt stimulation receptor cells in the olfactory mucosa are either silent during long periods of recording or display a spontaneous spike discharge. This spontaneous discharge can be described by a Poisson process (Getchell, 1974; van Drongelen, 1978*a*, *b*). When the olfactory mucosa is exposed to an odour at a certain concentration, a response can be observed. In the following we consider the cases in which stimulation results in an excitation of receptor cells. In the silent cells this response is manifested as an appearance of spikes after the onset of stimulation; in the

cells with spontaneous activity there is an increased number of spikes. At low stimulus concentrations the distribution of spike intervals approximately follows a Poisson process. Thus during the stimulation of a receptor cell, the stationary Poisson process is transformed into another Poisson process (van Drongelen, 1978*a*, *b*).

In a series of stimulations at low concentrations the stimulus is sometimes followed by a visible increase in firing rate; sometimes no response is evident. Even when there is no visible response, the suspicion of a response can be confirmed by adding up several traces. An example of the activity of a frog olfactory receptor cell is shown in Fig. 1. In this figure each of the six upper

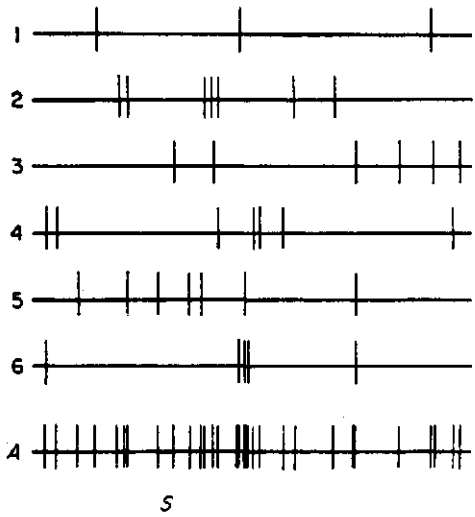

FIG. 1. Schematic activity of a frog olfactory receptor stimulated six times with cineole. The trace *A* gives the summated activity when superimposing spikes, and can be considered under certain conditions to represent the activity of a mitral cell.

traces represents individual recordings of odour trials. Superimposed spike activity is represented in the bottom trace. In the individual recordings no response is visible, a superimposition shows a clearly visible increased number of spikes after the onset of olfactory stimulation. Let us consider that the six odour trials in Fig. 1 originate not from the same cell but from six different cells, and that these cells make contact with one secondary neurone (termed mitral cell in the following). The bottom trace in Fig. 1 will give the occurrence of impulses arriving at this mitral cell. Under the simplified assumption that each spike potential in the receptor axons give rise to a spike in the mitral cell, the bottom trace reflects the mitral cell activity.

The example given above indicates how convergence can increase sensitivity of a neural system. The high convergence ratio found in the olfactory pathways can be imagined to give an amplification that is more accentuated than the one shown in our example. In the following we shall describe a mathematical model of the increased sensitivity that can be realized by a system with convergence. To facilitate the description we shall use the term *response probability*, which is defined as the fraction of odour trials that is followed by a response.

(A) MODEL

Receptor cell activity during spontaneous discharge and response, is modeled by a Poisson process with a characteristic mean firing rate (Getchell, 1974; van Drongelen, 1978*a*, *b*). We define R_1 and R_2 as receptor cell-mean firing rates during spontaneous activity and during the response respectively. The variables m_1 and m_2 represent the same parameters for a connected mitral cell. In this study we have arbitrarily chosen the relation between mean firing rates of the mitral cell and the receptor cell to be 1 to 1. Different estimations of this relationship would lead to different numerical values. However the principles demonstrated in the following would remain identical. The time T during which neural spike activity is considered should theoretically depend upon the mean spike frequency of the receptor cells and the number of spikes of receptor cells, necessary to evoke one spike in a connected mitral cell. Because we lack such information and since we want to keep the model as simple as possible we shall chose convenient values for R_1T and R_2T without mentioning particular values for R_1, R_2 and T.

The real number of spikes generated in time interval T are indicated as N_r and N_m for the receptor cells and the mitral cell respectively. With the above mentioned definitions the probability to observe an activity, represented in the number of spikes, can be predicted with the Poisson distribution:

$$P(N = X) = \frac{(RT)^X}{X!}.e^{-RT}, \tag{1}$$

with: $X = 0, 1, 2, 3, \ldots$, N = number of spikes in time T, R = mean firing rate.

Silent receptors cells

In this model we consider K receptor cells to have identical properties. When the receptor cells are stimulated the number of spikes in a time interval T follows the probability density function represented in equation (1). In silent cells the value R_1 equals zero, and a response can be characterized by the appearance of at least one spike. The probability that spikes occur, i.e.

the theoretical response probability is given by:

$$P(N_r > 0) = 1 - e^{-R_2 T}. \tag{2}$$

The response probability for a mitral cell connected with K receptor cells can be calculated with the formula:

$$P(N_m > 0) = 1 - e^{-m_2 T} \tag{3}$$

with $m_2 = KR_2$.

In Fig. 2, mitral cell response probabilities are plotted against corresponding receptor cell response probabilities. Numerical values used in the graph of Fig. 2 are: $K = 1$, 10, 100, 1000; $m_1 = KR_1 = 0$. R_2T ranges from 10^{-5} to 10·00 "spikes".

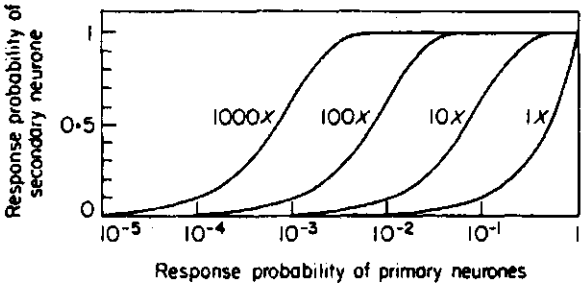

FIG. 2. Graphical representation of the relation between the response probabilities in the receptors and mitral cells at convergence ratios 1, 10, 100 and 1000. No spontaneous activity.

Spontaneously active receptor cells

In receptor cells which display a spontaneous discharge in the absence of overt stimulation, an excitatory response is experimentally recognized as an augmentation of the discharge frequency during a certain time. We will consider a response to be present when, during this time, the number of spikes is at least the mean of the spontaneous activity plus one standard deviation. During spontaneous activity, the mean number of spikes in a time interval T will be R_1T. A response in the same time interval will thus be at least $R_1T + \sqrt{R_1T}$ spikes. A theoretical expression of response probability of a receptor cell, is given by:

$$P(N_r \geqslant R_1T + \sqrt{R_1T}) = 1 - \sum_{X=0}^{R_1T+\sqrt{R_1T}} \frac{(R_2T)^X}{X!} e^{-R_2T}. \tag{4}$$

An addition of the response of these K receptor cells, which is an idealized picture of what happens in a mitral cell, shows Poisson processes with mean firing rates of $m_1 = KR_1$ and $m_2 = KR_2$ in the stationary and stimulated situation respectively. The theoretical response probability representing the

same requirement for the connected mitral cell as for the individual receptor cell is given by:

$$P(N_m \geqslant m_1T+\sqrt{m_1T}) = 1-\sum_{X=0}^{m_1T+\sqrt{m_1T}} \frac{(m_2T)^X}{X!}\, e^{-m_2T}. \qquad (5)$$

A numerical example of the relation between response probabilities of the receptor cells and the idealized mitral cell when connected to spontaneous active receptors is shown in Fig. 3. The values used in this example are: $K = 1$, 10 and 100 $R_1T = 1$; R_2T ranges from 1·001 to 10·000 "spikes".

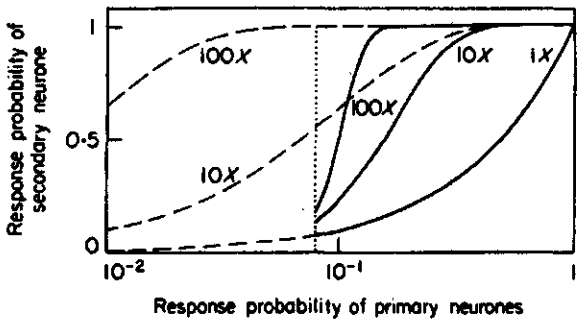

FIG. 3. Graphical representation of the relation between the response probabilities of the receptors and mitral cells when there is spontaneous activity. Convergence ratios 100, 10 and 1. Stippled lines show the response probabilities when there is no spontaneous activity.

A noticeable feature of the relation between response probabilities of the primary and secondary neurones revealed in this graph, is the steep augmentation in the response probability of mitral cells following a small increase in the response probability of receptor cells. Another feature revealed by the analysis is the lower limit of the response probability for the receptor cells below which the function in our case ceases to be meaningful. This phenomenon is due to the spontaneous activity. A significant augmentation of neural activity can occur when there is no stimulation, and there might be no significant augmentation when a stimulus is present. These errors become important for the detection of responses in the mitral cells when the response probability is low. Below certain response probabilities it is impossible to say if there is a response or not. In this region no objective criteria can determine whether an augmentation represents a fluctuation in spontaneous discharge or is caused by stimulation. The value for the response probability where these phenomena will occur is 0·08 in our example given and is marked by a vertical stippled line in Fig. 3. Other criteria for responses and other numerical values will lead to other values for this limit of response probability caused by the "error" fluctuations.

3. Sensitivity and Acceptor Distribution

The sensitivity of a neural system is dependent upon the convergence ratio. That ratio will anatomically be described by the number of receptor cell axons terminating on each secondary neurone. Functionally the convergence ratio will depend upon the fraction of these primary neurones that *ipso facto* can be excited, i.e., the overall receptor cell response probability. This probability is proportional to the number of encounters between an odorant and the receptor cells that give rise to one or several spikes. This number of successful encounters is in its turn dependent upon the number of "acceptors" on the receptor cell. In the following we shall use the term acceptor in a general way, postulating that there exists a structure at the cell level with a physico-chemical affinity to an adequate stimulating molecule (odorant). A successful fit between the odorant and the acceptor gives rise, via unknown process, to spike generation in the receptor cell.

A recent study of the properties of frog olfactory receptors (Revial, Duchamp & Holley, 1977; Revial, Duchamp, Holley & MacLeod, 1977), gives evidence indicating that the receptor cells carry a number of different types of acceptors corresponding to different odour groups. One might consider two different types of distributions of acceptors over the receptor cells; in one modality a receptor carries only one type of acceptor (unimodal sensitivity), in the other modality the receptors carry a combination of different acceptors (multimodal sensitivity). What are the consequences of these distributions for response probabilities of individual receptor cells and of secondary neurones? Examples of unimodal and multimodal sensitivities are shown in Fig. 4. It should be noted that the total number of acceptors are the same in the two modalities. If the receptor cells are stimulated with one of the four adequate stimuli *a*, *b*, *c*, or *d*, only one receptor cell will respond in the case of unimodal sensitivity. In the case of multimodal sensitivity *a* may excite one receptor *b* two, *c* three and *d* all four cells. Since we considered the response probability of a receptor cell to be proportional

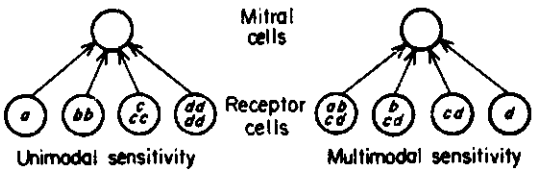

FIG. 4. Schematical representation of unimodal and multimodal sensitivity of receptor cells in the olfactory system. The letters a, b, c and d indicate the "acceptors" for the odours *a*, *b*, *c* and *d* respectively. In both cases the four types of cells converge onto one mitral cell.

to the number of acceptors per cell, the cells in the multimodal distribution will have a lower response probability than the cells in the unimodal distribution for odours *b*, *c*, and *d*. More appropriate, in view of the convergence, is the response probability of the connected mitral cell. The four types of receptor cells shown in Fig. 4 are in both cases connected to one mitral cell. Stimulating with one of the odours *a*, *b*, *c*, or *d*, the response probability of the mitral cell in the unimodal distribution equals the one found in one sensitive receptor cell. In the multimodal distribution, mitral cell response probability depends upon one or several receptor cells following the rules of probability calculation. In order to calculate mitral cell response probability in both types of distributions we shall chose a general model in which n receptor cells are connected with one mitral cell. The relation between the mean number of successful events per cell (m_i), the number of acceptors per cell (a_i) and the stimulus concentration (C) can be expressed as:

$$m_i = ka_iC, \tag{6}$$

k represents a reaction constant reflecting the affinity between acceptor and stimulant molecules. The real number of successful events in an arbitrary time interval may be described by a Poisson distribution. The probability for cell i P_{Ri} can be calculated by:

$$P_{R_i} = 1 - e^{-m_i}. \tag{7}$$

The response probability of a mitral cell (P_m) connected with n receptor cells with a response probability P_{R_i} is given by:

$$P_M = 1 - \prod_{i=1}^{n} (1 - P_{R_i}). \tag{8}$$

If the value for the P_{R_i} as given in (7) is substituted in formula (8) one has:

$$P_M = 1 - \exp\left[-\sum_{i=1}^{n} (ka_iC)\right]. \tag{9}$$

Thus the response probability of a mitral cell will be dependent upon the total number of acceptors to which the secondary neurone is connected via its receptor cells (n, a_i), the stimulus concentration (C) and the reaction constant (k). Thus the modality of sensitivity distribution over the receptors will not necessarily affect mitral cell response probability.

4. Conclusion

The main purpose of the present study was to demonstrate the amplification which can be obtained in a neural system with convergence as found in the olfactory system. At odour concentrations which commonly do not elicit an obvious response in the receptors one might find a conspicuous

response in the secondary neurones. Thus the convergence in the olfactory system is one possible way of obtaining the sensitivity for which this sensory apparatus is renowned. The high convergence ratio found in the olfactory system of all vertebrates and also in the olfactory system of insects, indicates that an analogous development in response to a demand of high probability of detection of chemical cues has occurred.

In the present study we have considered each spike in the receptor axon arriving at the mitral cell to evoke a post synaptic spike. Under this assumption a mitral cell will become depolarized to its physiological limit at moderate stimulus concentrations. Examples of such neurones have been demonstrated in the frog olfactory bulb (Døving, 1964). To prevent depolarization beyond the physiological limit, a neural system with high convergence ratio needs inhibitory mechanisms. In the olfactory system there exist different feed back loops that can serve this function (Shepherd, 1972; Pinching, 1972). These mechanisms may provide the means by which the olfactory system can make qualitative discrimination over a large range of concentrations, by appropriately conserving the essential parameters for the secondary neurones.

The recent studies by (Revial, Duchamp & Holley, 1977; Revial, Duchamp, Holley & MacLeod, 1977), demonstrate that there is a multimodal sensitivity in frog olfactory receptors. Theoretically these receptors have a smaller response probability than receptors with unimodal sensitivity. The important factor determining the sensitivity of the system is the response probability of the secondary neurones. We have demonstrated that the response probability of secondary neurone depends upon the total number of acceptors contained in its connected receptor cells. However, the type of acceptor distribution does not affect the response probability of the secondary neurones.

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ABSTRACT

Caterpillars perceive their food's taste by a limited number of receptor cells. As is shown in several studies reported in literature, gustation plays a crucial rôle in both qualitative and quantitative aspects of feeding responses. In this thesis, a comparative investigation on gustatory perception of plant constituents in several *Yponomeuta* species is described.

The species studied, their associated hosts and stimuli applied are shown in Table 1 of the first chapter. Electrophysiological data were obtained by means of a tip recording technique. The sensory cells respond to stimulation by generating a train of action potentials (Fig. 1, chapter 1). A model which can explain the shape and magnitude of the recorded action potentials is described in chapter 6. Routinely the number of spikes during an arbitrary interval of stimulation served as response index. In addition some of the activities were analysed in greater detail. This type of analysis, taking into account the number of activities in each recording and their time courses is described in chapters 1 and 5. Several types of interspecific sensitivity spectra have been recorded and are represented in Table 2 and Fig. 6 in chapter 1. A classification of a complete array of responses was carried out by a cluster analysis and is described in the second chapter; it appears that most species can be distinguished on the basis of functional criteria (Fig. 1, chapter 2). As far as can be determined natural concentrations (Table 1, chapter 1) appear to be situated above receptor cell threshold levels (Fig. 6, chapter 1). General aspect on relationships between receptor cell sensitivity, natural concentrations and data processing by the central nervous system are described in chapter 7. Although the vertebrate olfactory system is used to illustrate the examples in the seventh chapter, the principles described are also valid for the insect gustatory system. In larvae of *Y. cagnagellus* and *Y. evonymellus* behavioural responses to some of the stimuli used in the electrophysiological experiments were determined (chapter 3). To study inheritance of gustatory sensitivity, F1 offspring of reciprocal crosses between *Y. cagnagellus* and *Y. malinellus* was tested for sensitivity to solutions of dulcitol, phloridzin, prunasin and sorbitol (chapter 4).

SAMENVATTING

Fytofage insekten selecteren hun voedsel ondermeer door middel van smaak. Larven van verschillende soorten stippelmotten (Lepidoptera: Yponomeutidae) vormen een passende groep om de relatie tussen smaakgevoeligheid en waardplantkeuze op vergelijkende wijze te bestuderen; de soorten vertonen een duidelijke waardplantpreferentie, welke in de meeste gevallen onderling verschilt (Tabel 1, hoofdstuk 1). In het hier beschreven onderzoek zijn gegevens te vinden over de fysiologie en de bouw van de smaakorganen van de rupsen. De smaakprikkels die zijn getoetst werden uitgekozen op grond van de samenstelling van het natuurlijk voedsel (Tabel 1, hoofdstuk 1). De zintuigcellen reageren op een adequate smaakprikkel door een aantal actiepotentialen te genereren (Fig. 1, hoofdstuk 1). Deze actiepotentialen worden in de basis van de zintuigharen opgewekt en doorgeseind naar het centrale zenuwstelsel. Daarnaast wordt iedere actiepotentiaal waarschijnlijk ook antidroom voortgeleid naar de top van de haar. Deze voortgeleiding in twee richtingen en de bouw van de zintuigen kan de grootte en de bipolaire vorm van de gemeten actiepotentialen verklaren (Hoofdstuk 6). In dit onderzoek is meestal het aantal actiepotentialen per tijdseenheid gebruikt om de zintuigreactie te kwantificeren. Daarnaast is een fijnzinniger analyse uitgevoerd waarbij het tijdsgedrag van de zintuigreactie bekeken is (hoofdstukken 1 en 5).

Stoffen die voedselopname van fytofage insekten stimuleren of remmen zijn reeds lang bekend. De twee typen van reagentia zijn terug te vinden in de perifeer gemeten gevoeligheid van de verschillende bestudeerde *Yponomeuta* soorten (Tabel 2, hoofdstuk 1). Ter illustratie van het bovenstaande worden enige gevoeligheden hier besproken. Als een stof de voedselopname bevordert, mag men verwachten dat gevoeligheid wordt aangetroffen bij soorten die voorkomen op een waardplant welke die stof bevat. Hierbij wordt uitgegaan van een gelijksoortige neurale werking van zintuigreacties bij verschillende soorten. De activiteiten gemeten tijdens stimulatie met sorbitol (een suikeralcohol) leveren een mooi voorbeeld bij deze veronderstelling. Verschillende zintuigactiviteiten gemeten tijdens het toedienen van dulcitol (een stereo-isomeer van sorbitol) voldoen niet direct aan de bovenbeschreven verwachting. Larven die zich voeden met bladeren van *Euonymus europea* zijn gevoelig voor deze stof, maar *Y. evonymellus* en *Y. padellus* reageren ook terwijl deze stof in hun waard niet voorkomt (hoofdstuk 1). Gedragsonderzoek wijst uit dat dulcitol de voedselopname bevordert van zowel larven van *Y. cagnagellus (Euonymus)* als van *Y. evonymellus* (Prunus) (hoofdstuk 3). Stoffen die de voedselopname remmen ("deterrents") zullen waarschijnlijk alleen zintuigen van larven stimuleren die op een waard leven welke deze stof niet of nauwelijks bevat. De interspecifieke gevoeligheid voor phloridzin (een secundaire plantestof) voldoet aan deze verwachting (hoofdstuk 1). Wanneer een phloridzin oplossing wordt toegevoegd aan het natuurlijke voedsel van *Y. cagnagellus* en *Y. evonymellus* blijkt dit inderdaad minder aantrekkelijk, maar uitsluitend wanneer bladeren van de eigen waard aanwezig zijn. Wanneer *alleen* het met phloridzin gemodificeerde voedsel wordt aangeboden blijkt dit voor beide genoemde soorten even "aantrekkelijk" als het natuurlijke voedsel (hoofdstuk 3). Alle bestudeerde soorten blijken gevoelig te zijn voor prunasin, een stof die in bladeren van enige Prunoidae voorkomt. Met name in het blad van *Prunus padus*, de waard van *Y. evonymellus*, komt een hoge concentratie prunasin voor. In dit geval lijkt een eenvoudig uitgangspunt, zoals prunasin stimuleert of remt de voedselopname van alle soorten, niet op te gaan. Echter de kwantitatieve smaakgevoeligheid blijkt voor verschillende soorten sterk te verschillen. Larven van *Y. malinellus* en *Y. evonymellus*

zijn minder gevoelig voor de stof dan de andere soorten (hoofdstuk 1). Het effect van prunasin op de voedselopname is bestudeerd in een keuze-experiment: rupsen van *Y. cagnagellus* en *Y. evonymellus* zijn voor de keuze gesteld tussen neutraal voedsel waaraan uitsluitend dulcitol was toegevoegd en waaraan een mengsel van dulcitol+prunasin was toegevoegd. Larven van *Y. cagnagellus* vertoonden zeer duidelijk een voorkeur voor het voedsel met de dulcitol oplossing, *Y. evonymellus* vertoonde geen voorkeur (hoofdstuk 3). Daarnaast is een type interspecifiek gevoeligheidsspectrum als gevonden voor (+)-catechin (een secundaire plantestof) niet te verklaren met bovengenoemde eenvoudige veronderstellingen. Wanneer men de compleet gemeten reeks van kwalitatieve smaakgevoeligheden van de verschillende soorten in Tabel 1 van hoofdstuk 2 beschouwt, is het niet mogelijk alle soorten van elkaar te onderscheiden. Wel is het mogelijk de soorten in vier groepen in te delen, dit is in de laatste kolom van deze tabel weergegeven. Rekening houdend met de kwantitatieve gevoeligheid is het mogelijk een fijnzinniger klassificatie op te zetten. In dit geval zijn van elke soort vijf reacties op verschillende concentraties van elke stof bekeken. Elke reeks reacties kan worden weergegeven als een vector in een multi-dimensionale ruimte. Vervolgens kan men de zo verkregen punten indelen met behulp van een "cluster" analyse. Het resultaat van zo'n analyse is te zien in het dendrogram in Fig. 1 van hoofdstuk 2. Hierin vindt men de bovengenoemde vier groepen terug in de hoofdclusters. In slechts één geval, *Y. padellus*, is een van de soorten in het "onjuiste cluster" gekomen.

Het blijkt dat bij de rupsen de kwantitatieve zintuiggevoeligheid in de buurt van de natuurlijke concentraties van de smaakstoffen ligt (hoofdstuk 1). Het is waarschijnlijk dat dit voor zintuigcellen nog al uitzonderlijke verschijnsel gecorrelleerd is met de centrale verwerking van de zintuigsignalen. Met name het aantal zintuigcellen welke één bepaalde smaakstof waarnemen is bij lepidoptere larven gering. In het algemeen worden de relaties tussen zintuiggevoeligheid, natuurlijke prikkelsterkte en verwerking van zintuigcellen uitgewerkt in hoofdstuk 7. Daarnaast wordt in dit hoofdstuk ingegaan op de relaties tussen specificiteit van zintuigreacties en de gevoeligheid welke men in het gedrag mag verwachten.

Daar vele kruisingen tussen verschillende soorten *Yponomeuta* onderling fertiele nakomelingen opleveren, is een begin gemaakt met de analyse van overerving van smaakgevoeligheid. Beide reciproke kruisingen tussen *Y. cagnagellus* en *Y. malinellus* zijn ingezet; de alzo verkregen F1-nakomelingen zijn opgekweekt op beide ouderlijke waardplanten. Op deze wijze werden 4 experimentele groepen verkregen: nakomelingen van ♀♀ *Y. cagnagellus* x ♂♂ *Y. malinellus* opgekweekt op *Euonymus* en *Malus* (afgekort als $^{cxm}_{E}$ en $^{cxm}_{M}$); nakomelingen van ♀♀ *Y. malinellus* x ♂♂ *Y. cagnagellus* eveneens opgekweekt op *Euonymus* en *Malus* (afgekort als $^{mxc}_{E}$ en $^{mxc}_{M}$). De oudersoorten werden als controle groepen opgekweekt op de eigen waard (afgekort als $^{c}_{E}$ en $^{m}_{M}$). In de groep $^{cxm}_{M}$ bereikte geen der larven het vijfde stadium, noodzakelijk voor het meten van de smaakgevoeligheid. Voor de andere groepen zijn de gemiddelden van de reacties op prikkeling met dulcitol, phloridzin en prunasin weergegeven in Fig. 1, hoofdstuk 4. In de meeste gevallen is aan vijf individuen gemeten, wanneer een ander aantal is gemeten, is dit in Fig. 1 van hoofdstuk 4 aangegeven. Omdat vijf individuen een gering aantal is voor het bepalen van erfelijke eigenschappen is de kruising $^{cxm}_{E}$ tweemaal ingezet. Op deze wijze was het mogelijk meer individuen te toetsen. De uitkomsten van het groter aantal metingen verschilde niet significant van de eerder uitgevoerde 5 metingen aan vijf individuen.

Binnen het genus *Yponomeuta* blijken een aantal smaakgevoeligheden moeilijk te verklaren uit de samenstelling van de waardplanten. Bijvoorbeeld larven van

Y. evonymellus en *Y. padellus* blijken gevoelig voor dulcitol typisch voor Celastraceae (de plantenfamilie waartoe *Euonymus* behoort). Dit type gevoeligheid lijkt gezien de waard niet adaptief; mogelijk is hier sprake van pre- of post-adaptatie. Op grond van deze veronderstellingen kan men enige evolutionaire relaties binnen het genus *Yponomeuta* schetsen (hoofdstuk 1). Uitgaande van post-adaptatie is het waarschijnlijk dat de voorouder zich heeft gevoed met Celastraceae. Deze hypothese sluit aan bij de verdeling van waardplanten in het genus *Yponomeuta*. Zoals is te zien in de tabel bij het voorwoord, bevindt zich in elke taxonomische groep minstens één waard behorend tot Celastraceae. Echter, een alternatieve hypothese kan uitgaan van de Rosaceae als vooruderlijke waard. Vertegenwoordigers van deze familie komen niet in elk van de taxonomische groepen voor, maar zijn buiten het genus *Yponomeuta* zeer algemene waardplanten.

CURRICULUM VITAE

De auteur werd op 30 januari 1953 te Vlissingen geboren. Hij behaalde in 1970 het eindexamen HBS-B en begon in hetzelfde jaar zijn studie Biologie aan de Rijksuniversiteit Leiden. Maart 1977 behaalde hij het doctoraal examen Biologie cum laude, met de hoofdvakken Biologie en Dierfysiologie en het bijvak Diermorfologie.

Van april 1977 tot augustus 1979 was hij in dienst in het kader van de Stichting BION van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek (ZWO) en was als gastmedewerker werkzaam bij de Vakgroep Dierfysiologie van de Landbouwhogeschool te Wageningen. In deze functie voerde hij de hier beschreven experimenten uit. Sinds augustus 1979 geeft hij fysiologieles aan de Twentse Academie voor Fysiotherapie.