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SENSORY ACTIVITY AND FOOD INTAKE: A STUDY OF INPUT-OUTPUT RELATIONSHIPS IN TWO PHYTOPHAGOUS INSECTS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
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STELLINGEN

I De invloed van een toegevoegde smaakprikkel op het vraatgedrag van insecten kan men zuiverder meten door de gegeten hoeveelheid testdieet op zijn minst te corrigeren door het in mindering brengen van de hoeveelheid opgenomen controledieet.

Dit proefschrift

II Naast de duur van afzonderlijke maaltijden kan ook de intensiteit van voedselopname van invloed zijn op de totale hoeveelheid opgenomen voedsel.

Ma, W. C., 1972. Dynamics of feeding responses in *Pieris brassicae*.—Med. Landbouwhogeschool Wag., 72-11: 1–162.
Barton Browne, L., 1975. Regulatory mechanisms in insect feeding.—Adv. Insect Physiol., 11: 1–116.

III Experimenten waarbij zintuigen worden uitgeschakeld leveren een wezenlijke bijdrage aan het inzicht in de werking van die zintuigen, mits een goede correctie wordt aangebracht voor mogelijke ongewenste effecten van de schade, die bij deze ablaties wordt veroorzaakt.

Dit proefschrift

IV Hoewel in de meeste gevallen het (vlinder)wijfje bij de oviposition een goede waardplant voor haar nakomelingen zoekt, blijkt de bij rupsen aanwezige specifieke smaakgevoeligheid in een aantal gevallen relevant voor het tussentijds zoeken van een nieuwe waardplant.

V Aangezien de periodes verschillen, die bij het smaakonderzoek bij insecten door verschillende auteurs gekozen zijn voor het quantificeren van de electrofysiologische respons, zijn de resultaten niet zondermeer te vergelijken.

VI Hoewel de individuele zintuigcellen in de smaaksensillen bij rupsen veelal specifiek gevoelig zijn voor bepaalde klassen van prikkels, zal de gedragsreactie op complexe stimuli als volledige planten afhangen van de relatieve vuurfrequentie van alle smaakcellen.

Dethier, V. G., 1971. A surfeit of stimuli a paucity of receptors.—American Scientist 59: 706-715.

VII Het idee dat lange mensen trager reageren omdat ze langere zenuwbanen hebben, berust op een overschatting van de bijdrage die de geleidingstijd over de zenuwbaan levert aan de totale reactietijd.

Janbroers, J. M., 1973. Modern basketball.—Nijgh en van Ditmar, Den Haag.

VIII Dat de mogelijke verkorting van de studieduur voor biologie tot vier jaar, gezien het feit dat de besnoeiing waarschijnlijk vooral zal plaatsvinden in het huidige postkandidaatsprogramma, moet leiden tot een generatie biologen die nauwelijks ervaring met en inzicht in biologisch onderzoek hebben, valt te betreuren.

IX Zuiver wetenschappelijk onderzoek is maatschappelijk relevant.

X De initiatiefnemers van de actie "Blij dat ik rij" zijn zelf niet zozeer blij als zij rijden, maar veeleer als alle anderen zo massaal mogelijk rijden.

Proefschrift van F. Blom.
Sensory activity and food intake: a study of input-output relationships in two phytophagous insects.

Wageningen, 15 december 1978.

ZINTUIGACTIVITEIT EN VOEDSELOPNAME: EEN STUDIE OVER INPUT-OUTPUT RELATIES IN TWEE FYTOFAGE INSECTEN

SAMENVATTING

In dit onderzoek is het verband onderzocht tussen de zintuigrespons en de gedragsreactie op dezelfde stimulus. Daartoe werden zowel zintuig- als gedragsreactie kwantitatief beschreven in afhankelijkheid van de aangeboden concentratie smaakstof. De correlatie van zintuig- en gedragsreactie, die kunnen worden beschouwd als input en output van het centrale zenuwstelsel, werd gebruikt om een globaal inzicht te verkrijgen in de verwerkingsprincipes van het CZS.

Deze benaderingswijze werd toegepast op twee rupsesoorten: *Pieris brassicae* L. en *Mamestra brassicae* L. Beide soorten worden in de natuur aangetroffen op vertegenwoordigers van Cruciferae (o.a. kool). *Pieris* is oligofaag en beperkt zich in zijn voedselkeuze tot Cruciferae, terwijl *Mamestra* meer polyfaag is.

Bij rupsen zijn drie typen smaakzintuigen bekend: een paar (een laterale en een mediale) sensilla styloconica op iedere maxille en twee epipharyngeaal sensilla aan de binnenkant van het labrum. De zintuigrespons in deze drie sensillen werd met elektrofysiologische technieken bepaald. Daarbij worden actiepotentialen geregistreerd die optreden als reactie op de aangeboden stimulus. Het aantal actiepotentialen in de eerste seconde van stimulatie werd geteld (N_{sp}) en gebruikt als maat voor de zintuigactiviteit. De gedragsrespons werd gemeten in een vraattest, waarbij iedere rups (na 24 uur hongeren) gedurende 24 uur een stukje testdieet kreeg, waarin de smaakstof was geïncorporeerd. Het drooggewicht van de faeces (Fp), die tijdens deze 24 uur werd geproduceerd, werd gebruikt als maat voor de hoeveelheid opgenomen voedsel. De zintuig- en gedragsrespons werden gecombineerd in een relatief input-output diagram (hoofdstuk 2). De resultaten van deze bepalingen zijn vermeld in hoofdstuk 3 voor *Pieris* en in hoofdstuk 4 voor *Mamestra*.

De reacties op een aantal stoffen zijn gemeten en vergeleken, te weten:

saccharose (voor beide soorten)
inositol, een suikeralcohol (voor *Mamestra*)
sinigrine, een vertegenwoordiger van de mosterd-olie-glucosiden, secundaire plantestoffen van o.a. de Cruciferae (voor beide soorten)
strychnine, een vertegenwoordiger van de chemisch heterogene groep van deterrents, secundaire plantestoffen die de voedselopname remmen (beide soorten)
en het aminozuur proline (alleen voor *Pieris*).

Bij de resultaten wordt steeds achtereenvolgens de zintuigrespons, de gedragsrespons en de samenhang tussen beide behandeld. Bij *Pieris* (en in mindere mate bij *Mamestra*) zijn de effecten van een aantal ablaties, waarbij een of meer van de zintuigtypen werd uitgeschakeld, op de gedragsreactie op saccharose bestudeerd.

In *Pieris* veroorzaakt saccharose een reactie (toename van de impulsfrequentie) in een zintuigcel in ieder van de drie zintuigtypen. Saccharose stimuleert de voedselopname. Een correlatie van de relatieve zintuigactiviteit in ieder sensillum afzonderlijk met de relatieve gedragsreactie leidt tot een niet-lineair verband, terwijl correlatie van de relatieve totale zintuigactiviteit (som van de activiteiten in alle drie de zintuigtypen) met de relatieve gedragsreactie resulteert in een lineair verband. Uit de ablatie-experimenten blijkt dat een bilaterale uitschakeling van ieder van de drie zintuigtypen de gedragsreactie op saccharose verandert. Alleen uitschakeling van alle drie typen tegelijkertijd leidt tot een volledig verdwijnen van de gedragsreactie op saccharose. Unilaterale ablatie daarentegen van beide sensilla styloconica heeft geen enkele invloed op de gedragsreactie.

Bij *Pieris* reageert één cel op sinigrine (in het laterale sensillum styloconicum). Sinigrine verhoogt de voedselopname. De gedragsreactie op mengsels van sinigrine en saccharose kan volledig verklaard worden met de reacties op de afzonderlijke stoffen, zodat geen synergisme wordt gevonden. Impulsfrequentie en voedselopname van dezelfde individuele rupsen blijken positief met elkaar gecorreleerd. Correlatie van de relatieve zintuigrespons met de relatieve gedragsrespons op sinigrine (populatiegemiddelden zoals bij saccharose) geeft een rechtlijnig verband.

Strychnine stimuleert bij *Pieris* twee cellen (één in het mediale sensillum styloconicum en één in het epipharyngeale sensillum). Het remt de voedselopname. De gedragsreactie werd gemeten op diëten die naast strychnine ook saccharose bevatten. Als de relatieve gedragsrespons (percentage remming) wordt gerelateerd aan de relatieve zintuigrespons, dan resulteert weer een rechtlijnig verband.

In *Pieris* veroorzaakte proline een reactie in een cel in het laterale sensillum styloconicum. De gedragsreactie vertoont een duidelijk optimum bij 10^{-2} M proline. De resultaten zijn niet volledig duidelijk over het al dan niet bestaan van een synergistische relatie tussen proline en saccharose.

In *Mamestra* kon geen elektrofysiologisch bewijs verkregen worden voor de aanwezigheid van epipharyngeale sensilla. Daarom wordt aangenomen dat deze zintuigen in *Mamestra* niet aanwezig zijn. Saccharose en inositol stimuleren bij *Mamestra* ieder één cel (voor saccharose in het laterale sensillum styloconicum, voor inositol in het mediale sensillum styloconicum). Beide stoffen verhogen de voedselopname. Na

ablatie van beide sensilla styloconica kunnen rupsen nog steeds onderscheid maken tussen een dieet dat saccharose bevat en een controle dieet. Aangezien deze soort waarschijnlijk geen epipharyngeale sensilla bezit moet worden aangenomen dat er andere, nog onbekende, smaakzintuigen aanwezig zijn. Correlatie van de relatieve zintuigrespons met de relatieve gedragsreactie levert voor saccharose en inositol niet-lineaire verbanden op.

Sinigrine en strychnine veroorzaken beide een response in beide sensilla styloconica. Deze stoffen remmen de voedselopname (op een dieet waaraan saccharose is toegevoegd) alleen bij betrekkelijk hoge concentraties. De correlatie van input en output resulteert voor sinigrine in een rechtlijnig verband, maar voor strychnine in een niet-rechtlijnig verband.

In hoofdstuk 5 worden de reacties van *Mamestra* met die van *Pieris* vergeleken. De verschillen worden besproken in het licht van verschillen in voedselkeuzegedrag. De volgende hypothese relateert de verschillen in gedrag met een mogelijk fysiologisch mechanisme: *Mamestra* rupsen eten een aanzienlijke hoeveelheid van ieder geschikt substraat, saccharose heeft een geringe modulerende invloed. Bovendien zijn ze vrij ongevoelig voor smaakvergallende stoffen. *Pieris* rupsen daarentegen eten alleen een behoorlijke hoeveelheid als ze voldoende gestimuleerd worden. Tegelijkertijd zijn ze veel gevoeliger voor smaakvergallende stoffen. De resultaten van de saccharose-reacties in *Pieris*, zowel de zintuigactiviteiten als het gedrag na ablaties, zijn gebruikt om op een aantal verschillende manieren inputs uit de drie sensillum typen en uit symmetrische sensilla te combineren en te vergelijken met de gedragsoutput. Er wordt een schema voorgesteld voor de verwerking van de inputs binnen het CZS. De reacties op verschillende substanties worden vergeleken, waarbij de beperkingen van een onderlinge vergelijking worden geformuleerd. Enkele eigenschappen van het informatie-verwerkende systeem worden besproken, zoals de aanwezigheid van verschillende input-kanalen, het bestaan van variabele drempels en variabele Fp/spike verhoudingen.

De conclusie wordt getrokken dat er voor een aantal stoffen bij *Mamestra*, die een niet-lineair verband geven in het input-output diagram, argumenten bestaan voor de veronderstelling dat er nog andere zintuiglijke input naar de hersenen wordt doorgegeven uit onbekende smaakzintuigen. Voor een aantal andere stoffen (saccharose, sinigrine, strychnine bij *Pieris* en sinigrine bij *Mamestra*) ontbreken dergelijke aanwijzingen. Deze stoffen laten een rechtlijnig verband zien tussen input en output. Tenslotte kan worden vastgesteld dat, in aanmerking genomen de vermoedelijk grote complexiteit van de verwerkingsprocessen in het CZS, de input-output relaties een verrassende eenvoud vertonen.

was found that bilateral removal of either one of the three sensillum types did affect the behavioural response to sucrose. Only when all three types of sensilla were ablated simultaneously the behavioural response to sucrose was completely suppressed. Unilateral ablation of both stylconic sensilla, on the contrary, did not affect the behavioural response to sucrose in any way.

In *Pieris* a receptor in the lateral styloconic sensillum responds to stimulation with sinigrin. Sinigrin stimulates food intake, and the behavioural responses to mixtures of sucrose and sinigrin are adequately explained by simple summation of the responsed to the single components. Thus, no evidence for synergistic effects could be found. Impulse frequency and food intake of the same individual larvae were shown to be positively correlated. Correlation of the relative sensory responses with the relative behavioural responses to sinigrin (using population means, as for sucrose) again resulted in a linear relationship.

Strychnine evokes a response in two sensilla (i.e. the medial styloconic and the epipharyngeal sensillum). Strychnine inhibits food intake, the behavioural response was therefore measured in diets containing sucrose. Plotting the relative behavioural response as a function of the relative sensory response leads to a straight line relationship.

In *Pieris* proline stimulates a cell in the lateral styloconic sensillum. Behaviour was measured on diets containing pure proline or a mixture of sucrose and proline. Behavioural responses showed a clear optimum at 10^{-2} M proline. Experiments were not conclusive about the question whether or not the response on the mixture was higher than the summated responses on the components.

In *Mamestra* no electrophysiological evidence could be obtained indicating the presence of some epipharyngeal sensilla. It is assumed, therefore, that *Mamestra* does not possess such receptors. Sucrose and inositol both evoke a response in one type of sensillum (sucrose in the lateral styloconic sensillum, inositol in the medial styloconic sensillum), and both stimulate food intake. Correlation of relative input and output leads to non-linear relationships. After ablation of both styloconic sensilla, the operated larvae still discriminate between a sucrose diet and the control. This suggests that sucrose induces sensory input in some receptors hitherto unknown.

Sinigrin and strychnine both elicit responses in both styloconic sensilla. The two substances inhibit food intake at relatively high concentrations. Correlation of relative sensory responses (summated reactions from both sensilla) and relative behavioural responses (percentage inhibition) results for sinigrin in a linear relationship, and for strychnine in a non-linear relationship.

In chapter 5 the results from *Mamestra* and *Pieris* are compared. The differences are discussed in view of their differences in feeding habits. The following hypothesis relates the behavioural differences to a possible physiological mechanism: *Mamestra* larvae eat considerable amounts of any suitable substrate, and sucrose has only a weak modulating effect. They are also fairly insensitive to feeding deterrents. *Pieris*, on the contrary, eats only a considerable amount when stimulated sufficiently by certain phagostimulants, e.g. sucrose, and is at the same time much more sensitive to feeding deterrents. With regard to the sucrose response in *Pieris*, the results of the ablation experiments and the sensory responses of the receptors are used to discuss some possible ways of combining the inputs from the three sensillum types and from symmetrical sensilla. A model is suggested explaining the way sensory information is processed in the CNS as a basis of feeding activity. The responses to different chemicals are compared, realising the limitations of such comparisons. Some characteristics of the information processing system are discussed, the existence of various channels, the presence of variable thresholds, and Fp/spike ratios.

It is concluded that in *Mamestra* for some substances, that give non-linear relationships in their input-output diagram, arguments exist which suggest the presence of additional sensory input from gustatory organs hitherto unknown. For some other substances (sucrose, sinigrin, strychnine in *Pieris*, sinigrin in *Mamestra*) there is no need for assuming such receptors, and these compounds show linear relationships between sensory input and behavioural output. In view of the presumably great complexity of data processing in the CNS, these relations are remarkable simple.

CONTENTS

1. INTRODUCTION

1.1. GENERAL INTRODUCTION

Insects are very suitable for research on sensory physiology and also for behavioural studies. Many phytophagous insects have characteristic feeding habits, and may show remarkable food preferences. The sense of taste is known to play an important rôle in respect to hostplant selection. Insects are also suitable for electrophysiological studies, because of their limited numbers of sensory cells, and the possibility of recording sensory activity from the primary neurons. Because of these features especially blowflies have frequently been used in studies on chemoreception. For a study of sensory regulation of food intake behaviour lepidopterous larvae seem even more suitable than flies, since their number of sensory cells is still smaller, and also because of their characteristic feeding habits.

In the present study two species of phytophagous larvae are used, *i.e. Pieris brassicae* L. (Pieridae) and *Mamestra brassicae* L. (Noctuidae). *Pieris* is an oligophagous species, depending largely on Cruciferae. Since it seemed interesting to compare results obtained of an oligophagous insect with those of a polyphagous species, *Mamestra* was chosen as a second experimental insect. Both species are relatively easy to grow on an artificial diet. Much research has been done on *Pieris* and MA's (1972) study in particular may be regarded as a basis for the present investigation. Less detailed information is available for *Mamestra*,

although some behavioural and electrophysiological research on this species has been done as well (MA, 1972, WIECZOREK, 1976).

1.2. APPROACH

When studying sensory regulation of food intake, two different approaches are possible in an attempt to analyse the principles of information processing within the central nervous system (CNS). The direct method requires that the chain of neurons, which are involved in processing the incoming information, is elucidated. A thorough knowledge of neuro-anatomy is essential, and techniques for recording the activity of known neurons in the ganglia must be used. Some molluscs have a number of relatively large neurons, which are individually recognizable. Thus in these animals the requirements are met, and this method has been successful. In insects less in known of the neuro-anatomy of the ganglia, which makes it more difficult to use this method. With regard to chemosensory research in insects an extensive study using this approach has been started recently by Boeckh and collaborators (BOECKH *et al.*, 1976).

The second method considers the CNS as a black box, and involves the quantitative description of input to the CNS (information from gustatory cells) and the behavioural output. Comparison of input and output makes it possible to infer on a hypothetical basis what processes occur in the CNS. This latter method will be followed in the present study.

1.3. CHEMOSENSORY ORGANS

Chemosensory organs in lepidopterous larvae are described by SCHOONHOVEN & DETHIER (1966) and details pertaining to *Pieris* by MA (1972). On each maxilla one pair of styloconic sensilla (a lateral and medial one) are known to be gustatory receptors, each provided with four chemosensory neurons. Olfactory responses have also been reported to occur in the lateral styloconic sensillum (STÄDLER & HANSON, 1975). The maxillary palps are thought to bear mixed olfactory and gustatory sensilla (SCHOONHOVEN, 1972b). On the inner-side of the labrum a pair of epipharyngeal sensilla was first described by MA (1972) for *Pieris* and *Manduca sexta*, and were proven to be gustatory sensilla. These were also recorded from by DE BOER, DETHIER & SCHOONHOVEN (1977) in *Manduca sexta*, and their sensitivity spectra were established. This sense organ contains three chemosensory neurons. The epipharyngeal sensilla are not present in all lepidopterous

larvae, and they are known to be absent e.g. in *Dendrolimus pini* (MA, pers. comm.). The presence of yet another sensory organ in the buccal cavity of *Spodoptera* is hypothesized (MA, 1976). The morphological structure of all these sensilla (except the hypothetical ones) is known. Two different techniques are available to record electrophysiologically the activity of primary neurons (HODGSON, LETTVIN & ROEDER, 1955, and MORITA & YAMASHITA, 1959).

The innervation of the sensilla mentioned above is also known. Maxillary palps and styloconic sensilla are innervated by branches of the maxillary nerve, which originates at the suboesophageal ganglion (SCHOONHOVEN & DETHIER, 1966). The axons of the cells in the epipharyngeal sensilla join the labral nerve, which is connected to the frontal ganglion (MA, 1972) or the tritocerebrum (BULLOCK & HORRIDGE, 1965; MA, 1976). The suboesophageal and frontal ganglia are connected with the cerebral ganglion. The exact location of the axon terminations of the sensory cells, as well as any detail about the secondary neurons and the neural circuit to which the sensory information is transferred, is wholly unknown.

Studies by SCHOONHOVEN (1969b) and MA (1972) have revealed the sensitivity spectra for the various cells in *Pieris*.

In the lateral styloconic sensillum the four cells are sensitive to: 1. sucrose/glucose, 2. glucosinolates, 3. some amino acids, 4. anthocyanins.

In the medial styloconic sensillum the cells are sensitive to:
1. sugars, 2. deterrents, 3. some glucosinolates, 4. salt.

In the epipharyngeal sensillum the cells are sensitive to:
1. sucrose/glucose, 2. deterrents, 3. salt.

The sensitivity spectra vary from narrow (e.g. only sucrose and glucose) to relatively broad (deterrent cells). It is important to note that most substances in the present study stimulate in any sensillum only one cell. This feature greatly facilitates the exact determination of sensory input.

WIECZOREK (1976) reported that in *Mamestra* the chemoreceptory cells in the lateral styloconic sensillum are sensitive to:
1. sugars, 2. cations, 3. anions, 4. glycosides.

He paid special attention to the sensitivity spectrum of the glycoside-receptor.

1.4. CORRELATION BETWEEN SENSORY INPUT AND BEHAVIOURAL OUTPUT

The relationship between the number of nerve impulses during the first second of stimulation and food intake during 24 hours is not

simple and requires some presuppositions. The amount of faeces produced (see section 2.2) is strongly correlated with the amount of food consumed. The amount of food consumed in 24 hours is sometimes assumed to be entirely dependent on the time spent eating (MA, 1972). Also other parameters of food intake behaviour, i.e. feeding intensity, could contribute to the amount ingested (see BARTON BROWNE, 1975).

The time spent eating is divided in discrete intervals or meals (MA, 1972; BARTON BROWNE, 1975). The total time spent eating seems to depend on the physiological state (hunger) of the insect, and positive and negative input (i.e. information from sense organs). Negative input may arise from internal proprioceptors (abdominal wall and gut stretch receptors), as has been established in the blowfly (GELPERIN, 1971). These receptors react to stretching of the gut as related to the amount of food imbibed, and their activity inhibit food intake. With regard to phytophagous insects these receptors are so far only assumed to be present in lepidopterous larvae and acridids (BERNAYS & CHAPMAN, 1974). Positive and negative inputs also come from chemosensory cells, depending on which cell is firing.

The chemicals are classified as feeding stimulants and feeding deterrents depending on their effect on continuous feeding. Other classifications are made with respect to the various behavioural phases of the response which are affected by chemical stimuli, e.g. orientation, start of biting (BECK, 1965). The behavioural responses to a certain chemical depend on which cell is excited. Sensory adaptation gradually reduces the input from the gustatory senses in the course of a meal. Moreover, responses to feeding stimulants may adapt faster than responses to feeding deterrents (SCHOONHOVEN, 1977). In the feeding situation the sensilla may not contact the stimulus source continuously (depending on the size of larvae), in which case adaptation presumably proceeds slower than when stimulation is not interrupted.

Inputs to the CNS may have only instantaneous effects, or perseverating effects (BARTON BROWNE, 1975). A meal may be ended when negative input predominates the positive input, either because negative input from the stretch receptors grows, or because positive input adapts faster, or both (SCHOONHOVEN, 1972, 1977; BARTON BROWNE, 1975).

The foregoing considerations indicate that it is difficult to derive a direct quantitative relationship between the impulse frequency during the first second of stimulation and frass production during 24 hours. But the fact remains that an insect shows a behavioural discrimination between various chemicals, and the ability to recognize different concentrations of these chemicals at concentration levels as they occur in acceptable foods. Information about the nature and quantity of

specific chemicals must come from sense organs sensitive to these chemicals, and which show a concentration dependence in the same range as the behavioural discrimination. In view of this it seems reasonable to compare sensory input with behavioural output.

In the present study it is assumed that the frass production (Fp) reflects feeding behaviour adequately. Furthermore it is assumed that the impulse frequency during the first second of stimulation is a good measure of sensory activity. This assumption requires that during adaption impulse frequency decreases proportionately to the starting frequency (see also BARTON BROWNE, 1975). Comparison of frequencies of the first second and the first 100 msecs makes it possible to establish whether the ratios of frequencies at different concentrations remain the same (see section 5.2).

To recapitulate, the purpose of the present investigation is to know how the sensory responses and the behavioural responses to some chemicals are related in order to get some insight into the regulatory processes of the brain. Moreover, a comparison is made between an oligophagous and a polyphagous insect species.

1.5. LITERATURE ON INPUT-OUTPUT RELATIONSHIPS

The approach to obtain insight into central processing systems by quantitatively comparing input and output, is, until now, little practised in insect physiology. An extensive study has been made of the sugar response in the blowfly. Sensory responses of labellar and tarsal hairs, and behavioural responses to sugars have been established (summarized by DETHIER, 1976). Most of the work has been undertaken to explore sensory processes, and the structure of the sugar receptors. Both input and output are quantitatively known in this case, but they are difficult to relate to each other. The only comparison made concerns the tarsal sugar threshold in behaviour experiments and the x-intercept of the concentration-response curve in electrophysiology for a number of different sugars (DETHIER, *loc cit*). VAN DER STARRE (1972) discussed the validity of a comparison of behavioural tarsal threshold and electrophysiological data from single tarsal hairs.

MCCUTCHAN's (1969) work on the blowfly discusses quantitative behavioural responses to various acids. Electrophysiology was performed on tarsal hairs and, due to large differences among hairs, only general correlations to behavioural output could be made. In addition to the quantitative responses, a special type of behaviour (regurgitating, hesitative proboscis extension) at low pH's appeared to be correlated with an irregular firing of many cells.

In the studies mentioned either input or output could not be quantified sufficiently, and only some cases are available, in which both input and output are sufficiently detailed to allow quantitative comparisons.

GETTING (1971) in his study of sensory control of motor output in fly proboscis extension, shows correlations of input and output in individual blowflies. Input (sucrose impulses in labellar hairs) and output (motor activity in extensor muscles) are determined on a quantitative basis, and are even recorded simultaneously. Input-output correlations are presented on absolute scales. It is concluded that, depending on deprivation time and the individual fly, responses are proportionally following a straight line or first linear, increasing up to a maximum output and after that no further increase.

Another example is found in MA's (1972) study on *Pieris brassicae*. Relative input-output diagrams are given for sucrose responses. He concludes that the relationship between the behavioural responses in intact larvae and relative sensory responses in the lateral stylconic sensillum or epipharyngeal sensillum follows a non-linear function. In maxillectomized larvae, however, the relative feeding intensities and the reaction of the epipharyngeal sensillum do fit a linear function.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL INSECTS

Pieris brassicae L.

Fifth-instar *Pieris* larvae were used usually within 24 hours after their last larval moult; only insects weighing between 70–170 mg were used. They were raised from eggs on a semi-synthetic diet as described by MA (1972). The larvae were kept in an incubator with a photoperiod of 16 hours light and 8 hours darkness. They were maintained at 25° C unless the expected time of their last moult was inconvenient to start an experiment. In that case their development was retarded by placing them temporarily at a lower temperature (16° C). They were kept in glass petri-dishes and the diet was changed every other day. *Pieris* eggs were obtained from a continuous laboratory culture or from the Department of Entomology. Larvae for maintaining the culture were raised at least partly on cabbage.

Mamestra brassicae L.

Sixth-instar larvae weighing between 160–340 mg, were used, approximately 24 hours after the last larval moult. They were raised from

eggs on a semi-synthetic diet. The diet was modified after a diet described by NAGY (1970) for *Ostrinia nubilalis* Hbn: 4 g Vitamin Diet Fortification Mixture and 4 ml linseed oil were added per 1000 g diet. Larvae were raised in an incubator with a photoperiod of 16 hours light and 8 hours darkness, at a temperature of 20° C. They were kept in glass bottles placed horizontally with a layer of diet on one side. Larvae, at the end of their fifth instar, were removed from the bottles and kept in plastic boxes until their final larval moult. A number of larvae were used for maintaining the culture by allowing them to pupate. For that purpose they were transferred to boxes containing sterilized soil and diet in a foil cup. Moths were kept in a paper cilinder and offered a 10% honey solution. They laid eggs on the walls and top of the cylinder. Eggs were treated with 10% formalin for 30 minutes before they were put in bottles. The culture has been kept on an artificial diet for many generations.

2.2. BEHAVIOURAL STUDIES

Food intake was measured by determining the dry weight of the faecal pellets (= fras production: Fp), produced in a 24-hour test period on a test diet. This is a reliable parameter according to WALDBAUER (1964) and MATHAVAN & PANDIAN (1974), who report a strong positive correlation between dry weight of faecal pellets and dry weight of food consumed. This parameter was also used by MA (1972).

In a standard procedure designed to measure feeding activity larvae were selected by weight some hours after the beginning of a starvation period, which lasted for 24 hours. In each experiment a group of 5 or 10 insects was tested on the same experimental diet and concurrently one group of insects was kept on a control (empty) diet. Insects were distributed over the groups in such a way, that the mean weight of the various groups within one experiment did not differ more than 5% (in different experiments varying between 100–120 mg). During the test, larvae were housed separately in one of 10 compartments (6 × 4 × 2 cm) of a plastic box. After the starvation period each insect was given a piece of about 1 × 4 × 0.5 cm of the test diet and the 24-hour test period started. After the test period they were taken from the diet and faecal pellets were collected. In the beginning of this research, the pellets of a group of 5 larvae on the same diet were dried and weighed together. Later, in the major part of this study, the pellets of each individual larva were dried and weighed separately. Pellets were dried at 80° C until constant weight. The test diets consisted of 20 ml distilled water with 4% (0.8 g) agar

and 4% (0.8 g) cellulose in which the test chemicals were dissolved at various concentrations. A diet lacking the chemical to be tested, served as a control (empty diet). The diets were heated to about 100° C before pouring them into specific compartments of a plastic box. The 20 ml of diet in each compartment was cut into pieces immediately before the experiment. For behavioural and electrophysiological studies, identical concentrations of the chemical concerned were employed. Both in the test diets and electrophysiological experiment solutions of the following chemicals were used: sucrose, sodium chloride, L(−)-proline, agar-agar powder, cellulose all from Baker; meso-inositol from Fluka; sinigrin monhydrate from Aldrich, and strychnine-HCl from K & K.

2.3. CAUTERIZATIONS

In some behavioural experiments one or more of the styloconic or epipharyngeal sensilla had to be eliminated. This was done by electrical cauterization, which leads to coagulation of proteins by heat. The apparatus used was a Birtcher Hyfrecator, used with one electrode (unipolar) which was shaped into a fine needle. This allowed contact with restricted areas. The insects were anaesthetized with CO_2 after cooling on ice. The success of cauterization was assessed visually, and in some experiments electrophysiologically. When the pulses were delivered, the tissue immediately turned less dense. When cauterization was performed with strong pulses, the tissue had turned black a day later. In most experiments only a sample of the treated larvae was investigated electrophysiologically. Responses could be obtained from untreated sensilla and ought to be absent in the treated ones. In the experiments where only both the lateral or both the medial sensilla styloconica had to be ablated, each individual larva was checked electrophysiologically afterwards.

2.4. ELECTROPHYSIOLOGY

To obtain an indication of sensory activity tip-recording from the chemosensory sensilla was used, a method initiated by HODGSON, LETTVIN & ROEDER (1955). In the present study action potentials were fed, via a silver wire, into a Grass P15 B preamplifier, and could be observed simultaneously on a Telequipment oscilloscope. Test chemicals were always dissolved in a 0.05 M NaCl solution in order to improve the conduction of the signals and pure 0.05 M NaCl served

as a control stimulus. The severed insect head was mounted on a capillary filled with 0.1 M NaCl. Before mounting the head, its brain and mouthpart musculature were destroyed. When recording responses from the epipharyngeal sensilla, the head was fixed upside down with pins to a wax layer and the labrum positioned in such a way that the capillary could contact the two sensilla. In these stimulations capillaries were used with smaller tips than those normally used on the styloconic sensilla, in order to avoid leakage when the tip touched the labrum surface. Between two stimulations the sensillum was washed with distilled water and subsequently allowed to disadapt for a period of 3 minutes. Immediately before each stimulation the test solution was sucked through the capillary with a piece of filter-paper to ensure that the tip contained the intended concentration.

Recordings were made at ambient temperatures which varied between 20–24° C. A dish with water was placed just under the head to increase the local relative humidity.

2.5. DATA COLLECTION AND COMPUTATIONS

Feeding intensity was measured by determining the dry weight of faecal pellets of individual larvae or, in some instances, of groups of 5 insects. A control group was used in each experiment to assess the mean Fp on a feeding substrate lacking the test chemical(s) (= mean control Fp). This value was subtracted from all other values of Fp of the individual insects in the concomittant test groups. Thus the zero value of Fp in figures, tables and calculations represents the mean control Fp and in certain instances (e.g. feeding deterrents), the Fp may drop to a level under this value and approach the absolute zero, which is lower than the control Fp. The mean control Fp value of each experiment is given in the figures at the left hand side. The standard error of the mean was calculated from the values of individual Fp's (after correction for the control value). In some cases however, faecal pellets of groups were weighed together and calculation of the standard error was not possible. The concentration-response curves are plotted on a semi-log scale.

In the recorded electrophysiological responses the spikes were counted for the period between 50 and 1050 msec after the onset of stimulation (N_{sp}). Figures for the periods of 50–150 msec were kept separately. The figures obtained from this period, after multiplication by 10, were compared with the figures obtained by counting the one second period. This comparison gives an impression of adaptation rates.

For each concentration of each test chemical, the response of 7–12 individual sensilla were used to calculate the mean response and the standard error of the mean. These two values are displayed in the concentration-response curves.

In addition to determining the concentration-response curves of both behavioural and sensory reactions, the two types of responses were combined in an input-output diagram. In these plots the relative behavioural responses (maximum response, 100%) are presented as a function of the relative sensory responses (maximum response, 100%). Points representing concentrations higher than a concentration where one or both of the responses reaches 100%, are not included in the diagrams.

3. RESULTS AND DISCUSSION *PIERIS*

3.1. SUCROSE

3.1.1. *Sensory response*

In *Pieris* sucrose evokes responses in three different cells: one in the lateral, one in the medial styloconic sensillum and one in the epipharyngeal sensillum. Test solutions with concentrations of sucrose ranging from 10^{-4} to 5.10^{-1} M were used in the recordings. Responses from all three cells show in their response regular interspike-intervals, that increase slowly but steadily due to adaptation. The concentration-response curves of the three cells are sigmoid (Fig. 1), including a section in which the response increases with increasing concentration, followed by an approximately constant maximal level of response at higher concentrations. Both the cell in the lateral styloconic sensillum and the cell in the epipharyngeal sensillum start to fire at a lower concentration than the cell in the medial styloconic sensillum. The former cells also reach their maximal level of response at lower concentrations than the latter. The lateral and the epipharyngeal cell show an increasing response with increasing concentration between 10^{-4} and 2.10^{-2} M, the medial cell between 10^{-2} and 5.10^{-1} M. The maximal response reaches a higher firing frequency in the lateral cell (maximum 200 spikes per second) than in the medial and epipharyngeal cell (both maximally 145 spikes).

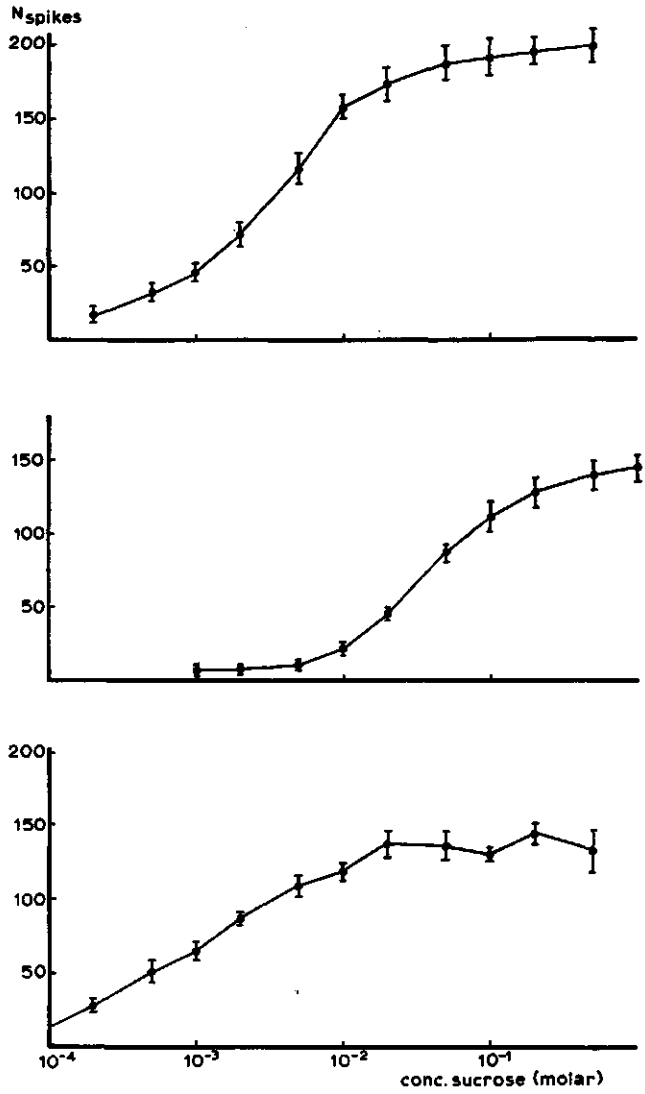

Fig. 1. *Pieris*, sensory responses on stimulation with sucrose in the lateral styloconic sensillum (upper trace), the medial styloconic sensillum (middle trace), and the epipharyngeal sensillum (lower trace). N_{sp} (number of spikes in the first second of stimulation) $\pm$ 1 $\times$ S.E.

3.1.2. *Behavioural response*

Sucrose at concentrations of 10^{-3} to 5.10^{-1} M was incorporated in test diets. Sucrose in this range stimulates food intake. The concentration response curve of this behavioural response is shown in Fig. 2, and will subsequently be referred to as the normal sucrose response. The response to sucrose starts at 10^{-3} M and increases with increasing concentration till the maximal response is reached at 2.10^{-1} M sucrose. At higher concentrations the response decreases, in accordance with the findings by MA (1972). At 2.1^{-1} M the maximal response corresponds to 7.7 × the control food (in Fig. 2 left of the ordinate).

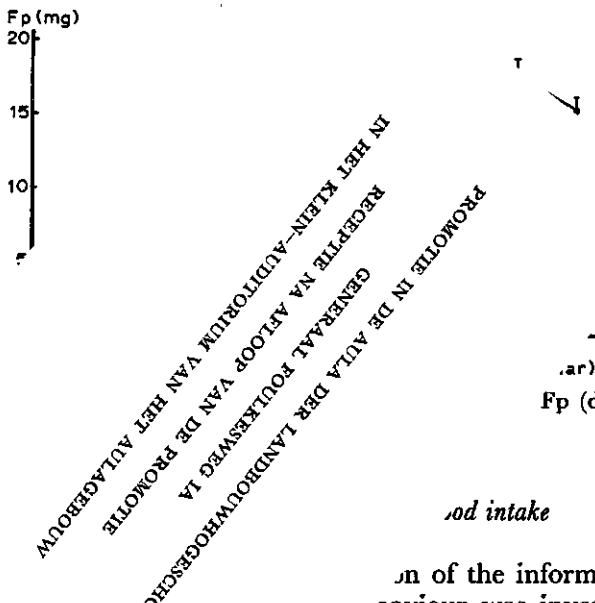

Fig Fp (dry weight of

od intake

n of the information from the naviour was investigated by ablati order to prevent that particular informati . In these operations it is always important (t) to differentiate between effects of the damage inh operations, and the intended effects of lost sensory infor am-operations were performed by fully anaesthetizing the an and treating them with a heat pulse directed at the spineret, which caused only slight damage. The following operations were performed:

a) *sham*-operations
b) *unilateral:* unilateral ablation of both styloconic sensilla
c) *lateral:* bilateral ablation of the lateral styloconic sensilla

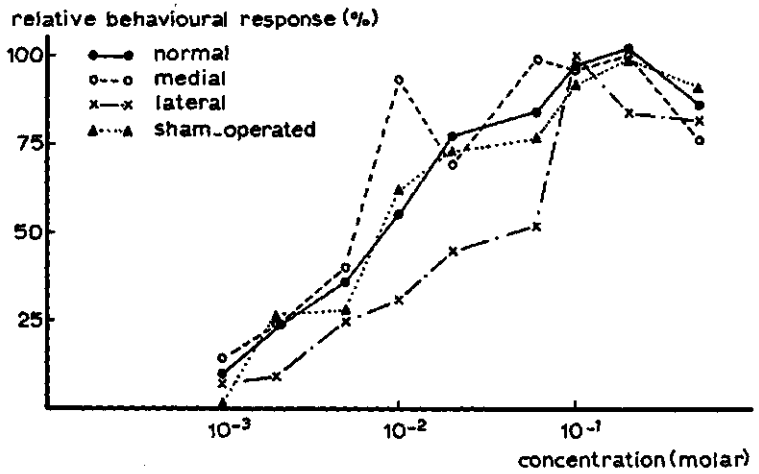

Fig. 3. *Pieris*, relative behavioural response of normal, medial, lateral, and sham-operated groups to diets containing sucrose. Behavioural response relative to the maximum.

most often at 2.10^{-1} M sucrose. The ratio between the maximal Fp and the mean control Fp (calculated from Table I) varies from 7.7 in the normal response to 1.4 in the palpectomy group. This variation is caused partly by a decrease of the maximal response and partly by an increase of the mean control Fp. All operated groups show a higher value of the mean control Fp than the normal (= intact) group. Therefore the higher control Fp value presumably reflects an effect

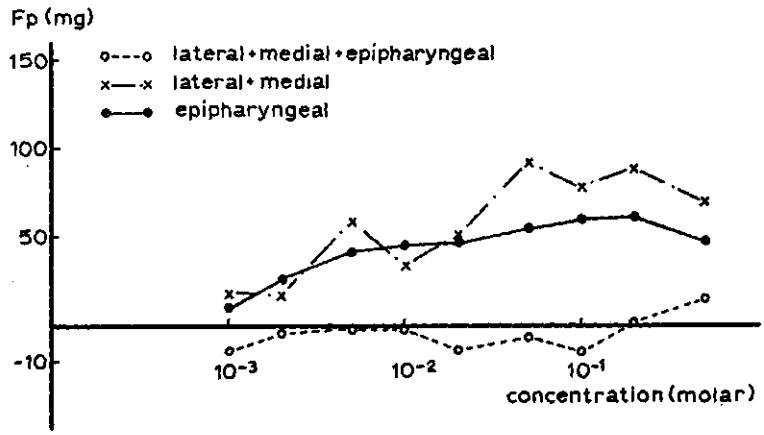

Fig. 4. *Pieris*, behavioural response of lateral + medial + epipharyngeal, lateral + medial, and epipharyngeal groups to diets containing sucrose. Fp $\pm$ 1 $\times$ S.E.

due to the operation. The small difference found in maximal responses of normal insects compared with sham-operated, lateral and medial insects might be explained by the higher control value, which was subtracted from the original values of Fp. If the maximal response of the normal insects equals the maximum that the insect is capable of eating (on the culture medium the same amount is eaten), then the increase in the control value can not be compensated by an even higher maximal response. Since higher control Fp values are subtracted from the same values on sucrose, the corrected Fp values of the operated insects are lower than those of the normal insects.

Effects of the various types of ablations

1) Sham-operated and unilaterally operated insects

The maximal response in normal insects seems larger than the maximal response in sham-operated insects, but due to the large variations, this difference is not significant. The maximal responses of sham-operated and unilateral groups are the same. The relative figures of normal and sham, or normal and unilateral groups do not give a significant difference. This means that there are also no differences in the ratios of the various responses at different concentrations.

It can be concluded that there is no significant change in behaviour after these operations. The slight difference in maximal response must be due to the operation effect. Between the normal and sham-operated group no differences, other than those resulting from the operation were expected. The fact that the unilaterally operated group showed no decrease in maximal response although the total number of spikes reaching the CNS is halved, could be attributed to either a learning process or to a special neural pathway in the central processing, which makes up for the partial loss of information. To investigate whether or not a learning process is involved, an experiment using a short test period (6 hours) was conducted, instead of the standard 24 hours. At a concentration of 2.10^{-1} M the feeding intensity is reflected by mean Fp values of 49 for unilaterally operated insects, 43 for sham-operated insects and 63 for normal insects. These values do not differ significantly from each other. Since the post-operative recovery period overlaps with the pretest starvation period, these animals have no possibility to get used to their missing information, and they must adapt their behaviour during eating. It therefore seems unlikely that the insects in this short test period have adapted completely to their new situation. It is concluded that one complete set of receptors suffices to induce a normal behavioural response, and probably neural connections within the CNS are such that compensation occurs for loss of the second set of receptors.

2) Lateral and medial groups

Maximal responses of lateral and medial groups do not differ from each other and are equal to the maximal response of sham-operated controls. Again the maximal response of the normal insects is slightly higher. The difference must be due to operation effects. When relative feeding intensities of lateral and medial groups are compared, significant differences are observed ($p < 0.02$). The relative figures of the lateral group are lower than the values observed in the medial group (see Fig. 3). Thus the intensity of the response of the medial groups increases more steeply with increasing concentrations than the response of the lateral group. The same holds if the lateral group is compared with normal insects ($p < 0.02$). Relative figures of the medial group show a tendency to lie above those of normal insects, but due to the very low value of Fp at 2.10^{-2}, this is not significant. It is concluded that ablating either both lateral or both medial styloconic sensilla does not influence the maximal response, but it does affect the ratios of responses at different concentrations. The two types of operations lead to different effects. At 10^{-2} M sucrose, for instance, Fp values of the lateral group are higher, and those of the medial group are lower than those of intact animals.

3) Lateral + medial group and epipharyngeal group

Lateral + medial and epipharyngeal groups both show an increased food intake on sucrose diets over the control diet. Maximal response of lateral + medial and epipharyngeal groups is significant lower than that of normal insects (epipharyngeal: $p < 0.001$; lateral + medial: $p < 0.05$) and than sham-operated, lateral, medial and unilateral groups. This might reflect an operation effect, but the damage when ablating both sensilla styloconica bilaterally does not seem to be more serious than when ablating only one of the sensilla styloconica bilaterally or both sensilla unilaterally. Ablating the epipharyngeal sensilla presumably inflicts more serious damage, as the whole innerside of the labrum is affected. The maximal response of this group is again a little lower than of the lateral + medial group. Thus in these groups the lower maximal response might be caused by a combination of operation effect in addition to intentional effects. The relative responses of lateral + medial and epipharyngeal insects do not show a significant difference. When compared to normal insects the lateral + medial group does not differ significantly, but the epipharyngeal group does. Relative Fp values of the group are higher than those of the normal insects. If ablation of the innerside of the labrum results in damage, which seriously inferes with normal feeding activities, this leads to a lower maximal response which is reached at lower sucrose

concentrations. This lower maximal response is used in computing the relative figures, and these are influenced in this way. Small differences in Fp give larger differences in the relative figures than if the maximal response had been larger.

4) Ablation of the maxillary palps leads to a decrease in the maximal response when compared to normal insects ($p < 0.01$), but not when compared to sham operated insects. The relative figures of normal and palpectomized insects do not show significant differences. This is the result of a balance in the figures at high concentrations, and a clear difference at low concentrations. At low concentrations the relative figures of palpectomized group are lower than those for normal insects. Control food intake is strongly increased after ablation of the palps. If uncorrected values of Fp are compared, food intake of palpectomy group at the lower concentrations (10^{-3}–5.10^{-3} M) is even higher than that of normal insects, though maximal food intake is lower than the maximum level reached in normal insects (see Table II). This agrees with MA (1972), who found that food intake at low concentrations of sucrose was enhanced after extirpation of the maxillary palps. In the present study the control food intake is always subtracted, resulting in lower calues of Fp at low concentrations. It is clear that palpectomy results in less discriminative eating of the animals: no increase is observed in food intake at 10^{-3} and 2.10^{-3} M over the control diet.

3.1.4. *Changing conditions*

In contrast to our standard experimental procedure the behavioural response to sucrose diets is reported here as measured in unstarved larvae. They were offered a test diet shortly after being taken from the culture medium (having been weighed in the meantime). The behaviour of these insects is shown in Fig. 5 (absolute figures) and Table III (relative values of Fp).

Although the maximal response (at 5.10^{-1} M) in unstarved insects does not differ significantly from the maximal response of normal (= starved) insects, the curve lays as a whole at a lower level than the normal response. To test for a difference in ratios of the responses at the different concentrations, the relative figures were tested in a Wilcoxon test, which indicated a significant difference ($p < 0.02$). This means that the relative figures of unstarved insects are lower than those of normal insects. Especially in the lower concentration range (10^{-3}–2.10^{-2} M) the unstarved insects eat proportionately less than normal ones from the same diet. The larvae do not discriminate be-

TABLE II

Fp values of normal (= intact) and palpectomized insects on sucrose diets.

Concentration	*Control*	10^{-3}	2.10^{-3}	5.10^{-3}	10^{-2}	2.10^{-2}	5.10^{-2}	10^{-1}	2.10^{-1}	5.10^{-1}
Palp group	72	62	70	91	155	137	160	171	159	148
Normal	23	41	66	87	120	160	171	199	200	176

TABLE III

Relative Fp values of starved (= normal) and unstarved insects on sucrose diets.

Concentration	*Control*	10^{-3}	2.10^{-3}	5.10^{-3}	10^{-2}	2.10^{-2}	5.10^{-2}	10^{-1}	2.10^{-1}	5.10^{-1}
Unstarved abs.	28	−1	18	43	61	106	136	149	156	157
relative		0	11	27	39	68	87	95	99	100
Normal (= starved) relative	23	10	24	36	55	77	84	97	100	86

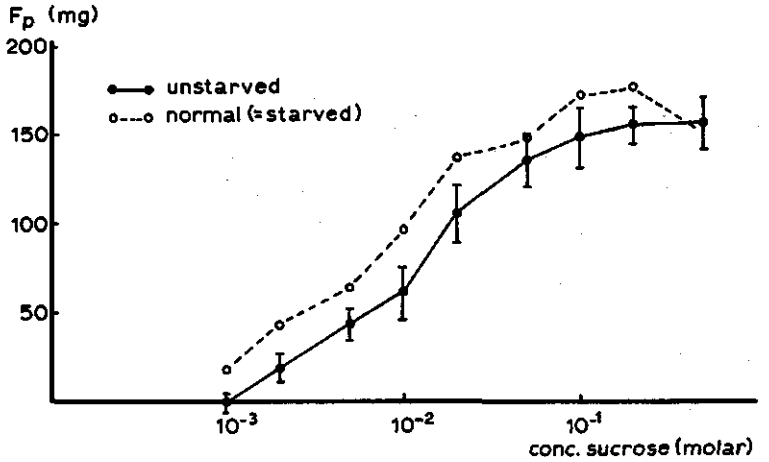

Fig. 5. *Pieris*, behavioural response of unstarved insects to diets containing sucrose, the dotted line represents the behavioural response of normal (= 24 hours starved) insects. Fp $\pm$ 1 $\times$ S.E.

tween the 10^{-3} M sucrose diet and the control diet. Consequently the insects show a reduced discrimination in food intake. In order to check whether or not starvation influences receptor sensitivities, the sensory response of larvae, that had been deprived of food for 24 hours, was measured. Sucrose concentrations of 10^{-1}, 5.10^{-2}, 10^{-2} and 10^{-3} M were used. The number of spikes in the first second from the lateral and medial styloconic sensilla were compared with those obtained from unstarved insects (the latter are used in the standard electrophysiological procedure throughout this work). For each concentration numbers of spikes for unstarved and starved insects were tested in a Mann-Whitney U test. For 5–9 sensilla of unstarved larvae the spike frequency for each concentration was established and compared to those for the normal (= starved) insects (10–12 sensilla). For each concentration the test variable U did not exceed the 10% critical value. From the results obtained it was concluded that starvation does not affect spike frequencies significantly.

3.1.5. *Correlations*

Sensory and behavioural responses are correlated in an input-output diagram. The relative behavioural responses are plotted as function of relative sensory responses.

To determine in which way the normal sucrose response depends on

sensory input from the three different sucrose sensitive cells, first the behavioural response is plotted versus the responses of each of the three individual cells (Fig. 6). The resulting groups of points for all three cells clearly deviate from a straight line. Response from the lateral styloconic sensillum as well as from the epipharyngeal sensillum give deflections below the line $y = x$. This finding agrees with MA's (1972, Fig. 76) observations. The response of the medial styloconic sensillum, on the contrary, gives a deflection above the line $y = x$. These results could be predicted on the basis of a comparison of figure 1 to 2. Both the lateral stylconic sensillum and apipharyngeal sensillum start responding at a concentration which is lower than the concentration required for a behavioural response. They reach, however, their maximal activity at a concentration, at which the behavioural response is only 55% of its maximum. The medial styloconic sensillum shows the opposite relationship. At low concentrations the receptors do not reacht, while feeding behaviour increases with increasing sucrose concentration. From 10^{-2} M and upwards the response of the medial styloconic sensillum increases, reaching a maximal response at the same value as the behavioural response.

From these results it may be inferred that the responses in the lateral styloconic sensillum and the epipharyngeal sensillum cause the increase in behavioural response at low sucrose concentrations, whereas at high concentrations the increase in feeding response is predominantly due to activity in the medial styloconic sensillum. For this reason, and because the ablation experiments indicate that input from all 3 sensilla is essential, a total sensory response was calculated by simply adding the number of spikes from the three different cells (L + M + E) at each concentration. The relative behavioural response is plotted versus this relative total sensory response (Fig. 7). Since the points suggest a linear relationship, the regression line ($Y = 1.25\ X - 21.81$) was calculated from values based on sucrose concentrations of 10^{-3} to 2.10^{-1} M. From the correlation coefficient (0.99 for these variables) it can be seen that the points fit closely to the regression line. This line does not go through the origin, but cuts the absissa at 17.5%. This means that when fewer than 17.5% of 473 spikes, i.e. 82 spikes per sec. are initiated, no behavioural response will occur. It is known (MA, 1972) that some sugars other than sucrose and glucose evoke action potentials in the medial styloconic sensillum without giving any increase in food intake. The number of spikes evoked by these sugars in the medial receptor appears to be always lower than 82, as could be determined from MA's (1972) data.

A maximal (100%) behavioural response is reached at 97% sensory response. This simple relationship, however, does not prove that the

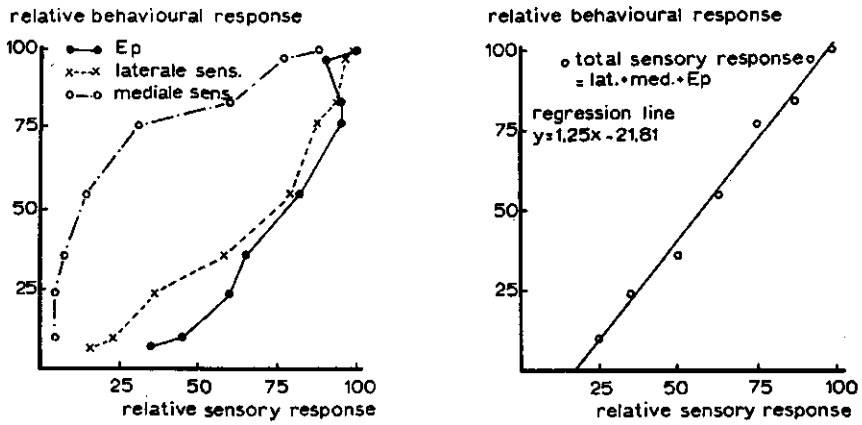

Fig. 6. *Pieris*, correlation between the relative sensory response of each of the three sucrose sensitive cells and the relative behavioural response to sucrose.

Fig. 7. *Pieris*, correlation between the relative "total sensory response" of the three cells summated (L + M + E) and the relative behavioural response to sucrose.

total sensory input is really being computed and used in the CNS merely according to such a simple procedure (see section 5.3).

As stated before the ablation experiments indicate that input from all three sensilla is essential. This is concluded from the fact that ablation of each one of the three types of a sensillum has an effect on behaviour. None of the ablations of a single sensillum type leads to a complete suppression of increase in food intake. This only occurs after removing all three sensilla.

The relative behavioural response of palpectomized insects is plotted versus the relative total sensory response (Fig. 8). Points for this group also show a linear correlation (correlation coefficient = 1.00). Compared to the regression line of sucrose response in intact insects (dashed line) the former line is rotated, regression coefficient 1.92 compared to 1.25 for normal response, and shifted to the right. This results in almost no difference between the operated and the intact insects at high responses (*i.e.* sucrose concentrations), and a large difference at low responses (*i.e.* low sucrose concentrations). The behavioural response in palpectomized insects thus starts at sensory response levels which are higher than in normal insects. Although ablating the maxillary palps results in a changed behaviour, especially at lower sucrose concentrations, the palps themselves do not suffice to give a higher food intake than on the control diet (lateral + medial + epipharyngeal group), in electrophysiological recordings from the palps (from the total palp as well as from individual sensilla) differences

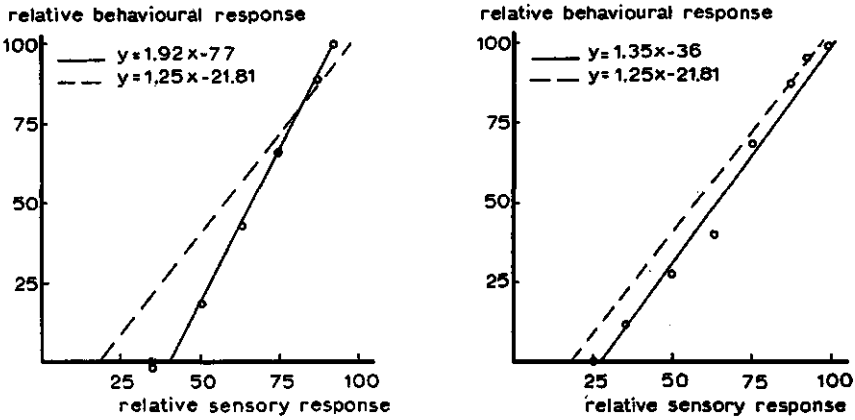

Fig. 8. *Pieris*, correlation between the relative "total sensory response" of the three sucrose sensitive cells summated (L + M + E) and the relative behavioural response of palpectomised insects to sucrose. Dotted line for normal (= intact) insects.

Fig. 9. *Pieris*, correlation between the relative "total sensory response" of the three sucrose sensitive cells summated (L + M + E) and the relative behavioural response of unstarved insects to sucrose. Dotted line for normal (= starved) insechts.

between stimulations with sucrose solutions or 0.05 M NaCl were never seen. The individual sensilla on the palp are small and with the equipment in use barely visible. Recordings were performed on individual sensilla, but it is uncertain if all types of sensilla were reached. Clearly, the palps do not contribute to the specific sensory input with regard to sucrose (see also MA, 1972). The palps probably affect food uptake in a more general way by exerting an inhibitory effect (ISHIKAWA *et al.* 1969).

Behavioural responses of unstarved larvae to sucrose were changed in comparison to the normal sucrose response in starved larvae. In Fig. 9 it is investigated how starvation affects the input-output relation. The relative behavioural response of unstarved insects is plotted as function of the relative total sensory response. The points fit the regression line that was calculated from the relative figures (correlation coefficient: 0.94). The regression coefficient of the line for the unstarved insects is not significantly different from the one of the line for the starved insects (1.35 versus 1.25). The regression lines are thus almost parallel, the line for the unstarved group is shifted statistically significant to the right. Thus the same relative behavioural response is only reached at a higher relative sensory response.

3.2. SINIGRIN

3.2.1. *Sensory response*

Sinigrin evokes a response only in the lateral styloconic sensillum of *Pieris* larvae, and neither the medial styloconic sensillum nor the epipharyngeal sensillum show a reaction to sinigrin. A concentration response curve of sinigrin for the lateral sensillum is shown in Fig. 10.

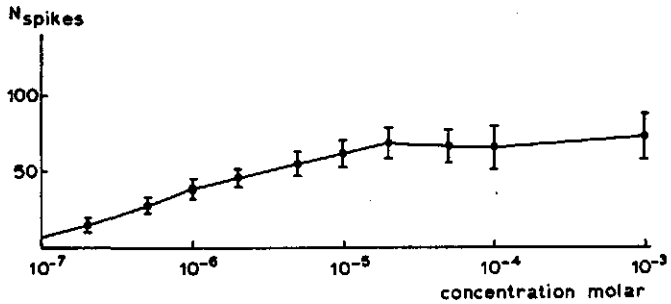

Fig. 10. *Pieris*, sensory response on stimulation with sinigrin in the lateral styloconic sensillum. N_{sp} (number of spikes in the first second of stimulation) $\pm$ 1 $\times$ S.E.

The sensory threshold occurs at about 10^{-7} M sinigrin, and the response increases with increasing concentration up to 2.10^{-5} M sinigrin. At higher concentrations the response remains at a maximal level of about 70 spikes during the first second (much lower than the maximal response of the sucrose cells). The rising phase of the curve is approximately linear. In contrast to stimulation with sucrose or strychnine stimulation with sinigrin induces firing with irregular intervals. Groups of short and long intervals clearly alternate (see section 5.2). To investigate whether or not sinigrin and sucrose interact at the receptor level, mixtures of sucrose (2.10^{-3}, 10^{-2}, 5.10^{-2} and 10^{-1} M) ad sinigrin (10^{-5}, 10^{-6} M) were used to stimulate the styloconic sensilla. In a sign test observed numbers of spikes on mixtures were compared with expected numbers, based on observations using single compounds. For the lateral styloconic sensillum the observed number of spikes was compared with the summated number of spikes for the constituting concentrations of sucrose and sinigrin, resulting in 12+, 16−, 2= (+ if observed < summated number) for all concentrations taken together. For the medial styloconic sensillum the observed number of spikes elicited by the mixture was compared with the number on sucrose alone (since pure sinigrin does not induce any response), resulting in 5+, 5− (+ if observed > expected number) in the sign

test. These values, of course, do not indicate a significant effect. Therefore, it is concluded that no interaction occurs at the sensory level between sucrose and sinigrin.

3.2.2. *Behavioural response*

When in behavioural tests diets are offered, which contain only various concentrations of sinigrin, an increase in Fp over that on control diet can be measured (for instance on 10^{-5} M sinigrin original not corrected values of Fp tested to the control values, $p < 0.001$). Maximal Fp on sinigrin diets is not nearly as high as on sucrose diets, since it reaches only 1.9 × the mean control Fp. A behavioural response starts at 10^{-7} M sinigrin, and the maximal level is reached at approximately 10^{-5} M sinigrin (see concentration-response curve: the lower curve in Fig. 11). MA (1972) found some food intake on pure 5.10^{-3} M

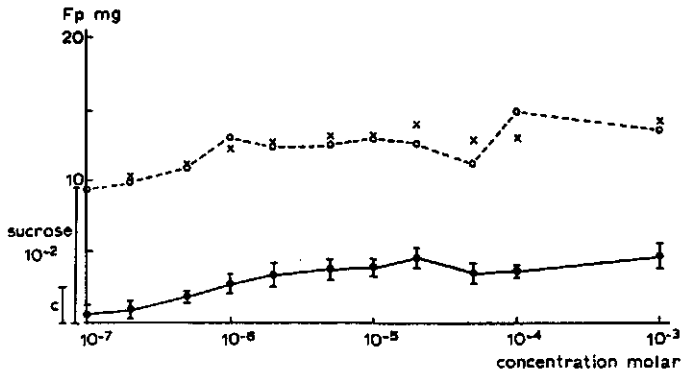

Fig. 11. *Pieris*, behavioural response to diets containing sinigrin (lower curve) and to diets containing sinigrin combined with 10^{-2} M sucrose (upper curve). Fp ± 1 × S.E. (for lower curve). Behavioural response to pure 10^{-2} M sucrose left of ordinate. Crosses are the expected values obtained by adding the response to 10^{-2} M sucrose to the lower curve.

sinigrin, using the Fp parameter, but no effect of pure sinigrin on his T_s, T_1 and F_i parameters (duration of meal, duration of first eating period, number of interruptions during a meal). Thus for the Fp parameter our results are in agreement. The fact that sinigrin, when applied pure, does not influence T_s, T_1 and F_i parameters, as compared to a control diet, might mean that either the amount of food intake (reflected in Fp) does not solely depend on duration of feeding but also on the rate of ingestion, or the frequency of meals during the

24-hours. In addition to measuring behavioural responses on diets containing only sinigrin, the responses to mixtures of sinigrin and sucrose were determined, in order to investigate whether Fp represents the mere summated response to both constituents or whether a synergistic interaction between both compounds lead to increased reactions (MA, 1972). Sucrose at a standard concentration of 10^{-2} M was combined with a concentration series of sinigrin. Also a single concentration of sinigrin (10^{-3} M) was combined with a complete sucrose series. Behavioural responses to these two series of mixtures are plotted in Fig. 11 (upper curve) and Fig. 12 respectively. In Fig. 11 (upper

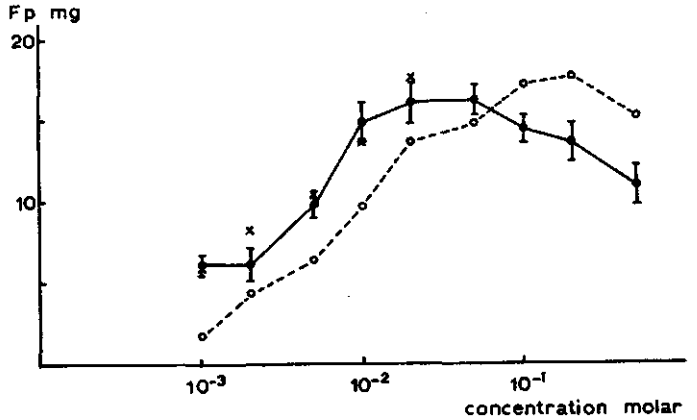

Fig. 12. *Pieris*, behavioural response to diets containing sucrose combined with 10^{-3} M sinigrin. Fp $\pm$ 1 $\times$ S.E. Dotted curve represents the behavioural response to pure sucrose, crosses are the expected values obtained by adding the response to 10^{-3} M sinigrin to the dotted curve.

curve) the behavioural response starts at 10^{-7} M sinigrin and increases with increasing concentration up to 10^{-6} M. Higher concentrations of sinigrin do not seem to increase food intake any further. In the figure expected values of Fp on sucrose-sinigrin mixtures, calculated by adding the Fp value on a 10^{-2} M sucrose diet (left of ordinate) to the Fp values on the various concentrations of sinigrin alone (Fig. 11, lower curve), are given as crosses near the points of the observed values for the mixtures. Observed and expected values for the various concentrations (2.10^{-7} — 10^{-3} M) are treated as pairs in the Wilcoxon matched-pairs signed-ranks test. Since this resulted in a T-value of 15 (N= 10), it is concluded that no significant differences do exist between observed and expected values.

In Fig. 12 the responses to mixtures (solid line) are compared to those on pure sucrose diets (dotted line) and on a diet containing

only 10^{-3} M sinigrin. Feeding responses to mixtures are greater than to pure sucrose diets at concentrations between 10^{-3} and 5.10^{-2} M sucrose, but at higher concentrations they are decreased. The maximum value of Fp on mixtures (163 mg at 5.10^{-2} M sucrose) does not significantly differ from the maximum value reached on a pure sucrose diet (177 mg at 2.10^{-1} M), which is in accordance with MA's (1972) observation. In the mixtures the feeding response reaches the maximum intensity at a lower sucrose concentration, and subsequently decreases at higher concentrations. The decreasing response part of the curve parallels the pure sucrose response at high concentrations. The fact that the responses to mixtures do not reach a higher maximum, may again indicate that this maximum is limited by other factors than sensory stimulation (see also section 3.1.3). Responses to the mixtures in the increasing phase of the curve at 10^{-3} — 2.10^{-2} M sucrose are compared to the expected values of Fp, obtained by adding the response to 10^{-3} M sinigrin and the response to various sucrose concentrations (crosses in Fig. 12). It may be seen that the observed and expected values match each other satisfactorily.

The two combination experiments, involving a fixed concentration of one compound (either sinigrin or sucrose) and a varying concentration of the other chemical, show that response of mixtures do not differ significantly from the summated response to the constituting components, and, therefore no synergism is inferred. This finding is in contrast to MA's (1972) conclusions.

3.2.3. *Direct correlation of behavioural and sensory response*

In the major part of the present investigation correlations have been studied using population means, i.e. comparing the mean behavioural and sensory responses of a group of insects (taken to be representative for the population). In this section behavioural and sensory responses of the same individual larva are measured and correlated for a group of individuals. Thus individual variation in sensory response will be correlated with individual variation in behavioural response. This was done by first measuring the individual score on Fp in a behavioural test, and subsequently recording the sensory response of the same individual from both lateral styloconic sensilla. Both responses were measured using 10^{-3} M sinigrin as a standard stimulus. In this way Fp values and spike frequencies (mean of the two sensilla) were obtained from the same individual insects. The two parameters were combined for computing the Spearman rank correlation coefficient, which appeared to amount to 0.76 ($n = 14$). This value indicates a

significant ($p < 0.01$) correlation between the individual sensoty input and its behavioural output. The group includes 2 insects, missing one of the lateral sensilla. For these individuals the sensoty response of the remaining sensillum was used in computations. From this experiment it is concluded that behavioural and sensoty responses are correlated in such a way that insects producing a large number of spikes also score high values of Fp. Variations in sensory responses are thus correlated with variations in behaviour. The interindividual variation in behaviour is, at least partly, caused by interindividual variations in sensory responses.

3.2.4. *Correlation (population means)*

Just like for the response to sucrose (section 3.1.5), behavioural as well as sensory responses to various concentrations of sinigrin are correlated in an input-output diagram. The relative behavioural response to pure sinigrin is plotted versus the relative sensory response of the lateral styloconic sensillum (Fig. 13). Points show a linear relationship (correlation coefficient is 0.99). The calculated regression line is reflected by: $y = x+2$. Of course this line is parallel to the theoretical line $y = x$ (see section 1.5). Besides, it can be shown that the y-intercept does not significantly differ from zero. We may, therefore, conclude that the observed regression line does not differ significantly from the theoretical line $y = x$.

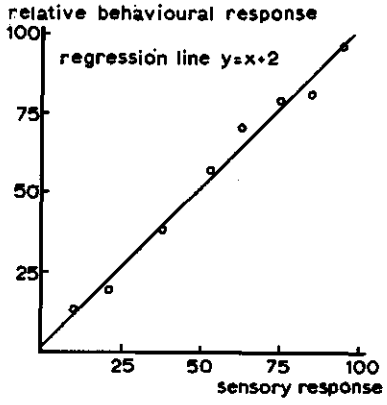

Fig. 13. *Pieris*, correlation between the relative sensory response to sinigrin and the relative behavioural response to sinigrin.

3.3. STRYCHNINE

3.3.1. *Sensory response*

Pieris larvae possess two cell types responding to stimulation with strychnine: one is located in the medial styloconic sensillum, and the other in the epipharyngeal sensillum (Fig. 14). The cell in the medial styloconic sensillum starts its response at 10^{-7} M strychnine, whereas the epipharyngeal cell has its threshold at 5.10^{-7} M. At 10^{-4} M strychnine both cell types reach their approximate maximal level of spike activity. The maximal number of spikes in the medial styloconic cell amounts to 175 in the first second, as compared to 117 spikes in the epipharyngeal cell.

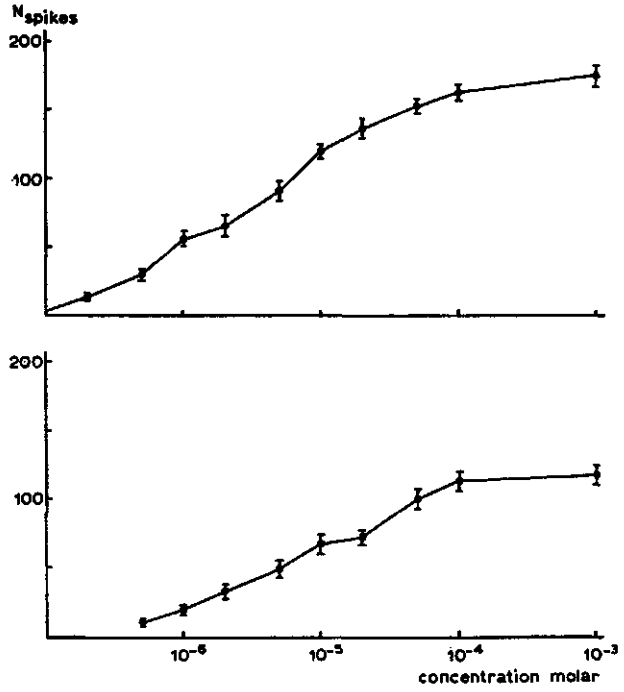

Fig. 14. *Pieris*, sensory responses on stimulation with strychnine in the medial styloconic sensillum (upper trace) and the epipharyngeal sensillum (lower trace). $N_{sp} \pm 1 \times$ S.E.

It was already shown by MA (1972) that strychnine and sucrose do not interact peripherally, i.e. each of the cells is neither influenced by the presence of the other compound nor by the activity of the other cell.

3.3.2. *Behavioural response*

Since the behavioural response to a feeding deterrent compound is a decrease in food intake, it is not easy to measure the behavioural response on diets only containing strychnine. Food intake on the substrate without any addition is low, and this level of control Fp (which is in all figures subtracted to obtain the corrected zero level) leaves only a small margin for showing feeding-inhibiting effects by strychnine. In order to measure a sizeable effect over a wide concentration range the diets have to be made more attractive. This is achieved by adding 10^{-2} M or 2.10^{-1} M sucrose to the diets, in addition to the various strychnine concentrations. Fig. 15 shows three concentration response curves: the upper one represents strychnine mixed in diets containing 2.10^{-1} M sucrose, the middle one strychnine with 10^{-2} M sucrose, and the lower one responses to pure strychnine diets. This last curve lays at negative ordinate values and does not show much detail.

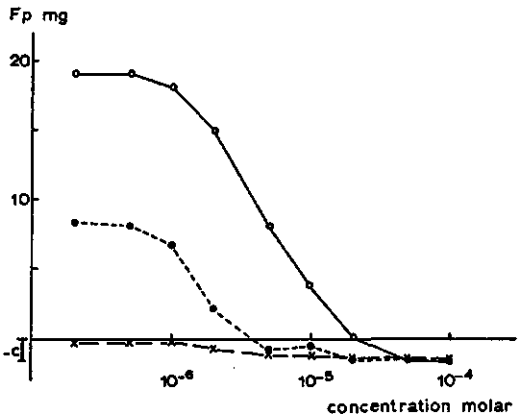

Fig. 15. *Pieris*, behavioural response to diets containing strychnine (lower curve), to diets containing strychnine combined with 10^{-2} M sucrose (middle curve), and to diets containing strychnine combined with 2.10^{-1} M sucrose (upper curve).

The curve lays about on zero level (responses equal to the mean control Fp) until a decrease sets in at 2.10^{-6} M strychnine. The drop is completed at 5.10^{-6} M (the absolute zero level is reached, no more decrease is possible). The two other curves start declining at 5.10^{-7} M, cutting the abcissa at 5.10^{-6} M for the 10^{-2} M sucrose diets and 2.10^{-5} M for the 2.10^{-1} M sucrose diets, further declining till 2.10^{-5} M and 5.10^{-5} M respectively (at absolute zero). Comparison of the curves shows clearly that when the strychnine containing diet is made more attractive, the concentration at which the decrease in Fp value starts

does not seem to be much affected by the presence of different sucrose levels, but the strychnine concentration at which the decrease is completed is shifted to a higher molarity.

Evidently, the insects tolerate higher amounts of strychnine in a more attractive diet, and in an otherwise acceptable diet complete feeding inhibition requires higher levels of a phagodeterrent than in marginal or neutral diets. This shows that the decision to eat or not is based on a balance of positive and negative input.

3.3.4. *Correlations*

Feeding responses to the pure strychnine diets cannot be used for correlations with sensory responses, because of lack of detail. Therefore the behavioural response on diets containing strychnine supplemented with 10^{-2} M or 2.10^{-1} M sucrose are used. Fig. 16 (for diets containing 2.10^{-1} M sucrose) and Fig. 17 (for diets containing 10^{-2} M sucrose) show the relative behavioural response to strychnine plotted versus relative sensory responses. The relative behavioural response is calculated as percentage feeding inhibition, a complete inhibition being

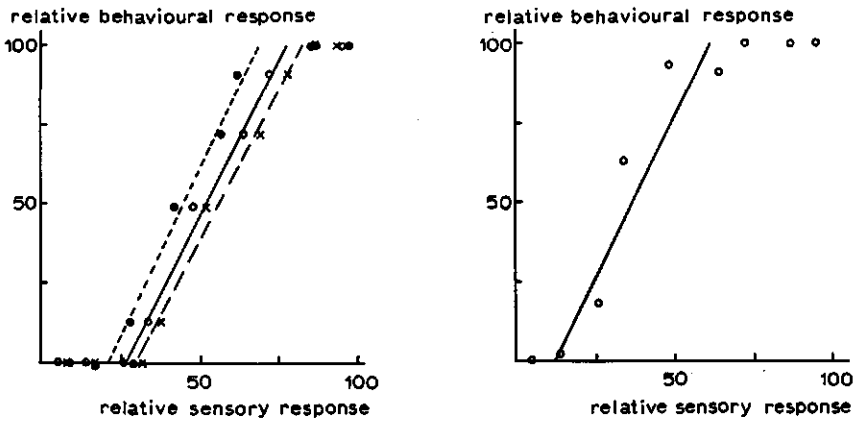

Fig. 16. *Pieris*, correlation between the relative sensory response in the medial styloconic sensillum (crosses), the relative sensory response in the epipharyngeal sensillum (closed circles), and the relative summated sensory responses (open circles) to strychnine and the relative behavioural response to diets containing strychnine combined with 2.10^{-1} M sucrose.

Fig. 17. *Pieris*, correlation between the relative summated sensory response in the medial styloconic sensillum and the epipharyngeal sensillum to strychnine and the relative behavioural response to diets containing strychnine combined with 10^{-2} M sucrose.

100%, and the Fp on the sucrose diets without strychnine being 0%. In Fig. 16 the relative sensory response to strychnine is calculated in three ways: (a) only the number of spikes in the medial styloconic sensillum is taken into account, (b) the same for only the number of spikes in the epipharyngeal sensillum, and (c) determining the summated number in both sensilla. It can be seen that all three alternatives lead to simple linear relationships with behavioural parameters. In all cases the points fit their regression line well, with a correlation of 0.99 in all three cases. The three regression lines are approximately parallel. This is due to the fact that there exist no essential differences in the relative responsiveness of the two strychnine sensitive cell types, since the shape of their concentration-response curve, and their threshold sensitivity as well as the concentration which stimulates maximally are quite similar. Thus, using both cells alone or their summated response makes not much difference in the relative figures of sensory activity, and the same holds for any other combination of the responses of the two cells. In Fig. 17 only the summated responses are used. The regression lines depicting the relationship between feeding behaviour and summated sensory responses shown in Figs. 16 and 17 are almost parallel (regression coefficients 1.97 and 1.98 do not differ significantly). The line in Fig. 17 (10^{-2} M sucrose diets) is shifted to the left compared with Fig. 16 (2.10^{-1} M sucrose diets). A shift to the left means that lower levels of sensory input suffice to evoke the same (relative) inhibition of feeding activity.

3.4. PROLINE

Proline is an amino acid that is known to be a strong stimulus for *Pieris* larvae (SCHOONHOVEN 1969c, MA 1972).

3.4.1. *Sensory response*

The lateral styloconic sensillum of *Pieris* larvae contains a cell, which responds to the presence of proline by increasing the number of spikes (Fig. 18). The threshold response occurs at a concentration of 5.10^{-4} M proline. Cell activity increases with increasing concentrations up to 5.10^{-2} M proline, at which point the curve begins to level off to a maximal number of impulses.

To investigate whether or not a peripheral interaction exists between sucrose and proline, mixtures of these compounds were tested in addition to recordings with single compounds. In these experiments

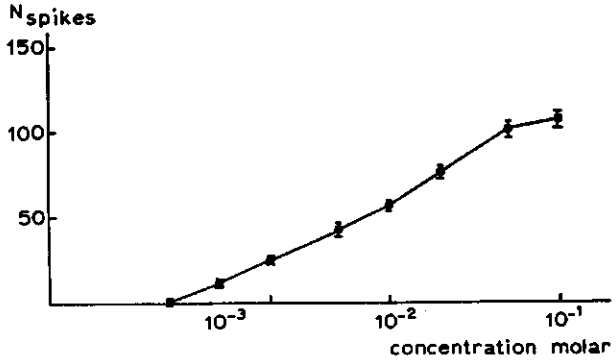

Fig. 18. *Pieris*, sensory response on stimulation with proline in the lateral styloconic sensillum. $N_{sp} \pm 1 \times$ S.E.

sucrose was tested at 10^{-1} and 10^{-2} M, and proline at 10^{-2} and 5.10^{-2} M. The 4 possible combinations were also used to stimulate both styloconic sensilla. In the medial sensillum no significant interactions are observed, when both stimuli are presented together. The lateral sensillum presents some difficulties. Although sucrose spikes are larger than those elicited by proline, analysing the recordings obtained with mixtures does not give unequivocal results, due to the occurrence of additions of both spike types. Sucrose spikes, being the most prominent and at the highest frequency, can be counted with reasonable accuracy. Proline spikes, however, present more difficulties. Therefore in addition to determining the number of spikes for sucrose only the total number of spikes (taking into account spike additions) was used. These numbers were compared (by means of a sign test) with that on pure sucrose and the number obtained sy summating the responses on pure sucrose and pure proline respectively. Both methods give comparable results: the responses to mixtures of 10^{-2} M sucrose + 10^{-2} M proline, 10^{-1} M sucrose + 10^{-2} M proline, and 10^{-1} M sucrose + 5.10-2 M proline do not differ significantly from the values expected when no interactions occur. However, the response to 10^{-2} M sucrose + 5.10^{-2} M proline show fewer sucrose spikes than expected: mean 120 impulses compared to 135 expected (sign test 8—, 0+, p = 0.004). Thus proline at high concentrations seems to inhibit the sucrose sensitive cell in the lateral sensillum.

3.4.2. *Behavioural response*

To measure the behavioural response, diets containing various concentrations proline were offered. Diets were used containing only

proline or containing proline mixed with 10^{-2} M sucrose. Fig. 19 presents the responses to pure proline diets in the lower curve and to proline diets, to which 10^{-2} M sucrose has been added, in the upper curve. The concentration-response curves, both with and without 10^{-2} M sucrose, seem of a different shape than those for, e.g. sucrose and sinigrin. Although there is a distinct optimum in the lower curve at 10^{-2} M proline, none of the pure proline diets caused a significant

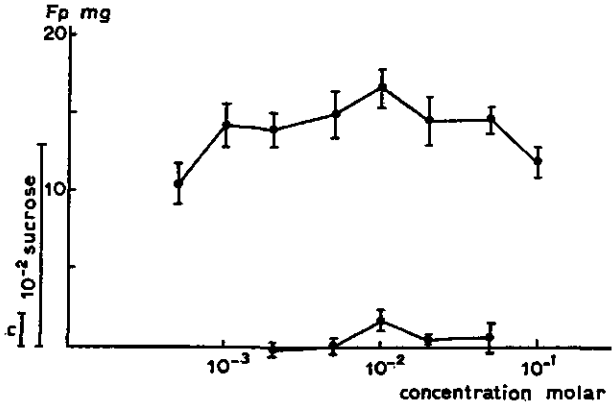

Fig. 19. *Pieris*, behavioural responses to diets containing proline (lower line) and diets containing proline combined with 10^{-2} M sucrose. Fp $\pm$ 1 $\times$ S.E.

increase in Fp over the control diet. The uncorrected values of Fp for 10^{-2} M proline, when compared to Fp values on the control diet, did not show a significant difference. The upper curve has a similar shape as the lower one, with again an optimum at 10^{-2} M proline, and lower Fp values at higher and lower concentrations. These responses have to be compared to Fp values for a diet containing 10^{-2} M sucrose only (left of ordinate). Feeding intensity on a diet containing 10^{-2} M proline and 10^{-2} M sucrose is significantly increased when compared to a diet containing 10^{-2} M sucrose only ($p < 0.01$). Fp on 5.10^{-2} M proline is slightly lower (bot not significantly) than on 10^{-2} M proline. The Fp value for the mixed diet of 10^{-2} M proline + 10^{-2} M sucrose (mean Fp = 166 mg) is compared to the value, obtained by adding the responses on the pure 10^{-2} M proline diet and the pure 10^{-2} M sucrose diet (mean Fp = 17 + 126 = 143 mg). Individual values of Fp are tested in a Mann-Whitney U test, showing $P = 0.072$ (one tailed). Thus, although there is a clear trend for the mixture to induce a larger Fp than the summation of the pure diets, this does not reach the 5% level. These experiments are not very decisive about the

question if the response to the mixture is larger than the sum of the response to the constituing components, i.e. whether or not any synergetic effect can be found. MA (1972, Fig. 28) found a considerable increase in Fp, with an optimum at 10^{-2} M proline, in diets containing 4.10^{-3} M sucrose. Maybe lower sucrose levels are needed to show the effect optimally. The decrease of Fp values at 2.10^{-2} and 5.10^{-2} M proline is presumably due to other factors. Sucrose at a concentration of 5.10^{-1} M is likewise suboptimal. It is also conceivable that high concentrations of proline inhibit the lateral sucrose receptor.

3.4.3. *Correlation*

The relative behavioural response is plotted against the relative number of spikes in the first second in the lateral sensillum (Fig. 20). The Fp values on the diets containing 10^{-2} M sucrose and various concentrations proline were used, since the behavioural response to the pure proline diets did not show a significant increase in food intake over the control diet. Only values of concentrations up to 10^{-2} M proline were used, because at that concentration the maximum is reached, and because interactions might occur at higher concentrations. The experimental data do not fit the regression line as well as in some experiments described in earlier sections (correlation coefficient: 0.93).

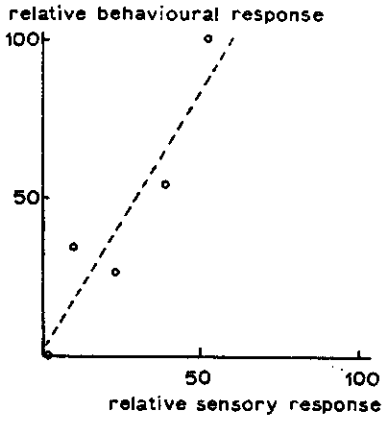

Fig. 20. *Pieris*, correlation between the relative sensory response in the lateral sensillum to proline and the relative behavioural response to diets containing proline combined with 10^{-2} M sucrose.

3.5. NACL

This section deals mainly with experiments designed to elucidate possible interactions between NaCl and sucrose. It is essential to know these interactions, if they exist, because 5.10^{-2} M NaCl was always added to all sucrose solutions used in the electrophysiological experiments.

3.5.1. *Behavioural response*

To investigate whether or not NaCl influences the Fp-values for sucrose diets, various concentrations NaCl (between 10^{-2} and 1 M) were added to a diet containing 10^{-2} M sucrose. The concentration-response curve shows that various concentrations of NaCl up to a maximum of 2.10^{-1} M do not affect food intake (Fig. 21). Fp values

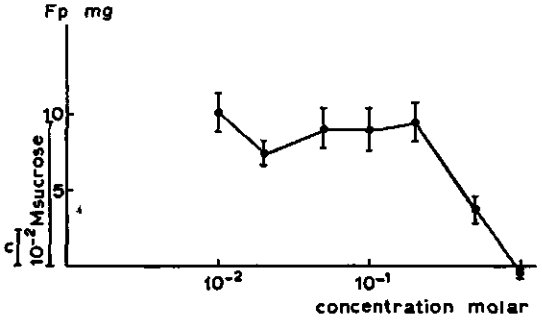

Fig. 21. *Pieris*, behavioural response to diets containing NaCl combined with 10^{-2} M sucrose. Fp $\pm$ 1 $\times$ S.E.

for this concentration range vary around the Fp value on a pure 10^{-2} M sucrose diet. When, however, NaCl in concentrations of 5.10^{-1} M and 1 M is added to a 10^{-2} M sucrose diet, the Fp value drops significantly below that on the pure sucrose diet. Thus, high concentrations of NaCl inhibit food intake, and the decrease is approximately linear. MA (1972), using other behavioural parameters and a 10^{-1} M sucrose diet, found NaCl inhibiting food intake at the same high concentrations, when added to the diet. In another experiment (using the Fp parameter) he found an increase in Fp values, when small quantities of NaCl are added to a 4.10^{-3} M sucrose diet.

3.5.2. *Electrophysiological response*

This section does not deal with responses to pure NaCl solutions, but rather discusses possible interactions between sucrose and 5.10^{-2} M NaCl at the receptor level. NaCl was added (at a concentration of 5.10^{-2} M) to all electrophysiological solutions and possible effects of this procedure needs elucidation. Responses to pure NaCl solutions at 5.10^{-2} M (controls during the recordings of other compounds) are weak and only impulses with a very low amplitude (sometimes even disappearing in the noise) could normally been seen. Their frequency usually varied between 3–13 in the lateral styloconic sensillum, 4–18 in the medial styloconic sensillum, and 7–25 in the epipharyngeal sensillum. Whether or not they represent a genuine response to NaCl is unknown, since they might also reflect spontaneous activity. Neither is it known which particular cells give rise to these spikes. To establish if a peripheral interaction exists, sucrose solutions lacking the 5.10^{-2} M NaCl were used in electrophysiological experiments. With the equipment described in section 2.4 it proved only possible to measure responses to sucrose solutions without NaCl when high spike frequencies were induced. This goes together with high amplitudes and is obtained in the lateral sensillum by 10^{-2} and 10^{-1} M sucrose, whereas the medial sensillum requires 10^{-1} M sucrose. Even at these high concentrations signals were not as good as in normal solutions i.e. containing 5.10^{-2} M NaCl, which is demonstrated by a reduced signal-to-noise ratio.

Numbers of spikes elicited by sucrose in the presence or absence of 5.10^{-2} M NaCl obtained from the same sensillum were treated as a pair in the sign test. At the concentrations used no significant differences were observed either in the lateral or the medial sensillum. It is concluded that 5.10^{-2} M NaCl does not influence the responses to sucrose.

3.6. SUCROSE + SINIGRIN + STRYCHNINE

Behavioural responses were measured to mixtures of sucrose, sinigrin and strychnine as well as to these compounds singly. Each compound was tested at two concentrations: sucrose at 10^{-2} and 10^{-1} M;sinigrin at 10^{-5} and 10^{-4} M and strychnine at 10^{-6} and 10^{-5} M. Table IV gives the mean Fp values on the various diets.

In general adding strychnine to either sucrose or sucrose + sinigrin decreases the Fp. Strychnine at low concentration (10^{-6} M) is less effective than at high concentration (10^{-5} M), and sometimes even gives no effect. When sinigrin is added to either sucrose or sucrose +

TABLE IV

Fp values on diets containing mixtures of sucrose, sinigrin and strychnine.

	No sucrose			*10^{-2} M sucrose*			*10^{-1} M sucrose*		
Sini.→ ↓ Strych.	None	10^{-5}	10^{-4}	None	10^{-5}	10^{-4}	None	10^{-5}	10^{-4}
None	—	60	72	122	166	187	164	185	183
10^{-6}	—	—	—	48	168	174	145	169	177
10^{-5}	—	—	—	−12	68	66	76	106	82

strychnine Fp increases. There is not much difference between the effects evoked by 10^{-5} and 10^{-4} M sinigrin, as both concentrations give approximately the maximal Fp for sinigrin (see also Fig. 10). In retrospect it would be preferable to select a wider range of sinigrin concentrations. Addition of sinigrin to 10^{-1} M sucrose gives only a slight increase of Fp to the maximal value possible. Diets containing sucrose + sinigrin tolerate more strychnine, than diets with sucrose only.

4. RESULTS AND DISCUSSION MAMESTRA

4.1 SUCROSE

4.1.1. *Sensory response*

In *Mamestra* a cell in the lateral styloconic sensillum responds to stimulation with sucrose with an increase in spike frequency. The response starts at 10^{-4} M sucrose, and increases with increasing concentration up to 5.10^{-2} M sucrose, before levelling off (Fig. 22). The maximal number of spikes is 236 in the first second of stimulation with 5.10^{-1} M sucrose. Firing occurs with a regular pattern, and interspike-intervals do not vary much, besides a gradual increase in length due to adaptation.

In *Pieris* light-microscopic inspection of the innerside of the labrum does not show much detail of the sensilla present. Scanning electron-microscopy gives more detail. Since *Mamestra* was not studied with this technique, some doubt remains as to whether or not epipharyngeal organs do occur in this insect. But even if some structures would be found, the decisive evidence for a gustatory function (instead of mechanical function) must come from electrophysiological recordings. It was attempted to record sensory responses from possible epipharyn-

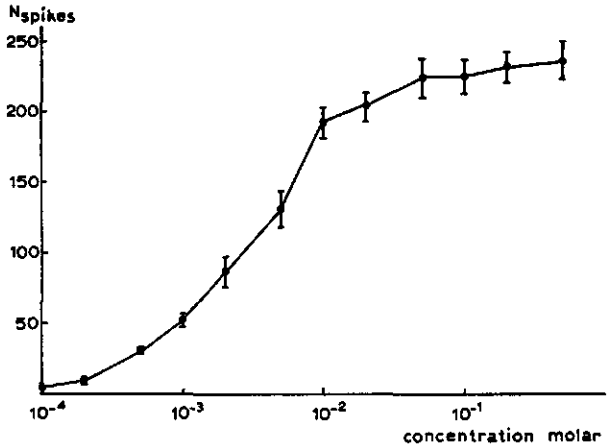

Fig. 22. *Mamestra*, sensory response to stimulation with sucrose of the lateral styloconic sensillum. $N_{sp} \pm 1 \times$ S.E.

geal sensilla at various places on the innerside of the labrum in *Mamestra*, using sucrose, inositol, and pure NaCl solutions, without any success, however. Thus no electrophysiological evidence could be obtained, indicating the presence of such sensilla in *Mamestra*.

4.2.1. *Behavioural response*

Mamestra larvae eat more from diets containing sucrose (at concentrations $> 10^{-3}$ M) than from the control diet (Fig. 23). The response increases slowly till a maximum at 2.10^{-1} M sucrose, and, like in *Pieris*, a decrease in food intake is found at 5.10^{-1} M. The maximal response (at 2.10^{-1} M), from which the control value has been subtracted, amounts to 1.44 $\times$ the mean control Fp.

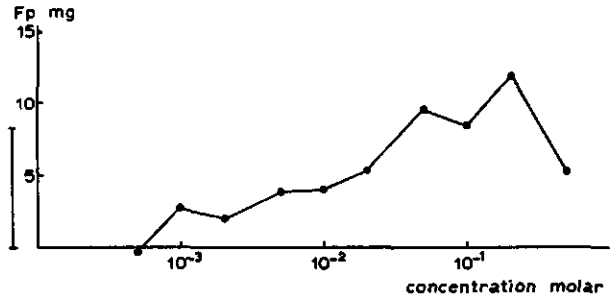

Fig. 23. *Mamestra*, behavioural response to diets containing sucrose.

To determine whether stimulation of the lateral styloconic sensilla represents the only relevant input for a behavioural reaction to sucrose, or whether other input channels are also involved, ablation experiments were performed. Of a group of insects both styloconic sensilla were bilaterally ablated. The Fp values on diets containing 2.10^{-1} and 5.15^{-3} M sucrose and the control diet were established for the bilaterally ablated insects. On 2.10^{-1} M the Fp value amounts to a mean of 140 mg (control value subtracted), on 5.10^{-3} M sucrose 55 mg and on the control diet 70 mg. It is clear that these insects still discriminate between the sucrose diets and the control diet, by eating more from the former. These results indicate that other sensory information from receptors hitherto unknown is involved.

4.1.3. *Correlation*

The relative sensory responses of the lateral styloconic sensillum to sucrose have been plotted versus the relative behavioural reactions (Fig. 24). It appears that the points do not fit a straight line; they are either on a curved line or on two straight lines with different slopes. From figs. 22 and 23 it can be seen that the sensory response reaches almost the maximal level (81%) at 10^{-2} M sucrose, while at this concentration the behavioural response is still rather low (34%). The major increase of the behavioural response occurs at a sucrose concentration range which only slightly affects the sensory response, whereas the initial part of the behavioural response curve is evoked by sucrose

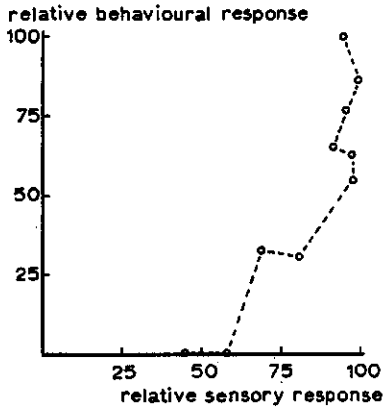

Fig. 24. *Mamestra*, correlation between the relative sensory response in the lateral sensillum to sucrose with the relative behavioural response to diets containing sucrose.

concentrations which have very pronounced effects on the sensory response. In section 4.1.2. evidence has been presented suggesting that other sensory inputs from sucrose are present, and the relationship between the input from the lateral styloconic sensillum and feeding response, in which the major increase in the latter seems almost unrelated to the former, seems to confirm this idea.

4.2. INOSITOL

4.2.1. *Sensory response*

In *Mamestra* larvae a cell in the medial styloconic sensillum increases its firing rate on stimulation with inositol. Responding starts at about 10^{-4} M inositol, and augments with increasing concentration up to 10^{-2} M, where it reaches the maximal level of response (Fig. 25). The maximal spike frequency which is elicited by 2.10^{-1} M inositol, amounts to 95 on the average. It is interesting to note that this is less than half the maximal value observed in the sucrose cell of the lateral sensillum.

Attempts to record from any epipharyngeal sensillum, if present, were, like in the case of sucrose, unsuccessful.

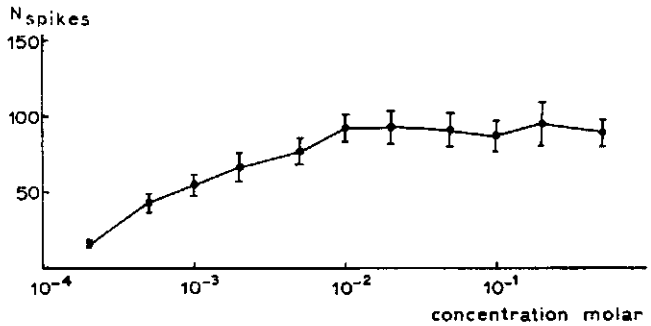

Fig. 25. *Mamestra*, sensory response on stimulation with inositol in the medial styloconic sensillum. $N_{sp} \pm 1 \times$ S.E.

4.2.2. *Behavioural response*

Inositol induces in *Mamestra* a positive food intake (Fig. 26). Responses start at 2.10^{-3} M inositol, and build up with increasing concentrations up to a maximal response at 5.10^{-1} M inositol. On 7.10^{-1} M inositol the behavioural reaction is decreasing again. The maximal Fp (after subtracting the control value) amounts to $1 \times$ the mean control Fp.

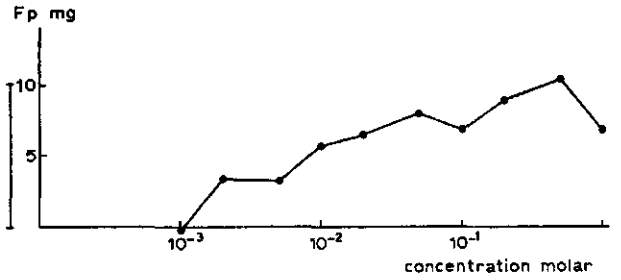

Fig. 26. *Mamestra*, behavioural response to diets containing inositol.

4.2.3. *Correlation*

When the relative behavioural response is plotted versus the relative sensory response in the medial sensillum, a group of points results which do not fit a straight line (Fig. 27). At low relative sensory responses the behavioural response increases proportionately, although the behavioural reaction starts at higher inositol concentrations than the receptor. At high relative sensory responses the points seem to be parallel to the ordinate, which indicates that behavioural reactions are unrelated to the sensory response.

Therefore, increases in behavioural responses do not depend on this sensory information (*i.e.* from the medial sensillum), and the shape of the figure may be interpreted as an indication that other (unknown, like for sucrose) sensory input is available to the CNS.

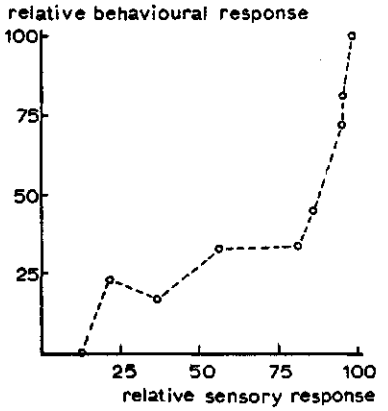

Fig. 27. *Mamestra*, correlation between the relative sensory response in the medial sensillum to inositol with the relative behavioural response to diets containing inositol.

4.3. SINIGRIN

4.3.1. *Sensory response*

Both in the lateral and medial styloconic sensilla spike frequencies increase when they are stimulated with sinigrin solutions (Fig. 28). In the lateral sensillum one cell fires with a regular pattern (regular, slightly increasing intervals, and constant spike amplitudes, agrees with WIECZOREK, 1976).

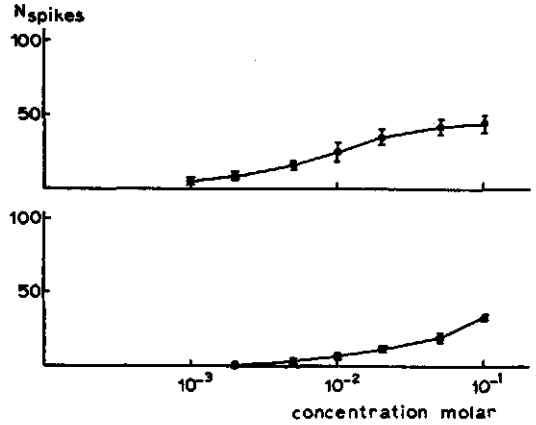

Fig. 28. *Mamestra*, sensory responses on stimulation with sinigrin in the lateral styloconic sensillum (upper trace) and the medial styloconic sensillum (lower trace). $N_{sp} \pm 1 \times$ S.E.

In the medial styloconic sensillum spikes due to sinigrin are sometimes difficult to distinguish from spikes evoked by NaCl, since their amplitudes overlap each other. If it was impossible to distinguish between NaCl spikes and sinigrin spikes, the number of NaCl spikes as counted in the controls was subtracted. Although firing occurs in a very irregular way, amplitudes do not show clearly different classes. Therefore it is uncertain if one or more cells are responding to sinigrin. For this reason the total number of impulses is used.

In the lateral sensillum responses start at 10^{-3} M sinigrin, and reach a maximal level at 5.10^{-2} M sinigrin. Maximal number of spikes in the first second is here 44 at 10^{-1} M sinigrin. Reactions in the medial sensillum start at 5.10^{-3} M sinigrin, and increases with rising concentrations, up to a maximal spike frequency of 33 at 10^{-1} M sinigrin. The limited solubility of sinigrin does not allow experiments at higher concentrations.

4.3.2. *Behavioural response*

Contrary to the situation in *Pieris* (section 3.2.2) sinigrin does not stimulate food intake in *Mamestra*. At high concentrations it even causes a marked reduction of food intake. Behavioural effects of sinigrin were therefore studied in diets fortified by 10^{-2} M sucrose, to which sinigrin was added at concentrations ranging from 2.10^{-3} M to 10^{-1} M.

At concentrations of 5.10^{-3} M sinigrin or higher the Fp values are lower than on a pure sucrose diet, and at 10^{-1} M even complete inhibition of feeding occurs (Fig. 29). Sinigrin concentrations which inhibit food intake in *Mamestra* are relatively high, whereas in *Pieris* stimulation of food intake starts at a concentration as low as 10^{-7} M. High levels of sinigrin do not affect feeding in *Pieris* negatively.

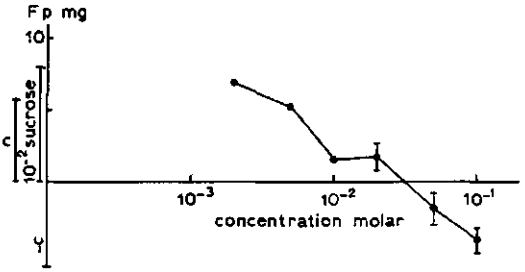

Fig. 29. *Mamestra*, behavioural response to diets containing sinigrin combined with 10^{-2} M sucrose.

4.3.3. *Correlations*

In Fig. 30 relative behavioural responses (inhibition of food intake varying from 0 to 100%) to sinigrin are plotted versus relative sensory responses. The sensory response of the lateral styloconic sensillum and the medial styloconic sensillum each alone as well as the summated response (spike numbers of both sensilla added) were used to calculate the relative sensory response. The points corresponding to the summated response (circles in Fig. 30) show the nearest fit to a regression line, correlation coefficient is 0.98. The regression line ($y = 0.99\,x + 0.53$) does not significantly differ from $y = x$. The points corresponding to the sensory responses of one of the sensilla types alone do not really fit a straight line, but give a better fit to a curved line. Since it is not likely that information from one of the cells is disregarded, the summated sensory response shows that in this case again a linear relationship exists between sensory input and behavioural output.

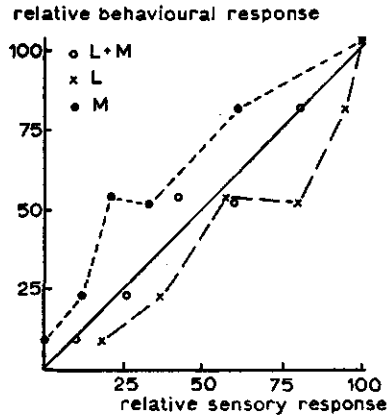

Fig. 30. *Mamestra*, correlation between the relative sensory response in the lateral sensillum (crosses), the medial sensillum (closed circles) and the summated response (open circles) to sinigrin. The relative behavioural response is based on diets containing sinigrin combined with 10^{-2} M sucrose.

4.4. STRYCHNINE

4.4.1. *Sensory response*

Stimulation with strychnine evokes an increase in firing rate in both the lateral and the medial styloconic sensillum. In the lateral sensillum one cell (constant amplitude, regular intervals) responds to strychnine, which agrees with the findings of WIECZOREK (1976). Responses begin at 2.10^{-4} M strychnine and increase until at 10^{-2} M strychnine the maximal number of 38 spikes per second is reached (Fig. 31). In the medial sensillum the responses show very irregular intervals (sometimes two spikes very close together), but no clear differences in amplitudes. Sometimes it was difficult to distinguish the NaCl spikes from the strychnine induced impulses, in that case the NaCl spikes as determined in controls were subtracted .In the case of this medial sensillum all calculations were done on the basis of total spike numbers. Responses in the medial sensillum start at 5.10^{-5} M strychnine and increase with rising concentrations until at 5.10^{-3} M the maximal level is reached. The maximal number of spikes is 96 at 10^{-2} M strychnine.

In *Mamestra* stimulation with sinigrin gives a higher maximal response in the lateral sensillum (Fig. 28: lateral 44 versus medial 33 spikes), whereas stimulation with strychnine gives a higher maximal response in the medial sensillum (Fig. 31: lateral 38 versus medial 96 spikes).

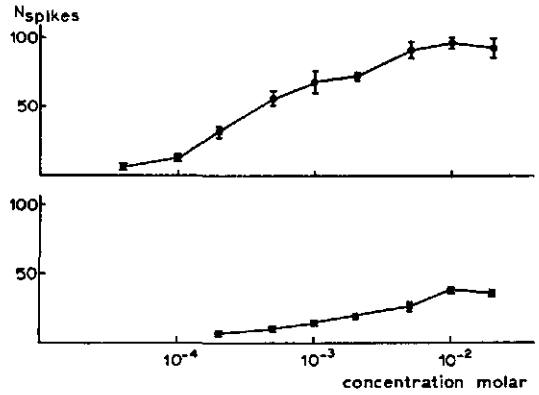

Fig. 31. *Mamestra*, sensory responses on stimulation with strychnine medial styloconic sensillum (upper trace) and in the lateral styloconic sensillum (lower trace). $N_{sp} \pm 1 \times$ S.E.

According to WIECZOREK (1976) strychnine and sinigrin stimulate the same cell in the lateral styloconic sensillum. To investigate this, cross-adaptation experiments were carried out on both the lateral and the medial styloconic sensillum. It appears that responses in both sensilla do adapt gradually, although at a slow rate. In the cross-adaptations experiment the responses to 10^{-1} M sinigrin and 10^{-1} M strychnine were measured after a 3 minutes disadaptation period, and within 10 seconds after a 3 minutes stimulation with the other compound. A sign test was used to compare spike numbers before (disadapted) and after the treatment, + of the subsequent number of spikes were smaller than before, and − if the subsequent number of spikes was larger. This resulted in a value of 10+ 2− ($p = 0.019$) for the lateral sensillum and a value of 7 + 5− ($p = 0.387$) for the medial sensillum. Thus the results of the cross-adaptation experiments lead to the conclusion that sinigrin and strychnine stimulate the same cell in the lateral sensillum, but that in the medial sensillum different cells are affected.

4.4.2. *Behavioural response*

As it was expected that strychnine would decrease food intake, behavioural responses were measured on diets containing 2.10^{-1} M sucrose, to which strychnine was added. Strychnine up to a concentration of 2.10^{-3} M does not significantly alter feeding intensity, but at higher concentrations a decrease of Fp values is observed. Fp decreases almost linear up to 2.10^{-2} M strychnine, which was the highest con-

centration used. At this concentration strychnine shows a strong inhibitory effect: it not only neutralizes the sucrose food intake, but the Fp values drop below the mean control Fp (below the abscissa in Fig. 32).

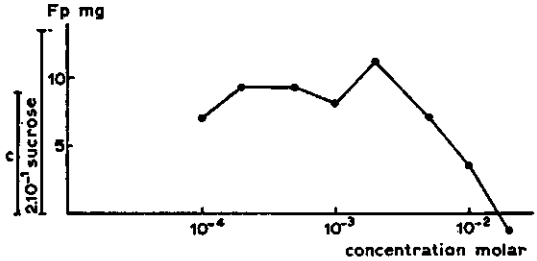

Fig. 32. *Mamestra*, behavioural response to diets containing strychnine combined with 2.10^{-1} M sucrose.

4.4.3. *Correlation*

In Fig. 33 sensorial input and behavioural output as affected by strychnine are correlated by plotting the relative behavioural responses (degree of inhibition of food intake in %) versus the relative sensory response. Numbers of spikes of either the lateral or the medial styloconic sensillum alone, and the summated numbers were used in calculations (Fig. 33). Neither of the groups of points seem to fit a straight line.

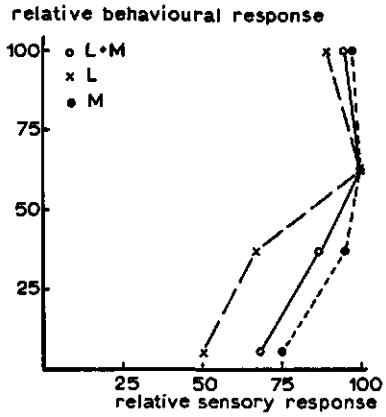

Fig. 33. *Mamestra*, correlation between the relative behavioural response to strychnine and the relative sensory response in the lateral sensillum (crosses), the medial sensillum (closed circles) and the relative summated response (open circles). The diets containing strychnine were fortified by 2.10^{-1} M sucrose.

The two highest concentrations of strychnine (*i.e.* 10^{-2} and 2.10^{-2} M) show no difference in sensory response, but a considerable difference in behavioural reactions. This part of the increase in the behavioural response is unrelated to a change in the sensory responses of both styloconic sensilla, and it causes the curve in the relationships of Fig. 33 to run parallel to the ordinate at high levels of sensory activity. This observation may be used as an argument to hypothesize the presence of other sensory input from strychnine in *Mamestra*.

5. GENERAL DISCUSSION

5.1. A COMPARISON OF MAMESTRA TO PIERIS

In this section the differences in sensory activities and behaviour of *Mamestra* and *Pieris* will be discussed in view of their difference in feeding habits. The major differences are summarized in Table V. Noticeable facts are that all attempts to record sensory activity from any possible epipharyngeal sensillum in *Mamestra* failed, but that evidence has been obtained for the presence of other unknown contact chemosensory organs. In chapter 4 arguments are presented favouring the idea that these unknown receptors are at least sensitive to sucrose,

TABLE V

A comparison of behavioural reactions and sensory activities of *Mamestra* and *Pieris*.

	Mamestra	*Pieris*
Control Fp	83 mg	23 mg
Sucrose	positive Fp, maximal 1.4 × control at 2.10^{-1} M	positive Fp, maximal 7.7 × control at 2.10^{-1} M
Inositol	positive Fp, maximal 1.0 × control at 5.10^{-1} M	no effect, neither in behaviour nor in electrophysiology (Ma, 1972)
Sinigrin	negative Fp, increasing from 2.10^{-3}–10^{-1} M	positive Fp, increasing from 10^{-7}–10^{-4} M
	Sensory activity in the same concentration range	
Strychnine	negative Fp, increasing from 10^{-2}–10^{-1} M	negative Fp, increasing from 10^{-6}–10^{-4} M
	Sensory activity in the same concentration range	
Epipharyngeal sensilla	Not present, but other chemosensory organs?	present

inositol and strychnine. The correlation of known input and output does not necessitate the assumption that this unknown organ also monitors the presence of sinigrin. Interestingly, MA (1976) proposed, on behavioural evidence, the presence of other gustatory organs in the buccal cavity for *Spodoptera exempta*. In contrast to these two species, there is no reason at all to imply the presence of additional sense organs in *Pieris*.

Comparing absolute amounts of food intake in the two species should be done with caution, because there were some differences in the conditions of the behavioural tests. Feeding experiments with *Mamestra* were performed at 20° C and with *Pieris* at 25° C, their respective optimal rearing temperatures. One experiment with *Pieris* was done at 20° C, resulting in lower Fp values for a 2.10^{-1} M sucrose diet (at 25° 180 mg, at 20° 147 mg, difference 18%). This reduction in feeding intensity was approximately the same as on the control diet, and the ratio between both was thus not affected. Furthermore, *Mamestra* larvae are heavier than *Pieris* larvae (*Mamestra* with an average of 240 mg compared to *Pieris* averaging 115 mg). Since this difference very likely also exists in wild larvae, which display different feeding preferences the weight difference does not interfere with the comparison of differences in feeding habits and differences of reactions in the behavioural tests. Some differences between the two species are now discussed briefly.

(a) A clear difference is the lower ratio of maximal behavioural response on sucrose to the mean control Fp in *Mamestra*, as compared to *Pieris*. *Mamestra* larvae eat a larger amount from the control diet, which strongly influences the ratio, because the mean control Fp is first subtracted from the sucrose response and thereafter used in calculating the ratio. In view of the absolute amounts eaten from the sucrose containing diet it may be concluded that *Mamestra* larvae eat considerable amounts of any suitable diet, and sucrose has a weaker modulating effect than in *Pieris*. In *Mamestra* sucrose raises food uptake only 1.4 fold as compared to the (relatively high) control whereas in *Pieris* the response to sucrose is 7.7 times the (low) control. This may indicate a higher internal drive to feed without positive sensory input in *Mamestra* than in *Pieris*.

(b) Sensory impulses elicited by sinigrin in *Mamestra* are interpreted in another way than in *Pieris*, because they have an opposite effect on Fp values. Sinigrin is used as a representative of the glucosinolates, which are secondary plant substances and characteristically occurring in Cruciferae. Some cruciferous species, e.g. *Brassica* species, are host plants both for *Pieris* and *Mamestra*. Sinigrin evidently does not signal a host plant to *Mamestra*, as it does to *Pieris*. The concentration of

sinigrin at which inhibition of food intake in *Mamestra* starts is 2.10^{-3} M, which is much higher than the concentration at which positive responses start in *Pieris* (10^{-7} M). At 10^{-4} M sinigrin the increase in food intake in *Pieris* reaches its maximal level, and at high concentrations ($> 10^{-3}$ M) the response remains at a constant level. Little is known of the quantitative aspects of glucosinolate contents of cruciferous leaves. Most research on these chemicals has focussed on their qualitative occurrence in various plant species or quantitative aspects of seed contents. JOSEFSSON (1967) studied the quantitative contents of some parts of *Brassica* species. By measuring the split products of enzymatic hydrolysis, it was found that leaves of various plants contain 5–52 mg of these split products per 100 gram fresh weight. The split products are from different glucosinolates with various molar weights. Since the M.W.'s of most glucosinolates vary between 250–500, it seems that the maximal value (52 mg/100 g fresh weight) found by JOSEFSSON (1967) corresponds approximately to $1–2.10^{-3}$ M, although one should handle this figure cautiously. Also it is unknown whether other glucosinolates are equally potent deterrents for *Mamestra* as sinigrin.

Roughly it can be stated that the maximal glucosinolates contents amount approximately to the concentration at which the behavioural effect in *Mamestra* starts. Furthermore, the possibility remains that wild *Mamestra* larvae, after growing on cabbage, got a reduced sensitivity for sinigrin, as compared to the experimental larvae which were fed on a semisynthetic diet. It is known that such differences in feeding history affect receptor sensitivity in some species (SCHOONHOVEN, 1969a, STÄDLER & HANSON, 1976).

(c) *Mamestra* is much less sensitive to strychnine than *Pieris*. The negative behavioural response of *Mamestra* to strychnine starts only at high concentrations (10^{-2} M). Strychnine was chosen as a representative of alkaloids, which are powerful deterrents to many insects. The lower sensitivity of *Mamestra* for deterrents was also observed by MA (1972) using a test with *Brassica* leaf discs with various concentrations of the deterrent ecdysterone. Thus, *Mamestra* is considerably less sensitive for two different feeding deterrents than *Pieris*, which presumably reflects a general insensitivity to secondary plant substances.

Summarising, it appears that *Mamestra* larvae may eat considerable amounts of any suitable substrate, and sucrose has a weaker effect than in *Pieris*. In combination with the low sensitivity to feeding deterrents this feature might form the basis of the more polyphagous feeding habits of *Mamestra*.

5.2. ELECTROPHYSIOLOGY

As mentioned before (section 1.4) it is important to know whether or not the concentration of the stimulus influences the adaptation rate of sensory responses, and that a comparison of spike numbers in the first second with those in the first 100 msecs could elucidate whether the ratio between the adapted response and the unadapted response is independent of stimulus intensity. Here the results of a comparison of relative values for the spike frequency during the first second of stimulation and the first 100 msecs are presented using a sign test. When the relative value for the first second is larger than for the first 100 msecs, a + is scored, whereas a − indicates that the relative value for the first second is smaller. The data of the sign tests for various receptors show that there exist no differences in the relative values of first second and first 100 msecs (Table VI), although the spike frequency in the first second is lower than in first 100 msecs. It is concluded that adaptation decreases the number of spikes per unit of time, but it does not change the ratios that exist between the responses to various concentrations. Because of this feature it seems reasonable to use the spike numbers in the first second as representative for the sensory activity during longer periods.

TABLE VI

Sign test on the relative values of the first second versus the first 100 msec for various receptors in *Pieris*.

receptor	+	−	=	*P (one-tailed)*
sucrose on lateral sensillum	3+	6−	2=	0.25
sucrose on medial sensillum	4+	6−		0.38
sucrose on epipharyngeal sensillum	4+	7−	1=	0.27
sinigrin on lateral sensillum	4+	7−		0.27
strychnine on medial sensillum	6+	2−	2=	0.15
strychnine on epipharyngeal sensillum	6+	2−	1=	0.15

In the present investigation electrophysiological information has been reduced to spike frequencies during the first second of stimulation, neglecting variations of spike intervals. It is, however, obvious that different receptors show different patterns of firing. Sucrose and strychnine responses in *Pieris* show regular intervals (slowly increasing due to adaptation), but responses to sinigrin exhibit irregular intervals, with alternating groups (1 to 3, Fig. 34) of short and long intervals (besides

Fig. 34. Sensory reaction in the lateral styloconic sensillum to stimulation with 10^{-4} M sinigrin (upper trace) and 5.10^{-4} M sucrose (lower trace).

an overall trend for increasing intervals due to adaptation). As an example a representative response to sucrose and one to sinigrin are compared. Sucrose elicits 112 spikes in the first second (mean interval 9 msecs), sinigrin elicits 41 spikes in the first second (mean interval 25 msecs). The largest difference between two successive intervals amounts to 5 msecs for sucrose and 26 msecs for sinigrin, compared to the mean interval this largest difference is 56% for sucrose and 104% for sinigrin. Thus for sinigrin there exists a much larger variation in the duration of successive intervals. This feature seems to be unrelated to spike frequency. It is unknown whether these interval variations play a rôle in the sensory coding and thus possess some information for the CNS.

5.3. SUCROSE IN PIERIS

In section 3.1.5. a straight line relationship is obtained when the relative behavioural output is correlated with the relative "total sensory input". This "total sensory input" is calculated by simple addition of spike numbers in all three cell types (L + M + E) for various sucrose concentrations. This is assumed to be the simplest way of taking into account all sensory responses of the three cell types. When the inputs from these three cells converge on one interneuron, simultaneous stimulation of this interneuron via different inputs may lead to summation, although conceivably different factors might hold for the three cells. Some other ways of combining the impulse flows from these cells also lead to straight line relationships in the input-output diagram, e.g. the summation of the responses of lateral and medial styloconic sensillum (L + M) only, or adding the response of the epipharyngeal sensillum to the averaged response of lateral and medial sensilla ($\frac{1}{2}$ (L + M) + E: Fig. 35).

Ablation experiments allow the following conclusions:

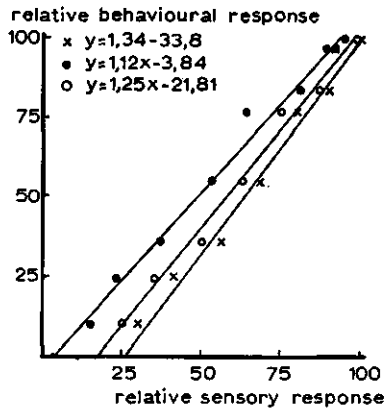

Fig. 35. *Pieris*, correlation between the relative total sensory response according to L + M + E (open circles), L + M (closed circles) and $\frac{1}{2}$ (L + M) + E (crosses) to sucrose and the relative behavioural response to diets containing sucrose.

1) ablation of either of the three sensillum types affects the behavioural response to sucrose.
2) only ablation of all three types abolishes the behavioural response to sucrose completely.
3) ablation of either the lateral or the medial sensilla has the same kind of effect (*i.e.* the maximal response is unchanged, but the ratios of the responses to different concentrations are changed), and ablation of the epipharyngeal sensilla has another kind of effect on the behavioural response (now the maximal response is changed).
4) ablation of the epipharyngeal sensilla has the same kind of effect as ablation of both pairs of styloconic sensilla (lateral + medial group).
5) unilateral ablation of both styloconic sensilla does not influence the behavioural response to sucrose in any way.

From these facts it can be hypothesized that input from the lateral and the medial styloconic sensilla is treated in the same way (has the same meaning for the brain), but the input from the epipharyngeal sensillum is treated differently. The input from the epipharyngeal sensillum is as important as the combined input from both lateral and medial styloconic sensilla. Ablation of either only the lateral or only the medial styloconic sensilla may be compared with the unilateral ablation of both styloconic sensilla: in both cases the maximal behavioural response is not affected by the operation. In the case of the unilateral ablation, the information from one of the two identical sets (left and right) of receptors is lost. The remaining set has the same sensitivity spectrum as both sets together. When either both lateral or both medial styloconic sensilla are destroyed, information from one

of the two pairs of different receptors (a lateral or a medial pair), with different sensitivity spectra, is lost. Now the remaining pair is not representative for both pairs. As a consequence, unilateral ablation results in unchanged ratios of the responses to various sucrose concentrations, but bilateral ablation of either both lateral or both medial styloconic sensilla changes those ratios. In the intact insect probably the input from both the left and right set of receptors is used, and behaviour is governed by the average. Also input from both the lateral and the medial styloconic sensilla is used, and an averaged sensory activity is processed further (in combination with input of the epipharyngeal sensilla). In these cases the two inputs must be combined in such a way that in the intact insect an average results, but when one of the two is lost (by ablation or a natural cuase) the weight of the input from the remaining one will increase. A combination of exitatory and inhibitory effects evoked by information from left and right, or information from the lateral and medial sensilla on each other could represent a suitable way of integration. A model based on such relationships is outlined in Fig. 36, in which information streams from the three types of sensilla are combined in a way that could explain the effects of the various ablations. Whether such a system exists as a neuronal network and where it should be situated can only be guessed.

Some other ablation experiments may provide additional indications about the relative importance of the information from the styloconic sensilla and the epipharyngeal sensilla for the behavioural response.

When either both pairs of styloconic sensilla or both epipharyngeal sensilla are ablated, the maximal Fp value on sucrose diets decreases (section 3.1.3.). Since the value of Fp seems to be linearly related to total spike frequency, the decrease in maximal Fp value may be compared to the amount of sensory input lost in the operated insects. The maximal behavioural response of lateral + medial group (both styloconic sensilla bilaterally ablated) is 93 mg, compared to 150 mg in intact insects, i.e. a reduction of 47%. The only remaining sensillum type is the epipharyngeal sensillum, which fires at a frequency of 137 spikes/sec., compared to a total of 469 spikes/sec from all three sensilla in the intact insect (when spikes are summated according to $L + M + E$). The styloconic sensilla thus account for 71% of total spike input. Clearly, their ablation has a much smaller effect than expected on the basis of simple spike totals, and input form the epipharyngeal sensilla can maintain the behavioural response at a higher level than expected. Therefore this input is more important. When the idea from the foregoing section is adopted, and the inputs from the lateral and medial styloconic sensillum are averaged before adding it to the input from the epipharyngeal sensilla (*i.e.* sensory input is $\frac{1}{2}$

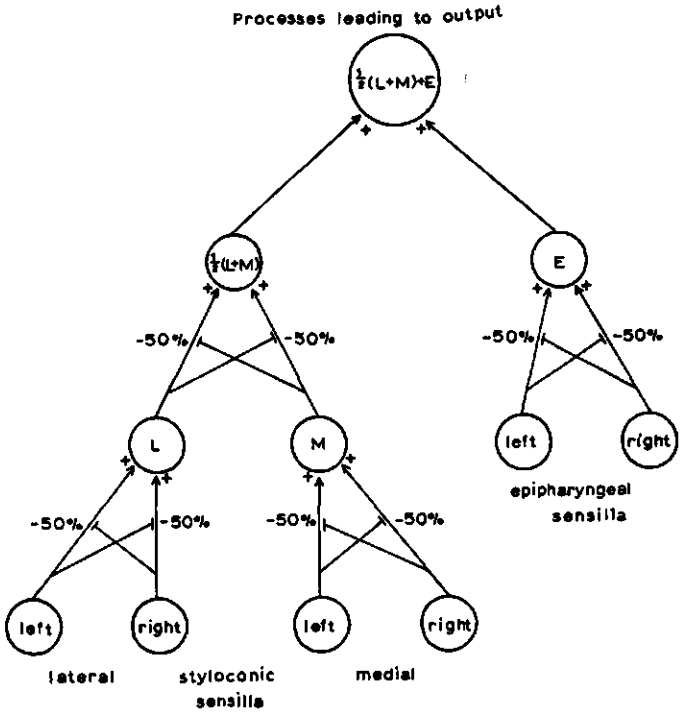

Fig. 36. Hypothetical stream of information from the three types of sensilla. A + indicates exitation, −50% indicates an inhibitory contact with half the strength of exitation.

(L + M) + E), the total number of impulses for intact insect amounts to 307, and the contribution of the styloconic sensilla to the total input would be only 55%. This method of combining the inputs attaches more importance to the input from the epipharyngeal sensillum and consequently approximates the behavioural figures better (47% reduction when the styloconic sensilla are ablated). Giving more importance still to the input from the epipharyngeal sensillum, will bring the figure for the sensory response closer to the figure for the reduction in behavioural response, e.g. combining the inputs according to ½(L + M) + 2E gives a total input of 452 impulses/sec, 39% of which is the contribution of the styloconic sensilla.

In insects lacking the epipharyngeal sensilla the maximal Fp of 61 mg corresponds to 34% compared to the intact insects. The remaining sensory input amounts to 69% when combining the inputs according to L + M + E, 53% when ½(L + M) + E is used and 36% for ½(L + M) + 2E.

Clearly, when applying the formulas $\frac{1}{2}(L+M)+E$ and $\frac{1}{2}(L+M)+2E$ results are obtained that come close to the figures from the behavioural responses of both groups. It must be recalled here, (see section 3.1.3) that the ablation could have an effect on the Fp value due to damage of structures important for feeding activity. This applies especially to the epipharyngeal group. Moreover, there exists a considerable variation not only in the behavioural responses (especially of the operated group due to the small number of animals), but also in the sensory reactions. Therefore, it does not make sense to look for a formula that exactly describes the behavioural response of one of these groups. For the time being we can only conclude that this type of formulas are suitable to describe the behavioural responses.

Evidence acquired from several experiments indicates that input from the epipharyngeal sensilla definitely has more effect on behavioural output than the inputs from the lateral and medial styloconic sensilla. The combination $\frac{1}{2}(L+M)$ as a formula describes a simple average, but is intended to represent a procedure as was described in Fig. 36. Recombining inputs in various ways, as has been done above (e.g. $L+M+E$, or $\frac{1}{2}(L+M)+E$ etc.,) does not change the essential properties of the input-output relationships for sucrose, since all formulas used lead to linear relationships (Figs. 7 and 35), neither of which go through the origin. In section 3.1.5 the input-output relationships are compared for e.g. the influence of starvation. In this case the combination $L+M+E$ was used for both starved and unstarved insects. Changing this formula to e.g. $\frac{1}{2}(L+M)+E$ does not influence the result essentially, two other straight relationships would result showing the same characteristic difference.

5.4. DIFFERENT CHEMICALS

Some difficulties arise when the responses to different chemicals are compared.

(1) The compounds may have different modes of action on food intake. For instance, sucrose affects the size of the first meal in *Pieris*, whereas sinigrin does not influence this parameter of food intake (MA, 1972). Both compounds do affect the Fp value (see also section 3.2.2).

(2) Different sensilla may adapt at different rates, an important characteristic which does not show up in the number of spikes in the first second. Therefore, the reactions during the first second to different compounds are not representative for the effects exerted by various compounds during longer periods of stimulation (see SCHOONHOVEN, 1976).

(3) In addition to impulse frequency other parameters of nerve activity could contain important information, such as variation in spike interval, latency, grouping of pulses etc. (see section 5.2).

Bearing these uncertainties in mind some results on sucrose, sinigrin and strychnine in *Pieris* will be discussed shortly.

Sucrose and sinigrin stimulate food intake, whereas strychnine inhibits food intake. These chemicals elicit a response in one cell (sinigrin), two cells (strychnine) or three cells (sucrose) of the receptors present in the three sensillum types. There exist no peripheral interactions among these chemicals (sections 3.2.1 and 3.3.1). For all three compounds the relative behavioural output is linearly related to the relative sensory input. These relationships are presented by their regression lines in input-output diagrams (Figs. 7, 13, 16 and 17). For sinigrin the regression line starts at the origin (or at least not significantly above it), which means that from the first spike on sensory input leads to behavioural output. For sucrose and strychnine, however, the regression lines intersect the abscissa at the right hand side of the origin, indicating that a certain amount of sensory input needs to be present before a behavioural effect is initiated. This threshold seems to be characteristic for a single compound under constant conditions, but can change when conditions are altered. Thus, for strychnine the threshold shifts to the right (to higher sensory input, Figs. 16 and 17) when the test diet is made more attractive by adding an increased amount of sucrose, which raises the stimulatory input to the CNS. For sucrose the threshold can also be changed, e.g. when the starvation period is altered (unstarved as compared to starved insects, Fig. 9). The threshold is also changed after palpectomy (Fig. 8). The palps are assumed to exert an inhibitory effect on the CNS (section 3.1.3, Ishikawa, Hirao & Arai, 1969), which is removed by palpectomy. These two examples show that the threshold observed in the input-output relation for some compounds is not a constant factor, but depends on levels of other stimulatory and inhibitory inputs to the CNS.

A linear relationship between sensory input and behavioural output gives a simple and fixed ratio between both parameters, i.e. spike frequency and Fp value. By dividing the maximal behavioural response by the number of impulses needed to get this maximal output (taking into account possible thresholds), a factor results which reflects the behavioural effect of one spike. This spike unit is representative for all impulses generated in this particular cell during 24 hours on a diet of a certain defined composition. For strychnine this factor depends again on the concentration of sucrose added to the diet (level of exitatory input). The behavioural effect of strychnine is the inhibition of the food

intake induced by the sucrose. When inhibition is complete the strychnine reduces the Fp on the 2.10^{-1} M sucrose diet from 188 mg to zero, and on the 10^{-2} M sucrose diet from 97 mg to zero. For the 2.10^{-1} M sucrose diet this inhibition starts (threshold) at 26.6% input and is completed at 77.3% input (Fig. 15). Thus it takes an increase of 51%, which corresponds to 148 strychnine spikes, to bring about the maximal inhibition of 188 mg, i.e. 1.28 mg/spike. For the 10^{-2} M sucrose diet the inhibition starts at 10.2% input and is completed at 60.7% input (Fig. 16). Thus it takes again an increase of 51% to bring about the maximal inhibition of 97 mg, i.e. 0.66 mg/spike. On the 2.10^{-1} M sucrose diet each spike above threshold has a larger inhibitory effect. So the level of stimulatory input interacts with the effect of inhibitory input, not only the threshold changes, but also the effect of supra-threshold impulses is changed.

For sinigrin the factor is 0.64 mg/spike (47 mg divided by 73 spikes). For sucrose the value of this factor depends on the way the summated sensory response is calculated (see section 5.3). It will vary from 0.46 mg/spike when the sensory inputs are summated according to $L + M + E$ (all spikes are equally important) to 0.77 mg/spike when the sensory inputs are combined according to $\frac{1}{2}(L + M) + E$ (spikes from the lateral and medial sensillum are less important than those from the epipharyngeal sensillum). When the ratio for sucrose is calculated for unstarved insects it appears to amount to 0.45 mg/spike as compared to 0.46 for starved insects. Thus, although starvation affects the threshold, it does not influence the weight of a supra-threshold spike. These examples, together with the complication mentioned above, clearly show that the effects of impulses evoked by different chemicals cannot be compared to each other without introducing complex conversion factors. Simple comparisons are only possible for each single chemical under different conditions.

Finally, some features which may emerge when making a model are presented:

(1) The presence of different input channels, e.g. stimulatory and inhibitory ones.

(2) The possibility of thresholds, which must be reached before additional sensory input initiates a behavioural response. These thresholds may be variable, depending on levels of stimulatory and inhibitory input.

(3) Factors, associated with each channel, that reflect the effect of each input spike on output in that channel. These factors may also be variable, depending on other inputs.

5.5. INPUT-OUTPUT RELATIONSHIPS

Input-output relationships for several compounds (sucrose, sinigrin, strychnine in *Pieris* and sinigrin in *Mamestra*) could be described satisfactorily by a linear function.

However, for some other chemicals (sucrose, inositol, strychnine in *Mamestra*) non-linear relationships were obtained. In the latter cases, conclusions derived from ablation experiments and the shape on the input-output relations (but not the non-linearity *per se*), indicate that other sensory information from hitherto unknown gustatory organs play a rôle. For the former compounds the total relevant input seems to be known (*i.e.* coming from the three sensillum types), or at least, there are no obvious reasons to assume other sensory channels. When part of the input is neglected, for example in the case of sucrose in *Pieris* or sinigrin in *Mamestra*, and the relative behaviour is correlated with the remaining part, non-linear relations appear (Fig. 6, Fig. 30). Such results resemble those observed for a number of compounds in *Mamestra*. It is concluded that when the total sensory input is known and compared to behavioural output linear relationships are found, whereas all non-linear relationships found so far, suggest a deficiency in our knowledge of the sensory message.

It might be argued that for sucrose in *Pieris* the linear relationship is the result of the addition of the inputs from the three cell types, whereas the processes in the CNS might follow a different pattern. This problem has been discussed in section 5.3. Whatever the conclusion in this case may be, obviously this argument does not hold for sinigrin and strychnine in *Pieris*. Therefore, it is concluded that for most compounds information processing in the CNS, including its translation into behavioural output is described by a linear function. Clearly, for some compounds such linear relationship only holds within a certain range of the sensory input. Below the behavioural threshold (section 5.4) and above maximal output the linear relationship is masked by other limiting factors.

Regarding the complexity of the processes which link output to input (as outlined in section 1.4), a linear relationship between both is surprisingly simple, and it suggests that the system involved functions on the basis of relatively simple principles.

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