A STUDY OF THE SENSORY BIOLOGY OF LABELLAR GUSTATION BY THE MALE QUEENSLAND FRUIT FLY, DACUS TRYONI (FROGGATT) (DIPTERA: TEPHRITIDAE) WITH RESPECT TO CUE-LURE

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ABSTRACT

Males of the Queensland fruit fly Dacus tryoni (Froggatt) are attracted to and readily ingest 4-(p-acetoxyphenyl)-2-butanone (cue-lure). The development of responsiveness of these flies follows a sigmoid curve: almost no flies respond in the first two days after eclosion, the proportion of responsive flies rises gradually to a peak (70-80%) 7 days post-eclosion and remains there for at least 21 days.

Light, scanning and transmission electron microscopy were used to study the morphology, distribution, and the innervation of the labellar sensilla of male Dacus tryoni. These were classified into six morphological types on the basis of their lengths: longest (LH), long (lH), medium long (MH), short straight (ssh), bristle (b), and very short bristle (sb) sensilla. Because of a dimensional overlap between lH and MH and the pattern of their distribution, these have been classified as subtypes of one group, the intermediate sensilla (iH). The total number of fringe sensilla had a positive correlation to the size of the labellum and to that of the fly. This has been suggested to occur within upper and lower limits, about a modal size characteristic of members of the species. Longest sensilla on larger labella were longer than corresponding sensilla on small labella.

Labellar fringe sensilla are in five rows, almost parallel to the marginal strip of the oral disc, the first or lowermost row being the marginal row. Bristle and short bristle sensilla occur on the marginal and fifth (uppermost) rows, but more of the "sb" are on the marginal row. The LH and iH are predominantly in the 3rd and 4th rows and, whilst ssh occur in all rows, the large proportion of them are in the marginal row. The marginal ssh are curved inwards over the marginal strip, towards the oral surface, such that when the labellum is everted their tips are in direct contact with food. Except for the shorter sensillar types on the upper rows, a large proportion of all the sensilla contact food substances when the proboscis is extended prior to and during ingestion.

The papillae on the oral surface numbering 56-66 were classified into two morphological types: the peripheral papillae (PP) and the actual interpseudotracheal papillae (IP). The PP occur, one at the origin of each pseudotrachea, all along and close to the marginal strip. The peg of these papillae is stout with either a pore or slit on the blunt tip and each has a wide ring-like bulging socket that may allow for multidirectional mechanosensitivity. The IP are in deep, narrow sockets located at the bases of the pseudotracheae, in the spaces between these and the interpseudotracheal plates. These papillae have sharp tips and are closely apposed to the pseudotracheae such that, the positioning of the pore allows monitoring of chemicals in food flowing in the tubes during suction. Their positioning seems to limit mechanical displacement.

Histological methylene blue staining showed that

papillae and fringe sensilla are innervated by two or more neurones. Ultrastructure of the innervation of the labellar sensilla revealed that, except for the short bristle sensilla which are innervated by two gustatory neurones only, all the other fringe sensillar types have 4 gustatory neurones and a putative mechanoreceptor. The papillae, PP and IP have 3 gustatory neurones in the single lumen of the peg and a putative mechanoreceptor.

Action potential frequencies were recorded from certain long and/or longest labellar sensilla when stimulated with a series of LiCl concentrations, and with cue-lure and five other closely related compounds. Dose/response plots and probit analysis showed that two neurones, labelled as P1 and P2, with medium-large and large spike amplitudes are positively sensitive to increase in LiCl concentration. A third cell, the n-neurone, had a smaller spike and a firing rate that negatively correlated with LiCl concentration; this corresponds to the classical "water cell" of Evans and Mellon (1962) and Rees (1970a). A fourth neurone (Lp), with a larger spike amplitude than P2, was also evident in a small number of the recordings with molar LiCl. Response characteristics of P1 in longest and long sensilla are similar for all LiCl concentrations; as also are the threshold and firing of P2, at concentrations less than 100 mM LiCl. However, at 0.5 M and molar LiCl, P2 had a significantly higher firing rate in long than in longest sensilla. 50 mM LiCl was chosen as the carrier electrolyte for cue-lure in electrophysiological tests, because of the relatively low firing rate of the n-neurone, very low or no response from the P neurones, and adequate conductivity.

A gustatory neurone, which was rapidly adapting to millimolar cue-lure and dose-sensitive to 4-phenyl-2-butanone and two of its derivatives {4-(p-acetoxyphenyl)-2-butanone and 4-(p-hydroxyphenyl)-2-butanone}, was recognised and denoted as the c-neurone. The electrophysiology of the c-neurone when stimulated with cue-lure, correlated well with the feeding behaviour data over the range of concentrations tested.

Further recording, using changing stimuli produced by a flow system, showed that at least one of the P neurones responding to 0.5 M LiCl has a different spike shape to that of the c-neurone.

Differences in responsiveness of the c-neurone to a range of molecules: 4-decanone (4-DCN), toluene (TLN), acetophenone (ACP), 4-phenyl-2-butanone (4-PBN), and 4-(p-hydroxyphenyl)-2-butanone (p-HPBN) relative to cue-lure (p-APBN), were used to identify some of the molecular characteristics that may be complementary to a hypothetical receptor site for the cue-lure molecule. ACP and TLN, with shorter molecules than 4-PBN, and the aliphatic 4-DCN were not stimulatory to the c-neurone. The results are discussed on the basis of the stereochemistry, hydrophobic and electronic charge characteristics of the functional groups of the substituents of the molecules tested. Possible application of these results to studies on the modification of lure potency by structure-activity modelling is considered.

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DECLARATION

The work presented in this thesis was carried out by the author in the Entomology Department of the University of Queensland. It is to the best of my knowledge and belief, original except as acknowledged in the text. This work has not been submitted in part or whole to this or any other University.

Peter G. Ng'ang'a Njagi.

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DEDICATION

To my parents Mr George Njagi and Mrs Esther Waithira Njagi, and to the memory of my late grandmother Mrs Mary Mumbi Gathuku.

"**NYŨMBA NYINYI NDĨĨGŨMAGĨRA. NGAI NĨWE MŨNENE.**"

I. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Many species of fruit flies of the family Tephritidae are well known fruit and vegetable destroying pests of economic importance in tropical, subtropical, and temperate regions of the world (Christenson and Foote 1960; Bateman 1972; Fletcher 1987). These species have been described as frugivorous because they exploit a broad range of pulpy fruits. Some of the non-frugivorous species that attack the vegetative parts of food plants are also pests of economic importance (Zwolfer 1983). Powerful chemical attractants for fruit flies have been made from naturally occuring organic substances and used for monitoring (e.g. Monro and Richardson 1969) and in some cases controlling and eradicating (in conjunction with insecticides) isolated populations of some of the pest species of economic importance (Bateman et al. 1966, 1973; Steiner et al. 1965, 1969; Cunningham et al. 1975; Ushio et al. 1982; Koyama et al. 1984).

While more than 80 species of Dacini have been documented in Australia, only a few of these are of economic importance as major pests of cultivated fruit (Fletcher and Bateman 1983). The Queensland fruit fly Dacus tryoni (Froggatt) is the most important, with a host plant record of over 117 species of both cultivated and wild plants spanning over 34 families (May 1953, 1957, 1960). Bateman (1979) listed over 150 species of host plants, including all common fruits except pinapples and strawberries, for this species. This indicates either an expansion of the host range or improved data gathering, or introduction of more hosts into Australia during

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the past two decades since the compilation of May's lists.

D. tryoni largely occurs on a relatively wide strip along the eastern coast of mainland Australia from far north Queensland (Cape York peninsula) to the northern parts of New South Wales, beyond which the distribution narrows down but continues southwards to a small region in eastern Victoria. The delimiting factors of this distribution are mainly the hot and dry weather further inland and the cooler winter in the southern states. Colonisation by this species has extended to off-shore islands: Papua, New Caledonia, the Society group and Easter Island during the past two decades (Drew 1982).

In their review of useful strategies for the management of fruit fly problems in Australia, Fletcher and Bateman (1983) asserted that protein hydrolysate baited "spot" sprays (usually protein hydrolysate plus malathion) are the most effective control measure, both at present and in the foreseable future. Cue-lure [4-(p-acetoxyphenyl)-2-butanone] is a powerful attractant for male flies of D. tryoni and of the melon fly, Dacus cucurbitae Coquillet. Cue-lure differs from protein hydrolysate and other food lures because it predominantly attracts male flies. Fletcher and Bateman (1983) suggest that cue-lure may be a suitable bait for administering chemosterilants in management programmes, assuming environmentally safe chemosterilants can be developed. This is based on the observation that male D. tryoni are not only attracted to cue-lure but also readily ingest it (Monro and Richardson 1969; Drew 1982). Other species of tephritids also feed on specific attractants, especially when available in relatively low concentrations: Dacus zonatus Saunders and Dacus diversus

Coquillet (Howlett 1915); Dacus dorsalis Hendel (Steiner 1952; Metcalf et al. 1975; Chambers et al. 1972); Dacus opiliae Drew and Hardy (Fitt 1981b). Steiner (1952) described the feeding of D.dorsalis males on methyl eugenol (3,4-dimethoxyallyl-benzene) as "compulsive".

Aliphatic amines and alcohols, tested electrophysiologically (Hodgson and Steinhardt 1967), were shown to block the electrical activity of neurones sensitive to water and sugar, without evoking action potentials in other neurones. This was suggested as a possible basis for the rejection of foods containing these amines and alcohols. Lure feeding behaviour (attraction and ingestion) of male D. tryoni has been used in the present work as the behavioural basis for a correlative investigation of the sensory biology of their labellar gustatory sensilla, which come into contact with the lure during ingestion. Three main approaches were used:

i. Study of the morphology, typology, numbers, and topographical distribution of the trichoid labellar gustatory sensilla and the interpseudotracheal papillae.

ii. Determination of the innervation of some of a range of the labellar gustatory sensilla representative of the different types.

iii. Determination of the sensitivity of the neurones innervating the gustatory sensilla, to find in what way they are sensitive to 4-(p-acetoxyphenyl)-2-butanone.

Variation of gustatory sensilla responsiveness with age of flies was also characterised, to enable choice of flies of known and stable responsiveness for the main experiments.

Classification of the labellar gustatory sensilla into different morphological types was a necessary preliminary to carrying out electrophysiological tests. Light and scanning electron microscopical studies gave details on topography, numbers and the distribution of the fringe labellar sensilla and interpseudotracheal papillae. Transmission electron microscopy (TEM) was utilised to acquire fine-structural details, particularly on the numbers and types of sensory neurones innervating the sensilla.

Electrophyiological tests were carried out to investigate the dose/response relationships of the labellar gustatory neurones. Tests were carried out with dilute LiCl concentrations, to determine a suitable concentration for a salt bridge in recording from gustatory neurones with the non-conducting cue-lure. One of the neurones responding to cue-lure had a spike amplitude similar to one responding to dilute LiCl. To determine whether it was the same neurone that is sensitive to both chemical stimuli, recordings in which test solutions were changed from 0.5M LiCl to millimolar cue-lure in 0.5M LiCl were carried out, using a flow system.

Willison's lure {4-(p-hydroxyphenyl)-2-butanone}; a possible degradation product of cue-lure (Keiser et al. 1973; Drew 1982) but less potent, was also tested electrophysiologically on some "longest" sensilla. Toluene, acetophenone, and 4-phenyl-2-butanone (the parent group of the two lures) and an aliphatic ketone, 4-decanone, were similarly tested. The latter two compounds have the same number of carbon atoms as 4-(p-hydroxyphenyl)-2-butanone, whilst the first two have fewer. This was to test how specific the neuronal responses to cue-lure are

and to obtain data, if possible, on the effective features of stimulant molecules, in the hope of revealing some of the characteristics of the acceptor sites of the gustatory dendrite membrane.

Longest (LH) and intermediate (iH) labellar gustatory sensilla were recorded from and comparisons made between the dose/response curves of their corresponding neurones; to LiCl alone (control) and to cue-lure in 50mM LiCl. The other lures and chemicals were tested on the longest sensilla only. Rice (1975) and Chapman (1982) have cautioned that, the study of responses of neurones in a sensillum is not sufficient to enable the behaviour likely to result from a stimulus to be predicted. This is because in the natural situation, individual sensory input into the CNS needs to be seen in the context of input from a large number of neurones. In addition, the internal state of an insect and environmental variables play important modulating roles. It was hoped that electrophysiological data from an array of different types of labellar gustatory sensilla might reveal a possible "across-fibre" coding for cue-lure.

Behavioural preference studies, on the cue-lure concentrations used in electrophysiological tests, were carried out in an attempt to identify possible correlations with gustatory cell properties.

1.2. LITERATURE REVIEW

1.2.1. Attractants for some tephritid species

Howlett (1912, 1915) discovered that Citronella and Bay oils, which contain methyl eugenol (3,4-dimethoxyallylbenzene) were attractive to Dacus (Batrocera) zonatus Saund. and D. diversus Coquillet, in India. Other organic chemicals attractive to some tephritids were made from naturally occuring substances and tested between the early 1950's and 1960's (Steiner 1952; Barthel et al. 1957; Beroza et al. 1960, 1961) (Table 1.1). Males of several species of tephritids usually respond to one lure and some of its analogues. Drew (1974) divided Dacini species collected in northeast Australia and 26 islands of the South Pacific into two major groups on the basis of their most potent attractant. Of the 79 species, 56 responded to cue-lure or its mixture with Willison's lure, whilst 23 were attracted to methyl eugenol. No members of a species responded to the two lures.

Female flies under certain environmental and physiological conditions or induced stress, have been observed to be attracted to the same lures as their conspecific males. Females of D. dorsalis were attracted to methyl eugenol in traps when the proportion of males in the wild population had been reduced to a very low level of an average catch less than one male/trap/day (Steiner et al. 1965). Mature virgin female Ceratitis capitata, (Wiedmann) were attracted to medlure [sec- butyl-4(or 5)-chloro-2-methyl-cyclohexane - carboxylate] and trimedlure (the tertiary derivative of medlure) when the number of male flies in an isolated population was reduced to a very low level (Nakagawa et al. 1970). Starved, mature female

Table 1.1 Attractants (lures) for some tephritid species of economic importance and the behavioural responses of adult male flies to them with age (d.pe: days post-eclosion); 0 no response; - repellent; + low response; ++ 20-50% response, increasing with age if less than 7 d.pe; +++ maximum 70-80% response. attained.

Tephritid sp.	Common name of lure	rel. or actual concentration	Responses: attraction (and feeding) 1-3 d.pe	4-7 d.pe	>7 d.pe	References
C. capitata	Medlure[1] / Trimedlure[2]	high		-(-)	-(-)	Beroza et al. 1961,
		low			+++(+++)	Beroza & Jacobson 1963.
D. cucurbitae	Cue-lure[3]	high	0,+(0,+)[5]		———	Beroza et al. 1960,
		low			+++(+++)	Iwahashi cited by Bateman 1982.
D. dorsalis	Methyl eugenol[4]	high	0(0)	++(++)	+++(+++)	Beroza & Jacobson
		low	0(0)	++(++)	+++(+++)	1963, Umeya et al. 1973.
D. neohumeralis	Cue-lure	high	0(0)	+(+)	+(+)	*
		low	0(0)	++(++)	+++(+++)	
D. opiliae	Methyl eugenol	0.3 ml on cotton wick	0(0)	++(++)	+++(+++)	Fitt 1981b.
D. tryoni	Cue-lure	1.31 mg/cm^2	———	———	+(+)	present study.
		30 µg/cm^2 (on filter paper)	0(0)	++(++)	+++(+++)	

1 sec-butyl-4(or 5)-chloro-2-methylcyclohexane carboxylate.
2 tert.-butyl-4(or 5)-chloro-2-methylcyclohexane carboxylate.
3 4-(p-acetoxyphenyl)-2-butanone.
4 3,4-dimethoxyallylbenzene.
5 Contradictory information on whether flies 1-3 d.pe are responsive or not.
* Drew (1982) suggested that this sibling sp. of D. tryoni has a similar response to cue-lure.

flies of this species were also reported to feed on trimedlure (Nadel and Peleg 1965). Mature, virgin females of D. tryoni are attracted to cue-lure in small numbers (Fitt 1981b).

1.2.2. Natural sources of some of the tephritid attractants

A question arises as to whether and how often male tephritids of these species are likely to encounter the lures, or any of their components, in their natural habitats. Flath and Ohinata (1982) reported the presence of minute quantities of 4- phenyl-2-butanone amongst other volatiles isolated from flowers of the orchid Dendrobium superbum to which male melon flies, D. cucurbitae are attracted. Although this indicates the presence of a component of the cue-lure molecule in the wild, there is no available information on how widespread its occurence might be in other species of this genus of orchids and other flowers. It is interesting to note that the region where D. tryoni is endemic in the tropical eastern coast of Australia, also supports about 230 species (in about 25% of the orchid genera) of the Australian orchid flora, with an abundance of Dendrobium species (Lavarack and Gray 1985). No reports have been found or any evidence of observations on D. tryoni males being attracted to flowers of orchids. Methyl eugenol has been extracted from roots, blossoms, leaves, stems and fruit tissue of a diverse range of plant species: oxygenated fraction of carrot Daucus carota L. root oil (Buttery et al. 1968); golden shower Casia fistula L. blossoms (Kawano et al. 1968); leaves of Zieria smithii (Andrews) (Fletcher et al. 1975); and almond hulls (Buttery et al. 1980). Dacus cacuminatus (Herring) males have been observed to aggregate in small numbers on leaves of Z.

smithii (Fletcher et al. 1975) whilst those of D. dorsalis attracted to petals of C. fistula continually dabbed the surfaces with their labella (Mitchell 1965). Couropita guianensis Aub. (Lecythidecea), Brexia madagascariensis Thou. (Saxifragaceae), Vriesea heliconioides Thou. (Bromeliacea), and dry leaves and twigs of Pelea anisata Mann (Ruteceae) are plants of different families cited by Kawano et al. (1968) as containing some volatile components that strongly attract males of D. dorsalis.

1.2.3. Some factors influencing responses of male tephritids to the lures

Some of the factors influencing responses of male tephritids to lures are largely known with regard to their potency in traps and cages in field. The effects of some of these factors have only been mentioned as observations or speculated on as directly or indirectly affecting numbers of flies collected in traps. Physiological changes in adult flies post-eclosion have also been shown to affect their behaviour to the lures (Table 1.1). Hence, these factors have been divided into two groups; exogenous and endogenous with respect to the flies.

Endogenous factors

These involve the changing physiology of flies with age and their nutritional status. Such effects have largely been investigated in the laboratory, field cages and in release/recapture trials with flies of known age. No direct assessment of age has been done on wild flies captured in traps because no

easy age-assessment techniques are available yet.

Age

In laboratory tests, D. dorsalis males were only attracted to methyl eugenol after nine days post-eclosion (Umeya et al. 1973). D. opiliae males are unresponsive to 0.3 ml of methyl eugenol on cotton wicks in the first three days post-eclosion at 25°C and 75% R.H. Maximum attraction (and feeding), of up to 80%, was attained nine days after eclosion and remained at that level up to three months (Fitt 1981b). The percentage of males of D. cucurbitae responding to cue-lure similarly increases in a sigmoid pattern reaching saturation (about 80%) fourteen days after eclosion, which was their average age at sexual maturity (Iwahashi cited in Bateman 1982). This contrasts with the statement of Beroza et al. (1960) that cue-lure attracts D. cucurbitae of all ages, even newly emerged ones (Table 1.1). Males of the medfly C. capitata respond to trimedlure as early as their first day of emergence (Nadel and Peleg 1965; Nakagawa et al. 1970).

Protein nutrition

Proteins are the source of essential amino acids for growth of the immature stages and development of reproductive maturity in adults (Johannson 1964). Drew et al. (1983) established that bacteria ingested from colonies of micro-organisms on leaf surfaces of host and non-host plants are a major source of proteins for D. tryoni, D. cacuminatus and probably other species in nature. Development of sexual maturity in males of some tephritids synchronizes with their response to the lures (cf. effects of age in Chapter 2 of this thesis); an indication

that protein ingestion may be necessary for both. The only report is that on protein availability to male D. opiliae: Fitt (1981b) observed twice as many (80%) protein-fed males of D. opiliae nine days and older responded to methyl eugenol, compared to those fed on a protein-deficient diet. Availability of the proteinaceous diet was crucial for the first three days post-eclosion.

Previous contact and ingestion of a lure

Male tephritids allowed to contact and feed on the lure become less responsive to the same lure for a duration which may be characteristic of each species and the nature of the attractant. Males of D. cucurbitae became unresponsive to cue-lure after 4-5 days' exposure (and probable feeding) to various dosages (0.05 - 5 ml) impregnated onto container paper bags (Chambers et al. 1972). Unexposed flies were 3-8 times more responsive to the lure in subsequent tests. This refractoriness persisted for at least three weeks. D. opiliae males that had ingested methyl eugenol, when exposed for nine hours were significantly less responsive to the lure on the following day (fifteen hours later) than those exposed only to the odour for the same duration (Fitt 1981b). The length of the refractory period in this species was not estimated. However, it was confirmed that the actual contact and ingestion of the lure rather than exposure to the odour alone gives rise to refractoriness to the olfactory stimulation by lures. It is

probably a prolongation of the state of neuronal processing of gustatory and olfactory inputs during feeding. Ito and Iwahashi (1974) (cited in Fitt 1981b) also observed that males of D. dorsalis that consumed methyl eugenol in traps (and presumably were marked) never returned to traps baited with the same lure.

Exogenous factors

There is some literature available on the environmental conditions that have been observed to affect trap catches but in most studies, their impact does not seem to have been evaluated.

Environmental conditions: light intensity, temperature, humidity

Few D. tryoni and D. cacuminatus visited cue-lure and methyl eugenol baited traps on damp overcast days in summer (Brieze-Stegeman et al. 1978; Hill and Hooper 1984). From my observations on the number of male D. tryoni caught in two cue-lure baited traps in two localities in Brisbane (St. Lucia and Paddington) during March-May 1987, more flies were attracted to the traps on clear sunny mornings after a rainy previous day or night than when it was dry. It is not clear whether it is the high humidity of the surroundings after a rainy spell or the actual presence of some rain water in traps (which may dissolve some lure) that compounds with some or all the other factors to affect the "attention" of the flies (Rice 1988). However, Fitt (1983) calculated that 77.4% of all the daily fluctuations of response of male D. opiliae to methyl eugenol, in field cages in

northern Australia, could be explained by the humidity index (mean no. of hours/day with humidity 70% or over). Occurence of rainfall accounted for only 21.2%. Twice (80-90%) as many male flies responded to the lure 12-14 days after eclosion during the wet season (October - March with more than 10 mm rainfall) as those during the dry season (May - September). The linear regression analysis showed that temperature changes had a significant effect (up to 50%) on trap catches.

Male tephritids of some of the species have a daily periodicity of responsiveness to the lures. The time of day seems to vary depending on the ambient weather conditions. In Hawaii, male D. dorsalis were caught in methyl eugenol baited traps from one hour after sunrise to sunset with peak responses occuring at 10.00 - 14.00hrs (Nakagawa et al. 1970). Similarly, males of D. tryoni visited non-insecticidal, cue-lure baited traps as early as 04.30hrs attaining peak numbers at 06.30hrs during summer (Brieze-Stegeman et al. 1978). In spring these times shifted to about 07.00 to 11.00hrs. In the same study, D. cacuminatus were attracted to methyl eugenol slightly earlier than 07.00 and at 07.00hrs in summer and spring respectively, with peak response at 10.00 and 13.00hrs. For both species, flies stopped visiting the traps in the late afternoon and some of those in the traps left, leading to a decline in their numbers, to a minimum at 18.00 - 20.00hrs. In laboratory experiments, D. opiliae had a fluctuating response to methyl eugenol throughout the day at 25 ^{o}C and a natural light regime (Fitt 1981b). However, a marked peak response (90%) occured at 10.00 - 11.00hrs and declined slowly during the afternoon, eventually dropping sharply to a low level (25%) at

dusk (19.00hrs). These daily rhythms of response to the lures seem to be evoked by the increasing light intensity and temperature, from early morning through midday and early afternoon which switches the "attention" of the flies' "internal programme" to lure feeding behaviour during this period (Rice 1988). Decline in response may also trail declining light intensity in the late afternoon, "attention" being switched to mating activities that peak at dusk for some species: D. opiliae (Fitt 1981a,b); D. tryoni (Myers 1952; Tychsen and Fletcher 1971; Tychsen 1977); D. cacuminatus (Myers 1952); D. cucurbitae (Kobayashi et al. 1978; Suzuki and Koyama 1980); D. dorsalis (Kobayashi et al. 1978). In contrast, mating of D. neohumeralis (Tychsen 1977) and C. capitata (Prokopy and Hendrichs 1979) occurs during mid-morning to early afternoon, the sequence of behavioural activities being also linked to increasing light intensity and/or temperature.

Feeding and other behavioural activities of D. tryoni are influenced by light intensity and the duration of the photoperiod: Barton-Browne (1956) observed that populations of D. tryoni in laboratory cages were significantly less active and fecund at 120 lumens/sq. ft (1291.2 lux) than at 60 and 240 lumens/sq. ft (645.6 and 2582.4 lux respectively). Female flies illuminated in all photoperiod regimes at 120 lumens/sq. ft and for 2 and 4 hours at 240 lumens/sq. ft had low fecundity. This was attributed to a low rate of egg development in the ovaries and delayed mating resulting from decreased feeding activity.

Concentration of the lure

The relative concentration of odour molecules around

a source depends on the volatility of the chemical, diffusion rate, and the ambient air currents. High concentrations of some of the attractants repel male tephritids, olfactorily and when contacted (Table 1.1). C. capitata avoid direct contact with areas of paper or cotton wick treated with trimedlure but instead congregate around the edges where there are lower quantities of the lure (Beroza et al. 1961; Beroza and Jacobson 1963; Chambers et al. 1972). In container traps with 0.9 ml trimedlure on a cotton wick, about 2.5 times the number of C. capitata were attracted, compared to traps with an almost 11-fold initial concentration (9.7 ml) of the lure (Nakagawa et al. 1971). The higher concentration was suggested to be repellent. Cue-lure is slightly repellent at high concentrations and only a small number of D. cucurbitae males were observed to make direct contact with the chemical on treated surfaces (Chambers et al. 1972). Concentrated methyl eugenol does not repel males of D. dorsalis and the flies contact and feed upon the undiluted lure (Beroza and Jacobson 1963).

1.2.4 Locating the source of lure

Olfactory sensilla, and tarsal and labellar gustatory sensilla are sequentially involved prior to ingestion of a lure. In the initial stages of the male fly's response to a lure, both long and short range detection of the chemical seems to depend on the antennal and palpal olfactory receptors (Metcalf et al. 1975).

1.2.4.1. Olfactory receptors of tephritids

Antennectomy studies by Metcalf et al. (1975) showed that male D. dorsalis performed zig-zagging movements,

approaching methyl eugenol from the side with an intact antenna. Males without both antennae did not orientate to the lure but instead moved in circles.

A scanning electron microscopy study of the olfactory sensillar types on the antennae of male and female flies of D. tryoni showed five multiporous types: trichoid type I and II, clavate, basiconic, and styloconic (Giannakakis and Fletcher 1985). These were suggested to play a part in the detection of lures by males and mature virgin females, and the detection of male sex pheromone by mature females. Surgical and chemical ablation studies on various sensory organs of mature female D. tryoni have shown that, sensilla on the antennae and the maxillary palps are the most important in the detection of the sex pheromone (Giannakakis and Fletcher 1981). Scanning electron microscopical and ultrastructural studies of the funicular sensilla of D. oleae showed lack of sexual dimorphism, in sensillar types and their innervation which are similar to the morphological types of D. tryoni (Hallberg et al. 1984). Each funiculus has a single olfactory pit which houses the poreless (NP) sensilla that are innervated by three neurones. Two of these neurones were suggested to be putative thermo/hygroreceptors and the third a mechanoreceptor. On the general surface are another three, multiporous, dually-innervated types: the long-single walled (LSW), the short-single walled (SSW), and the double-walled (DW) sensilla. These three types were suggested to be olfactory receptors (Hallberg et al. 1984).

In locating an odour source, odour-modulated positive anemotaxis and zig-zagging and casting in the wind borne odour plume are important. Frequency and amplitude of

the latter manouvres depend on the concentration gradient of odour molecules at a given distance from the source and the nature of their distribution (David et al. 1982; Kennedy 1983: review; Baker et al. 1984). In a wind tunnel experiment (Jones et al. 1981) C. capitata male flies flew straight upwind (positive anemotactic flight), when trimedlure odour uniformly permeated the airstream; whereas zigzags were superimposed on the positive anemotactic flight, when the odour was released in intermittent plumes created by highly turbulent air passing over the source.

After landing on the substrate containing the lure or food material, tarsal gustatory sensilla contact stimulant chemicals and the neurones therein are stimulated. Extension of the labellum and subsequent stimulation of the labellar sensilla follow.

1.2.4.2. Gustatory sensilla of tephritids

Tarsal sensilla

Structural ablation and behavioural tests (Prokopy and Spatcher 1977) showed that receptors of the oviposition-deterring pheromone (ODP) in females of Rhagoletis pomonella (Walsh), are present in large numbers on all tarsi. The D-chemosensilla on the 2nd, 3rd, and 4th tarsomeres were proven electrophysiologically to be equally and highly sensitive to the ODP (Crnjar et al. 1978; Crnjar and Prokopy 1982). It was concluded that deterrence by ODP is specifically mediated by a high frequency neuronal response, elicited in the paired D tarsal sensilla on the distal ventro-lateral portions of the second, third and fourth tarsomeres of all tarsi. A slightly

smaller number of D-sensilla on the fifth tarsomere were sensitive to the ODP, compared to those on other tarsomeres. The higher influence on behaviour by the D-sensilla on the prothoracic tarsi was suggested to arise from a possible ranking of the sensory inputs at the central processing level and/or their higher probability of contacting the ODP first, due to their forward-most position. The presence and characterisation of the responses of an ODP-sensitive neurone, in the tarsal D-sensilla, was further confirmed by the detailed electrophysiolo-gical study of Bowdan (1984). From experiments with a range of concentrations of: sucrose (0.01- 2M); NaCl (0.1 - 3M); crude extract of the ODP (25 - 400 dragging bout equivalents (d.e.)/ml); and the three mixtures of all pairings of these chemicals at 0.05M sucrose, 100 d.e./ml ODP, and 0.5M NaCl. The ODP-sensitive neurone was shown to have distinct dose/response and long-term adaptation characteristics, different from those of the sugar- and salt-sensitive neurones.

Tarsal gustatory sensilla of Stomoxys calcitrans L. are innervated by 4 chemosensitive neurones and a mechano-receptor (Adams et al. 1965); as are those of Phaenicia serricata Meigen (Matsumoto and Farley 1978). The 4 chemo-sensitive neurones may include a classical water cell, two salt-sensitive neurones, and a sugar-sensitive neurone (Dethier 1976). If tarsal sensilla of R. pomonella have a similar number of gustatory neurones, the presence of a recognisable spike for the ODP-sensitive neurone (Crnjar and Prokopy 1982; Bowdan 1984) implies that the pheromone might act on the second salt-sensitive neurone, or that the sugar-

sensitive neurone has a receptor site for the pheromone.

In a brief study on the tarsal and labellar gustatory sensilla of C. capitata, Angioy et al. (1978a) used three of each alkaline and alkaline-earth chlorides, five sugars and two ammonium salts. At 0.1M of all the alkaline chlorides (NaCl, KCl and LiCl), magnesium chloride, barium chloride and the ammonium salts (sulphate and the chloride), only the water-sensitive neurone was active in the tarsal sensilla. While at 0.1M and higher concentrations (for ammonium salts only) spikes from at least another two neurones were evident. All sugars except lactose were stimulatory to the tarsal sensilla at 0.5M.

Behavioural experiments on the oviposition of D. tryoni (Eisemann 1980; Eisemann and Rice 1985) led to the conclusion that tarsal gustatory sensilla of female D. tryoni respond to fructose, in exudates on fruit surfaces acting as markers of breaks in the fruit skin through which the ovipositor can be inserted. In contrast, molar glucose and 0.5M sucrose did not increase the number of eggs laid relative to a sugar-less control; they therefore concluded that the oviposition behaviour of D. tryoni depends on sensory input from neurones that have furanose sites only. Electrophysiological tests are planned to test this suggestion (Rice pers. comm.).

Labellar sensilla

The labellar gustatory sensilla of several labellate dipteran species have had their topography, distribution, and numbers described. Wilczek (1967) presented a detailed inventory of both the fringe gustatory sensilla and the interpseudo-tracheal papillae of the blowfly Phormia regina Meigen. Wilczek

used dimensions (for some sensilla types) and locality (for others) as the basic criteria for classification of the labellar gustatory sensilla into five major groups: largest; large; intermediate; marginal and different. A similar classification was adopted in studies on the labellar gustatory sensilla of Calliphora vicina Robineau-Desvoidy (Maes and Vedder 1978) and Protophormia terraenovae Robineau-Desvoidy (Wieczorek 1980). In these studies, the distinguishing dimensions of labellar gustatory sensilla of different types operate within a given species and not between species. In the above where distribution was used as a criterion for classification (e.g. "marginal" and "different" sensilla), a range of dimensions are included in each topographical group.

The distribution of labellar taste sensilla is apparently in rows that parallel the marginal strips, along the sides of the labellum. Each row contains sensilla of at least two morphological types, intermingled in unequal numbers. This seems to hold for individuals of species with relatively larger body size. P. terraenovae has three rows of sensilla (Wieczorek 1980) whilst the smaller Drosophila melanogaster Meigen, has only two distinct rows and fewer sensilla (Falk et al. 1976).

Mixing of morphologically (probably physiologically different too) types of sensilla, on the labellar lobes may enable a wider range of stimulus situations to be monitored by each part of the organ. This would ensure that the system has no directional bias in both mechano- and chemosensitivity, because stimulation of a group of sensilla on one part of the labellum would involve a small number of sensilla of each type. This may also facilitate repositioning of the labellum on the food

material for proper ingestion. Yetman and Pollack (1987) observed that directionality of labella extension of Ph. regina depends on the labellar gustatory sensilla stimulated. Sensilla on the anterior of the labellum elicited anterior extensions, mid-region sensilla lateral extensions, whilst those on the posterior gave rise to posterior extensions. However, they point out that the positioning of the labellum is not exclusively governed by sensory input from the labellar sensilla since the initial orientation depends on input from tarsal gustatory sensilla.

The development of the electrophysiological tip-recording technique (Hodgson et al. 1955) and later the side wall technique (Morita 1959) led to a stream of information especially on Ph. regina, the most studied insect species for gustatory chemoreception. The side-wall technique was a further improvement on the tip-recording technique in that non-ionising test chemicals are applied directly onto the sensillum tip, without having to be in an electrolyte. The recording electrode in a glass micropipette containing an electrolyte contacts the dendrites through a puncture in the sensillum shaft. However, puncturing of the sensillum sometimes creates injury and neurones usually start firing before application of test solutions. No side-wall recording has yet been done on gustatory sensilla in tephritids.

Those electrophysiological studies on gustatory chemoreception in tephritids reported in the literature, cover a limited number of species and some are not rigorous. Most of them simply demonstrate that neurones in the labellar and/or

tarsal gustatory sensilla are sensitive to pure salts and/or sugars. Gothilf et al. (1971) experimented on six, long, marginal, labellar gustatory sensilla of the Mediterranean fruit fly C. capitata, with eight sugars, sugar alcohols (mannitol and inositol), monovalent chlorides (NaCl, KCl, and NH_4Cl) and two divalent chlorides ($MgCl_2$ and $CaCl_2$). Sugars and sugar alcohols were subdivided into roughly four groups on the basis of their stimulatory effectiveness: highly stimulating (sucrose, fructose, glucose); intermediate (inositol, galactose, D-arabinose, maltose); low (mannose, L-arabinose) and the nonstimulatory (mannitol and lactose). For, presumably an equimolar concentration of these substances, the spike frequency of the adaptation curves for the first two and a half seconds was in the order: sucrose > fructose > glucose >> inositol > galactose > D-arabinose = maltose > L-arabinose = mannose >> mannitol = lactose. A similar trend was shown to hold for consumption of 5% solutions and survival of flies. Neurones sensitive to sucrose and NaCl had definite dose-dependent adaptation characteristics, the spike frequency falling to 50% of the initial after the first second and progressing to a slow adapting phase thereafter. Threshold for sucrose was at 0.031 - 0.125M and saturation at about 0.75M; while for NaCl threshold was at 0.1 - 0.5M and maximum spike frequency at 3M. Calcium chloride at 0.01 and 0.1M inhibited the activity of a neurone sensitive to fructose; at higher concentrations (0.5 and molar) only spikes of small amplitude were recorded.

The marginal labellar sensilla of C. capitata had a pattern of neuronal activity similar to that of the tarsal sensilla of the same insect elicited by $MgCl_2$, $BaCl_2$ and

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ammonium sulphate (Angioy et al. 1978a). However, at 0.01M and higher concentrations, the alkaline chlorides and ammonium chloride had at least one additional neurone firing. This indicates a tenfold lower threshold for salt-sensitive neurones (cf. Angioy et al. 1978 for NaCl) in the marginal labellar sensilla of C. capitata considering the report by Gothilf et al. (1971), that 0.1M NaCl was nonstimulatory. The marginal labellar sensilla of C. capitata presumably corresponds to the marginal short straight and/or intermediate sensilla of D. tryoni in the present study. In the labellar gustatory sensilla of Dacus oleae Gmelin, 0.1M NaCl was stimulatory to the salt-sensitive neurones whereas 0.01M was not (Angioy et al. 1978, 1978b). As in the tarsal sensilla, all sugars except lactose were stimulatory to the labellar sensilla at 0.5M. No dose/response and/or adaptation curves were presented in these studies because with the exception of the ammonium salts, only one or two concentrations of each chemical were tested (Angioy et al. 1978; Angioy et al. 1978a,b).

The ovipositor sensilla

There is some evidence that the ovipositor gustatory sensilla of tephritids may play a role in lure detection by mature virgin females. Mature virgin females of D. tryoni and D. aquilonis May (responding to cue-lure), and D. sp. A May and D. tenuifascia May (responding to methyl eugenol) probed filter papers containing these lures, with the tips of their ovipositors (Fletcher and Giannakakis 1973; Fitt 1981a). Because a similar behaviour has been observed when female flies respond to the sex pheromone, it is most likely that ovipositor

gustatory sensilla are used in detection of both the lures and the nonvolatile components of the male pheromone. Fletcher and Giannakakis (1973) suggest that detection of the sex pheromone by ovipositor receptors, elicits extension of the ovipositor by the female in a direction of high pheromone concentration. Because the male fly takes a place behind the female prior to copulation, this facilitates contact of the genitalia and introduction of the aedeagus into the female genital opening. Further experimental work on these observations may provide useful information on the possibility of utilisation of lures or some of their components in the mating systems of some tephritids.

Morphological studies of the aculeus of the ovipositor of female D. tryoni showed 4 pairs of sensilla in the ventro-lateral groove close to the tip (Eisemann 1980; Eisemann and Rice 1985). Although these sensilla did not seem to play a role in detection of sugars in the oviposition substrate, they were able to discriminate monovalent cations, divalent calcium ions and water (Eisemann and Rice, in press). Marchini and Wood (1983) observed 5 pairs of B-type sensilla on the ventral part of the tip of the aculeus of the ovipositor of C. capitata, which may play a role in monitoring pH in fruits: pH 3 was shown to be most favourable whilst pH 6 was the least. Quinine Hydrochloride (0.01M), copper sulphate (0.5M), molar NaCl, molar KCl, and molar magnesium chloride in the oviposition substrate elicited rejection by female flies. Detailed SEM and TEM work on the ovipositor sensilla of R. pomonella by Stoffolano and Yin (1987) showed that the three

pairs of sensilla in the longitudinal grooves are uniporous and are innervated by 3 or 4 chemoreceptor type neurones and a mechanoreceptor. An extra pair of sensilla (Cs1) located outside the groove closer to the tip, are also uniporous and usually innervated by 4 neurones. These sensilla had been shown electrophysiologically (by placing a pipette over the whole ovipositor) to respond to the presence of sucrose, glucose, NaCl, and malic and quinic acids, though no dose/response curves were obtained (Girolami et al. 1986). Ovipositor gustatory sensilla may not only monitor fruit chemicals, but also a wide range of others, e.g.: oviposition-deterring pheromone; other chemical contaminants on the ovipositor; and the gland secretions of the females (Stoffolano and Yin 1987). Thus, such a complement of gustatory sensilla on the ovipositor, may play a role in the reproductive success of the species by ensuring deposition of eggs in a suitable chemical environment in the fruit pulp, as well as other functions.

Metcalf et al. (1975, 1979, 1981) have done extensive behavioural studies on the attraction and elicitation of feeding of male D. dorsalis by chemicals related stereochemically to the methyl eugenol (3,4-dimethoxyallylbenzene) molecule. The characteristic ingestion of the lure by flies has been used, as an important component of behaviour, for screening for candidate lures, in addition to the attractancy of the test chemical. Metcalf et al. (1975) tested 34 compounds, based on the isomerism of methoxyphenyl substituents, other substituents in place of the methoxy groups, and points of unsaturation (double bonds). Only the p-isomers were found to be attractive and they concluded that, at least one electron-rich oxygen atom and the

phenyl ring are important for the attraction and elicitation of feeding by methyl eugenol. Metcalf et al. (1979, 1981) tested 3,4-dimethoxybenzenes which are isomeric to methyleugenol as candidate lures for D. dorsalis. Ortho-substituted 3,4-dimethoxybenzenes were more attractive and elicited more feeding than the para- and meta- isomers, but to a lesser extent than methyl eugenol. The paired ether atoms were suggested to be important for the interaction of lure molecules with a lipo-protein patch at the olfactory receptor site (Metcalf et al. 1979). The hydrophobic (π) and electron donating (σ) properties of the primary substituent of the lure molecule positively correlated to the odour intensity (characterized by humans) and their effectiveness in eliciting behavioural responses (Metcalf et al. 1981).

Thus, behavioural screening of a large number of methyl eugenol-like compounds has provided important information on the characteristics of the lure molecule that may play a role at the receptor site. However, no other chemical equal to or more potent than methyl eugenol has been obtained. Similar studies have been done with cis- and trans- isomers of trimedlure (McGovern et al. 1986). Although trans-trimedlure is about 4 times more attractive to C. capitata than cis-trimedlure, the presence of up to 50% of the cis- isomer in a mixture of the two isomers does not affect the attractancy of trans-trimedlure.

Miller et al. (1983) reported that methyl eugenol is carcinogenic in mouse liver. Because of its wide occurence in food plants in relatively minute quantities and the possibility of being a health hazard to humans at relatively high doses,

Mitchell *et al*. (1985) carried out a further screening of chemicals for alternative lures for *D. dorsalis*. In the search for more potent and safe lures, the understanding of the basic interaction between lure molecules and the receptor is necessary and provides a means of assessing the suitability of other alternatives. In the studies cited above, no electrophysiological studies have been incorporated to investigate the presence of neurones sensitive to the lures. Such studies may provide extra knowledge on the characteristics of the responsive neurones, useful in modelling the nature of the receptor molecule.

The available literature on the sensory physiology of dipteran gustatory sensilla largely comprises of studies on a few species: *Ph. regina*, *C. vicina*, and *D. melanogaster*. Labellar sensilla of *B. peregrina* have also been widely used in studies on the presence of multiple receptor sites on the sugar-sensitive neurone (Morita and Shiraishi 1968; Shimada *et al*. 1974; Shimada and Isono 1978; Tinamura and Shimada 1981; and Shimada *et al*. 1985). In addition to the previously known pyranose and furanose sites (based on the alpha-glucosidic linkages in sugars) (Dethier 1955; Evans 1963; Morita and Shiraishi 1968; Omand and Dethier 1969; Jakinovich *et al*. 1971; Morita 1972; Hanamori *et al*. 1974), two other sites have been recognised recently: an "aryl" (Ar) site for aromatic amino acids and an "alkyl" (R) site for fatty acids, aliphatic amino acids, and small peptides (Shimada and Tinamura 1981; Shimada *et al*. 1985; Shimada 1987).

Considering the Queensland fruit fly *D. tryoni*, no

reports have been found on any electrophysiological studies on either the labellar or tarsal sensilla. The behavioural studies of Eisemann (1980) and Eisemann and Rice (1985) need electrophysiological tests to determine the sensory basis of fructose stimulation of labellar and tarsal gustatory sensilla of this species. Giannakakis and Fletcher (1985) reported on the preliminary mapping and typology of the funicular sensilla of male and female D. tryoni pending electrophysiological studies on their role in olfactory detection of conspecific sex pheromone and food lures. Even the funicular sensilla of D. oleae whose innervation has been documented (Hallberg et al. 1984) remain uncharacterised electrophysiologically with regard to the range of stimuli they respond to.

Therefore, the sensory biology of tephritids remains the least studied for both larvae and adults as compared to their general biology and ecology (reviewed by Rice 1989). The available information is scant and in most cases not detailed. It is the objective of the work reported in this thesis to provide some knowledge as a building block in filling this gap, which may facilitate opening up of further channels of research into this field for D. tryoni and other tephritid species. It may also generate interest in studies directed towards the understanding of the basic chemical ecology of the behaviour of males of tephritid species to lures. There are some implications for applied studies in that any improvements in the potency of the lures by steric modification or other means may be speeded up by comparative behavioural and electrophysiological studies, such as the work described here.

II. DEVELOPMENT OF RESPONSIVENESS OF MALE *DACUS TRYONI* TO CUE-LURE WITH AGE

2.1. INTRODUCTION

Male flies of a number of tephritid species readily ingest lures that they are attracted to (Howlett 1915; Steiner 1952; Jacobson and Beroza 1964; Chambers et al. 1972; Monro and Richardson 1969; Fitt 1981b).

The response (attraction and feeding) of male D. opiliae to methyl eugenol increases with age for flies 3 to 6 days post-eclosion. Maximum response is attained nine days after eclosion and remains at that level for up to three months (Fitt 1981b). There is variance between reports on the attraction of D. dorsalis to methyl eugenol and of D. cucurbitae to cue-lure with regard to the responsiveness of flies 1-3 days post-eclosion (Table 1.1 in chapter I). Male flies of these species have increasing responsiveness to their respective lures with age and a correlation with sexual maturity (Umeya et al. 1973; Ito and Iwahashi 1974 cited in Fitt 1981b; Iwahashi in Bateman 1982).

With regard to the response of male D. tryoni to cue-lure, mention has been made of flies being unresponsive during the first three days after eclosion and becoming more responsive thereafter (Monro and Richardson,1969). However, no data are available to show the extent to which the responsiveness (attraction and then feeding) varies with age and if there is a trend after the three days of unresponsiveness, or the age at which maximum response is attained. Such data backed up by field trials is important in trapping work for monitoring fly populations and control with lure/insecticide baits.

The observations presented show the effect of age on the feeding behaviour of male D. tryoni on cue-lure. This information is important because it enables choice of insects of a known age-range whose behavioural responsiveness to the lure is maximum and less variable, for use in electrophysiological and other behaviour studies.

2.2. MATERIALS AND METHODS

Male *D. tryoni* were taken from a laboratory culture kept at about 26 °C and 70% R.H in which emerging adult flies had been provisioned with sucrose, water, and protein hydrolysate. The protein hydrolysate was made available during the first week after eclosion. Flies eclosing over a period of 6-12 hours were taken to be of the same age-range.

Two groups of male flies were tested, those from a normal colony (MNC) and those isolated from the female flies (MIC). For the isolation, male flies of about six hours old were taken from eclosion cages and transferred to cages at least 0.6 m away and kept in isolation from female flies for the rest of the time. Sexually mature flies of the MIC group were unmated when tested. These were tested to see whether there would be any difference in responsiveness to the lure under such isolation, relative to that of flies from a normal colony, which were free to mate.

An average of 22 male flies of known age were put into each of four perspex cages (30 x 15 x 20 cm) on the eve of the day of testing. Observations were done during the period 08.00 - 10.00 hrs in the morning (Brisbane time). A 16 sq. cm filter paper disc (Watman no.1), impregnated with the lure by dipping it into a solution of one percent cue-lure in acetone and then air-drying, was stuck onto a 5.5-6.0 sq. cm glass plate with sticky tape and placed at the centre of each cage. This concentration of the lure had elicited a maximum feeding response in dose-response trials, when presented to the flies at an equivalent concentration of 30 ug of cue-lure per sq. cm of filter paper in preliminary trials. Each cage was placed on

white paper of a size corresponding to that of the bottom of the cage in such a way that, the filter paper disc impregnated with the lure was placed over the centre of a circle of 10.0 cm diameter marked on the paper. The outline of the circle was visible through the transparent bottom of the cage (fig. 2.1).

The light intensity at the centre of each cage was 2,400 - 2,600 lux. All male flies that flew from the walls or walked on the floor of a cage into the circle around the lure containing disc and remained there to the end of the scoring interval of the five minutes were recorded as those attracted (fig. 2.1). Among the attracted flies, those on the filter paper disc that were continuously dabbing the surface with their labella were scored to be those feeding on the lure during the same period. The feeding actiity of flies was accompanied by regurgitation of brown fluid onto the filter paper. Observations were done for a duration of 30 minutes from the time of onset of the light sources (two 200 watt flood lights). Mean temperature in the vicinity of the cages was $27.0 \pm 2.0\ ^{o}C$.

Mean percent response for the flies was calculated for each age of flies: 1-21 days for the normal colony (MNC); 1-14 days for the isolated males (MIC). Because of the number of flies of each age tested per day, tests were carried out with flies spanning four consecutive generations each lasting for a month. Duncan`s Multiple Range Test was used for analysis of the data on a SAS statistical package on a computer. The Student`s T Test was also used for the analysis.

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FIGURE 2.1 Top view diagram (not to scale) of the arrangement of filter paper disc (F) impregnated with cue-lure, placed in test cages. All flies within the outline of the 10 cm diameter circle (C) marked on white paper beneath each cage were recorded as those "attracted" while those feeding on the lure from the filter paper disc (diameter = 5.52 cm) were scored under "feeding". A glass plate (G) onto which the filter paper was glued prevented contamination of cages.

2.3. RESULTS

Male flies one and two days old did not show a significant response to cue-lure (mean response less than 5%). Feeding response on the third day was also low (15% for MNC and 2% for MIC) but significantly different between the two groups of flies ($t = 3.78, p = 0.05$) (Table 2.1). Mean values for the feeding response on consecutive days for the first five days differed significantly. However, there was extensivve overlap of the mean values from day five onwards.

Between three and six days, there is a transition period during which the responsiveness of flies to cue-lure (attraction and feeding) increased gradually but steadily with age, exceeding the 50% response mark after the fourth day. Maximum response was reached on and after day six at an average mean response of about 70% (close to the maximum response observed for the same concentration of the lure in a preliminary dose-response experiment).

For MNC flies seven to nineteen days old, differences between means of percent response on consecutive days were not significant (Table 2.1) except on day seven, between day twelve to fifteen and on day eighteen. This occured as a result of responses on days seven, thirteen, and fifteen being significantly higher than those on the preceeding and later days. Similary, for the MIC males, the responses of seven to fourteen days old flies were not significantly different on consecutive days except on days seven and thirteen (Table.2.1). Comparison of the means between the two treatments (groups) after the attainment of saturation did not show any significant

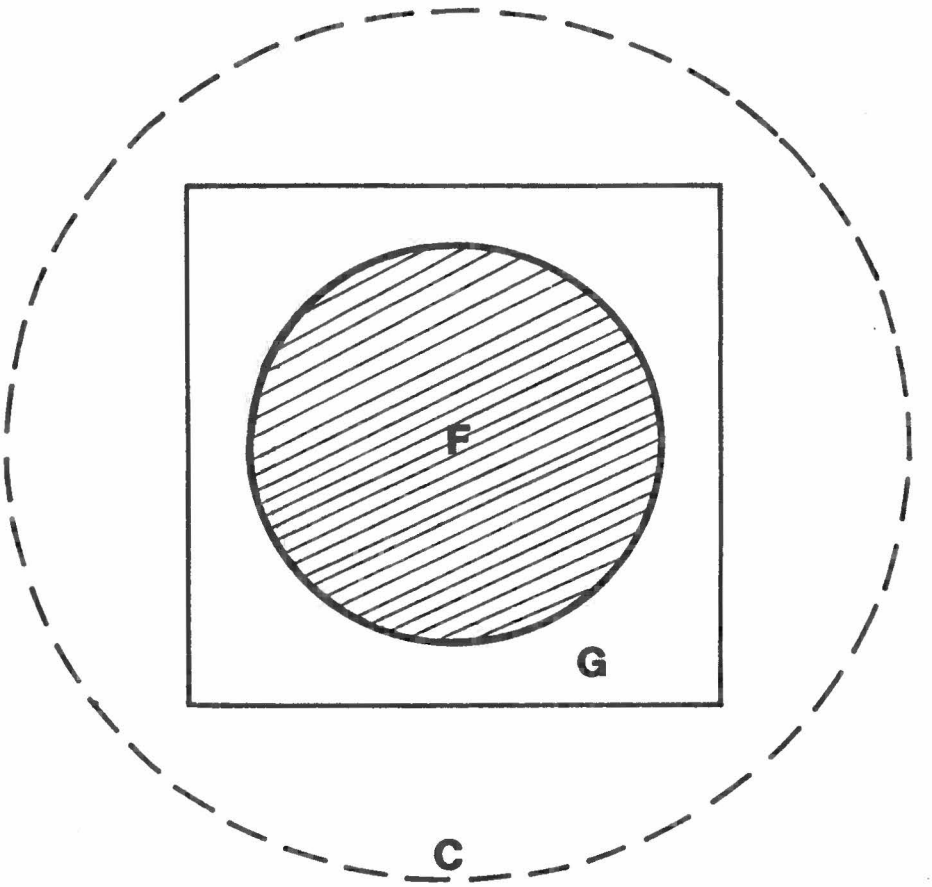


FIGURE 2.1

difference ($F_{1,13} = 0.13$, $p=0.05$).

For the MNC flies, the mean response recorded on days twenty and twenty one showed an apparent drop when compared to the mean peak response of about 70% over this period (Table 2.1, fig. 2.2).

Table 2.1 Mean percent response (± s.e.) of male D. tryoni attracted (A) and feeding (F) on cue-lure on each day after eclosion for flies from a normal colony (MNC) and isolated from females (MIC). Four replicates with 18-20 flies of each age per group.

	Mean percent response (± s.e.)	
Day	MNC: A(F)	MIC: A(F)
1	0 (0) g	0.21 ± 0.21 (0) g
2	3.37 ± 0.88 (3.38 ± 0.54) fg	0.42 ± 0.42 (0.21 ± 0.21) g
3	16.9 ± 3.17 (14.8 ± 3.34) f	2.23 ± 0.66 (2.03 ± 0.49) f
4	37.5 ± 8.03 (37.5 ± 8.03) e	30.9 ± 4.16 (30.2 ± 3.86) e
5	59.3 ± 1.80 (58.8 ± 1.75) bcd	60.0 ± 1.97 (58.9 ± 1.64) bcd
6	71.4 ± 7.68 (70.8 ± 7.85) abc	76.5 ± 4.28 (75.2 ± 4.06) abc
7	79.3 ± 0.82 (79.3 ± 0.82) ab	70.2 ± 2.86 (69.0 ± 2.88) ab
8	64.7 ± 2.46 (63.5 ± 2.26) bcd	76.1 ± 2.70 (73.3 ± 3.46) bcd
9	65.4 ± 5.50 (64.4 ± 5.37) bcd	68.0 ± 2.58 (66.8 ± 2.30) bcd
10	58.0 ± 1.99 (57.8 ± 2.02) bcd	70.5 ± 4.70 (69.9 ± 4.39) bcd
11	64.2 ± 7.38 (63.6 ± 7.47) bcd	62.7 ± 6.14 (61.0 ± 6.87) bcd
12	62.0 ± 5.04 (60.8 ± 4.68) a-d	80.8 ± 4.41 (80.8 ± 4.41) a-d
13	79.6 ± 2.26 (78.0 ± 2.84) ab	74.1 ± 1.95 (72.8 ± 1.82) ab
14	65.1 ± 3.51 (63.7 ± 3.40) bcd	62.9 ± 6.85 (62.2 ± 6.92) bcd
15	88.6 ± 1.77 (87.8 ± 2.05) a	
16	73.6 ± 6.64 (72.3 ± 6.58) a-d	
17	72.6 ± 3.23 (71.1 ± 3.37) a-d	
18	77.4 ± 3.20 (76.4 ± 3.18) ab	
19	63.4 ± 10.9 (62.1 ± 10.7) bcd	
20	50.5 ± 11.8 (49.2 ± 11.5) de	
21	52.2 ± 3.50 (51.1 ± 3.37) cde	

Table 2.1 (Cont.)

Means in the same column with the same letter are not significantly different (Duncan's Multiple Range Test; α=0.05, df=13, MSE=23.42).

FIGURE 2.2 Relationship between age and mean of percent response of pooled data (●) of males of D. tryoni from a normal colony (MNC) and those isolated from females (MIC) feeding on cue-lure from filter paper discs on each day during the first three weeks after eclosion. 70-80 flies from each group were used for each point. Only MNC flies were tested for age range 15-22 days post-eclosion (O). Vertical bars = ± 1.0 s.e.

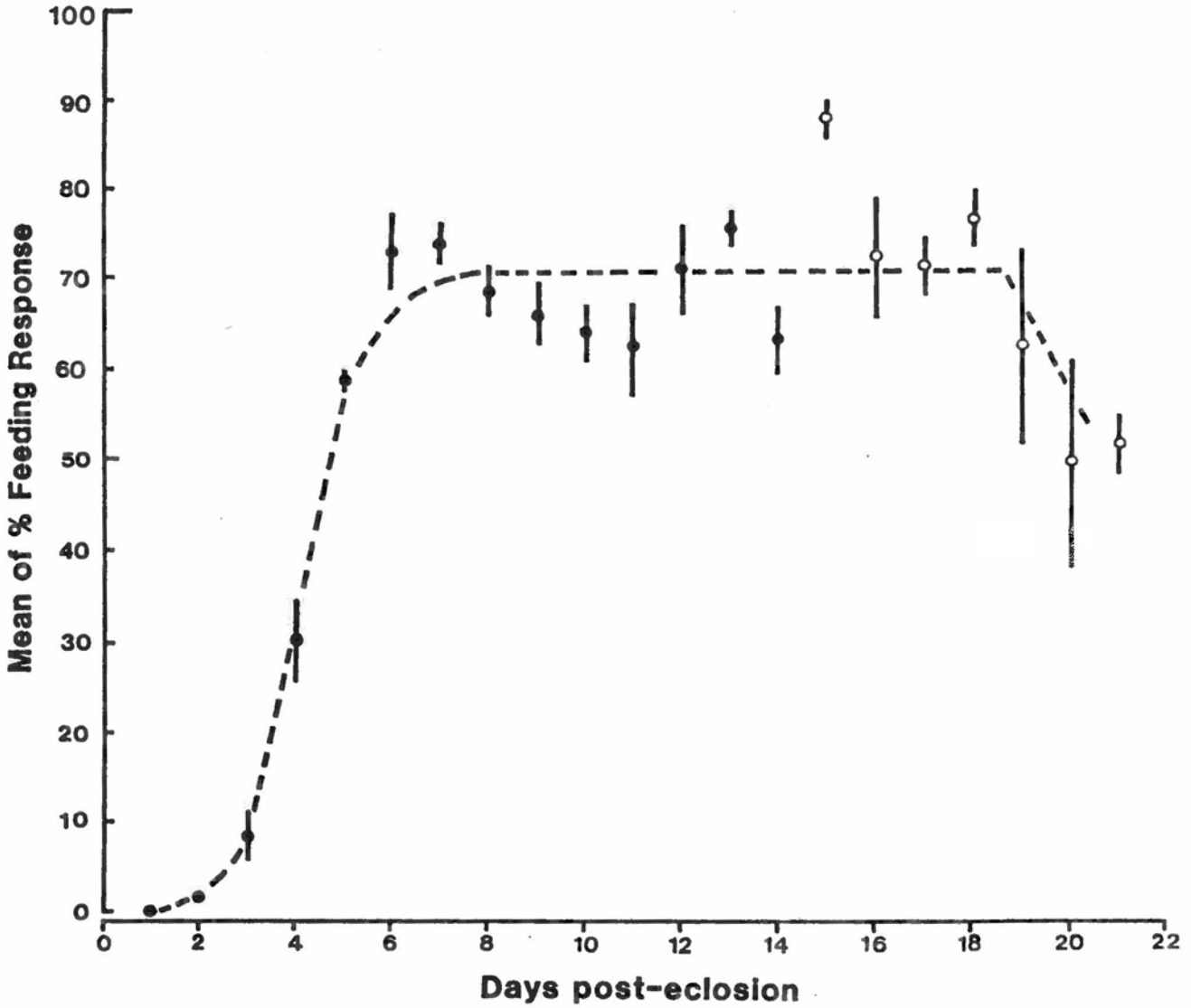

Mean of % Feeding Response
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
Days post-eclosion

FIGURE 2.2

2.4. DISCUSSION

Males of D. tryoni were unresponsive to cue-lure for the first two days after eclosion. The proportion of flies responding to the lure increased with age in the following 4 days attaining a maximum (70-80%) 7 days after eclosion and remaining at that level for at least 21 days. Feeding on the lure followed the same sigmoid developmental pattern as that of flies attracted, with almost all flies attracted ingesting the lure.

Responses on the third day showed a significant difference between the two groups of flies used. With regard to the response of the flies in the MNC group, the small number of males responding on the third day (up to a mean of 15% response), may have resulted from the unsynchronized eclosion of the adult flies which sometimes spreads over a period of more than twelve hours. The effect of this was found to be largely reduced by removing flies emerging within a period of six hours and keeping them in separate cages as was done for the second group of flies.

Mean percent response values on days thirteen and fifteen were significantly higher than those on either preceeding or later days. This may have arisen from variability in responsiveness of flies drawn from a later generation since the environmental conditions were the same. The drop in the mean percent response recorded for the first group of flies (MNC) on days nineteen, twenty, and twenty one probably is an indication of a decline in the responsiveness of the flies to the lure three weeks after emergence. A similar observation was recorded for flies twenty one to thirty days old which showed a lower

response level (55.80 ± 4.32, n = 8) compared to that (73.4 ± 5.15, n = 6) of those fourteen to twenty days old, in dose-response trials .

The pattern of response to cue-lure by males of D. tryoni seems to synchronize with both the development of their pheromone gland and sexual maturation. Fletcher (1969) reported a correlation between amount of pheromone in the pheromone gland reservoir and the onset of sexual maturity of flies. 40% of the flies started mating on day 6 when there was a large droplet of pheromone in the reservoir. The proportion increased to 98% on day 10 when 40% had reached stage 3 (pheromone reservoir two-thirds full). Mating started on day 5 followed by a sharp increase on days 6 and 7. Fitt (1981b) showed that, the development of the response of the male D. opiliae to methyl eugenol and the proportion of flies mating have sigmoid trends with slightly different levels. Mating and response to the lure did not occur during the first three days post-eclosion, after which a small percentage responded on the fourth day. The proportion of responsive and sexually mature flies increased gradually reaching a maximum of about 80% on the eighth day and remained at that level thereafter. The host-seeking behaviour of female Aedes aegypti also increases with age in the first 96 hours after eclosion, after which maximum responsiveness (ca. 80% of the females responding) is attained (Davis 1984). This correlated with electrophysiological data that showed a corresponding increase in the sensitivity of the antennal receptors that respond to lactic acid in this species. Male cockroaches (Periplaneta americana) become more responsive to the sex pheromone released by the female cockroaches with

increasing age (Schaller-Selzer 1984). The development of the response to the pheromone positively correlated with the development of neurones in the macroglomerulus in the deutocerebrum.

Almost all of the males of D. tryoni more than six days old, attracted to cue-lure of the concentration presented, ingested the lure. While the olfactory sensilla obviously play an important role in detection of the lure and the homing in of the flies on the source prior to feeding, part or the whole of the olfactory system (peripheral and central) is either still developing or gated out in teneral flies. This is clear from the observation that, even at close range within the confinement of the experimental cages, flies one and two days old did not show any oriented movements to the lure source.

The possibility of physiological changes taking place in the peripheral (olfactory as well as gustatory) sensory system, with the possible involvement of a humoral factor (a primer hormone) would be interesting to investigate. Foster (1967) reported that fed, allatectomised male Scatophaga stercoraria had a low tendency to mate with their female conspecifics, similar to starved males that had intact corpora allata. It was suggested that ingestion of food (prey), with adequate nutrients, led to the stimulation of the production and eventual release of juvenile hormone (gonadotrophic hormone), which may have released the male sexual behaviour from inhibition by acting on neural control centres in the thoracic ganglion. The corpora allata (on their own or under the influence of a neurosecretion from neurosecretory cells in the brain) have been shown to influence the sexual behaviour of a

number of orthopteroids and members of other families (review by Truman and Riddiford, 1974). Ecdysteroids have also been shown to affect mating behavoiur of some dipterans e.g. the house fly Musca domestica L., by regulating the production of the sex pheromone (Adams et al. 1984; Blomquist et al. 1984). Thus, initiation of the development of the pheromone gland, manifestation of mating activity, and the ingestion of cue-lure in male D. tryoni may be under a similar system of hormonal control. However, the functional significance of ingestion of the lure in relation to the development of sexual maturity is not clearly understood. It has been suggested that the lures to which tephritids respond may serve as markers for the rendezvous where mating takes place (Fitt 1981b). If there is a role played by cue-lure in the male-female interactions in D. tryoni, it may be complex and indirect. In field and laboratory colonies, the response of D. tryoni males to cue-lure and mating activity are influenced by the photoperiod, so that despite the similarity in the trend of development they are temporally segregated. While the former has been observed to occur early in the morning in summer extending to mid-morning in spring (Brieze-Stegeman et al. 1978), the latter takes place at dusk (Fletcher 1969; personal observations). Thus, it is possible that the lure has to mix with the gut contents after ingestion and may influence the behaviour after deposition in faeces, a concept which calls for further experimental work involving the female flies. In addition, Mass Spectrometry analysis of pheromone, from cue-lure fed D. tryoni, did not show the presence of any lure components (Bellas and Fletcher 1979).

Even for sexually mature male flies, not all the

flies responded to the cue-lure (an average of 70-80% at saturation of response). Similar limits of responses were presented in reports on several other species, cited above, for different behavioural activities. Various factors such as the physiological state, differences in individuals of a population stemming from their genetic make-up, or the effects of embryonic and post-emergence nutritional state (Wellington 1957; Foster 1967; Fitt 1981b) may have an immediate or an indirect influence on the response of the flies to the lure. However, the trend and the constancy of the maximum responses shown by populations of different species in different behaviours, may reflect on the extent to which these insects have adapted or learnt to associate the stimuli with some of their resources. The proportion of responsive individuals in a population may therefore depend on the period of co-evolution of the species and the chemical cue or its precursors in the environment (Metcalf et al. 1979). On the other hand, Brieze-Stegeman et al. (1978) reported that the arrival of D. cacuminatus and D. tryoni males in traps baited with methyl eugenol and cue-lure respectively was over a range of time, from early morning to early afternoon. Behavioural diversity in a population may simply be due to a proportion of the flies being unresponsive to the lure during the period of observation. These may respond at a different time of the day. Thus, responsiveness may be considered to show the proportion of the time spent by the flies of the sample population in responding to the lure, relative to other behaviours displayed during the trials.

III. MORPHOLOGICAL CLASSIFICATION AND TOPOGRAPHICAL DISTRIBUTION OF FRINGE LABELLAR GUSTATORY SENSILLA OF MALE *D. TRYONI*

3.1. INTRODUCTION

The sensory receptors involved in the feeding behaviour of some cyclorrhaphous Diptera have been shown to operate sequentially. Different types of olfactory, gustatory, and tactile sensilla are stimulated by vapours from, and contact with, food substances prior to ingestion. Once food material has been contacted, sensory input to the CNS from tarsal gustatory sensilla usually plays a part in eliciting extension of the proboscis. This usually brings the longer labellar gustatory sensilla into contact with the food material. The resulting sensory input leads to the CNS generating motor impulses necessary for the full extension of the probocsis, e.g. in Phormia regina (Dethier 1976). This exposes labellar gustatory sensilla of the shorter types to the food material which, if acceptable, results in an input to the CNS which then commands the opening of the labellar lobes to expose the interpseudotracheal papillae. Thus tarsal sensilla, longest, long, medium long, and short straight labellar sensilla and interpseudotracheal papillae are sequentially involved. A question arises as to the role of the bristle and very short bristle sensilla which, by virtue of their topography, might not contact food at this stage.

Males of the Queensland fruit fly Dacus tryoni are known to ingest cue-lure (4-(p-acetoxyphenyl)-2-butanone) (Monro and Richardson 1969; Chambers et al. 1972; Drew 1982; present work). In the present study the classical sequence of sensilla involvement has been seen to occur prior to ingestion of the lure. Therefore, the labellar gustatory sensilla of these flies would appear to have a role in controlling the ingestion of the

TABLE 3.1 Numbers of sensilla (±s.d.) of the six types on the labellar lobes of male *D. tryoni*; R, L for right and left labellar lobe respectively.

Specimen	1		2		3		4		5		Mean number of each type per labellum	
Labellum / Sensilla type	R	L	R	L	R	L	R	L	R	L	R (±s.d)	L (±s.d)
Short bristles (sb)	17	17	14	18	26	23	32	22*	26	29*	23.0±7.35	21.8±4.77
Bristles (b)	15	16	12	10*	15	9*	12	23*	14	15	13.6±1.52	14.6±5.60
Short straight (ssh)	43	40	46	46	37	37	44	43	37*	36*	41.4±4.16	40.4±4.16
Medium long (MH)	17	15	19	18	15	16	15	13*	22*	17	17.6±2.97	15.8±1.92
Long (lH)	22	22	14	16*	19	24*	20	17	16	18	18.2±3.19	19.4±3.44
Longest (LH)	11	11	11	11	11	11	11	10	12	11	11.2±0.45	10.8±0.45
TOTALS	125	121	116	119	123	120	134	128	127	126	125.0±6.52 range; 116-134	122.8±3.96 119-128

* For each column, examples where a decrease in number of sensilla of one type and an almost equal increase in another type were observed.

Instrumental errors in length measurement are in parenthesis.

3.3.1.1. Very short bristles (sb): $2.0 < l \leq 10$ ($\pm$ 2.5) µm

These were observed to occur along the marginal strip spanning the edge of the oral disc singly or, in groups of two or three, alternating with the other sensilla (mostly short straight sensilla) in the resulting marginal row (figs.3.1A, 3.1B, and 3.2). A small number of these sensilla are on the posterior part of each labellar lobe and others on and alongside the lateral process of the furca.

The number of "sb" sensilla on the right and left labellar lobes for five specimens are shown on Table 3.1. Variation in numbers of these sensilla may be, to an extent, attributable to their very small size making some of them unobservable in the light microscope.

3.3.1.2. Bristle sensilla (b): $10 < l \leq 30$ ($\pm$ 5.0) µm

Bristle sensilla are located in the middle region of each labellar lobe above the upper row of the longest (LH) sensilla between the sensilla LH6 and LH9, around the point where the lateral process of the furca terminates. Also, a small number of these sensilla were observed on the lower part of the labellar lobe, below the lower row of the longest sensilla between LH2 and LH8 and among the other sensilla in the marginal row (figs. 3.1A,B and 3.2).

In addition to the above distribution, the presence of bristle sensilla elsewhere on the labellum was rather random, so that their positions of occurence on all the labella observed were not consistent. Some were observed at points that had otherwise no sensilla, on some labella, or at positions

lure. The surest way to test this is by electrophysiology. Prior to a study of the electrophysiology, it is necessary to map the sensilla distribution, which itself requires classification of the sensilla on the basis of their morphology.

Previous studies on the morphology, ultrastructure, and electrophysiology of the labellar gustatory receptors of some cyclorraphous Diptera include those on: Ph. regina (Dethier 1955; Larsen 1962a,b; Wilczek 1967); Protophormia terraenovae (Wieczorek 1980; Wieczorek and Koppl 1982), C. erythrocephala (vicina) (den Otter 1971; Maes and Vedder 1978) and D. melanogaster (Isono and Kikuchi 1974; Falk et al. 1976; Fujishiro et al. 1984). However, electrophysiological studies on the labellar gustatory sensilla have only been reported in two tephritid species: C. capitata (Gothilf et al. 1971; Angioy et al. 1978, 1978a) and D. oleae (Angioy et al. 1978, 1978b). No other reports have been found on mapping and/or electrophysiological characterization of the labellar sensilla in these or other tephritid species including the Queensland fruit fly D. tryoni, apart from a preliminary study by Don Smith and Martin Rice. Therefore to establish such information for D. tryoni, four main aspects have been investigated:

1. Classification of fringe labellar gustatory sensilla into different morphological types.
2. Enumeration of sensilla of each morphological type.
3. Identification of patterns of distribution of the sensilla.
4. Checking for bilateral symmetry between right and left labellar lobes in 1-3.
5. Attempting correlation of the size of the labella with the

3.2 MATERIALS AND METHODS

Light microscopy

Male Queensland fruit flies D. tryoni, 1-21 days old were taken from a culture kept for twelve generations in the laboratory. These were originally propagated from pupae taken from an old culture at the Department of Primary Industries, Indooroopilly. Flies were bred and maintained at about 26 °C, 70% R.H. and a light regime of 14L:10D.

Probosci were excised and the oral disc bearing the labellar lobes were macerated in 10% KOH, washed and dehydrated through a series of increasing concentrations of ethanol prior to mounting on slides. For some specimens, the right and left lobes were separated and mounted on slides with Puri's medium without further processing. Preparations from either method were observed under a phase-contrast microscope at x200 and x450.

For the study on possible correlation between size of the labellum and both the length and total number of gustatory sensilla, two groups of flies were used. Measurements on the large (N) adult flies that eclosed from pupae of larvae reared at approximately the normal density (2 larvae/gm of medium) were compared to those of smaller adults from pupae of larvae reared at about 2.5 - 4 times the normal density. Fresh (wet) weight of pupae was taken four days after pupation, that of adults just after eclosion. The width (α) and breadth (β) of the labellar lobes were good indicators of the body size of the fly.

The sensilla were mapped on paper using a scale on a grid in one of the eyepieces of the microscope and each sensillum was numbered.

3.3 RESULTS: Types and distribution of labellar sensilla

Using the terminology of Graham-Smith (1930) for *C. erythrocephala*, the labellar lobes of male *D. tryoni* are on the average 1.0 mm in length and about 250-300 µm wide at the middle region between the marginal strip and the termination of the lateral process of the furca in the relaxed position (figs. 3; 3.1A, B; and 3.2). The labellar lobes are slightly narrower at the anterior, which has a lower density of sensilla.

The average of the total number of fringe labellar taste sensilla per male fly was calculated as Nt = 248 $\pm$ 10, (range = 235-262, n = 5). Data for the right and left labellar lobes are in Table 3.1.

3.3.1 MORPHOLOGICAL TYPES OF THE LABELLAR SENSILLA

Dimensions, distribution and numbers

Measurements showed lengths of labellar sensilla ranging from less than 5.0 µm for the short marginal bristle sensilla to 285.0 µm for the longest sensilla on the posterior half of the labellum. Similarly, the diameters of the basal sockets were between 4.0 µm and 15.0 µm . However, there was much overlap of the values of the diameter for most of the sensilla of different types and the ratio of the socket to shaft length was between 1.0 (for the short bristles) and 0.05 for larger sensilla of the other types.

The sensilla were classified into six types on the basis of the lengths of their shafts. For each type, the length (l) is shown to occur within a range of two limits: $x \leq l \leq y$ meaning that l is greater than or equal to x and less or equal to y. Similarly, $x < l = l$ greater than x, and $l < y = l$ less than y.

FIGURE 3

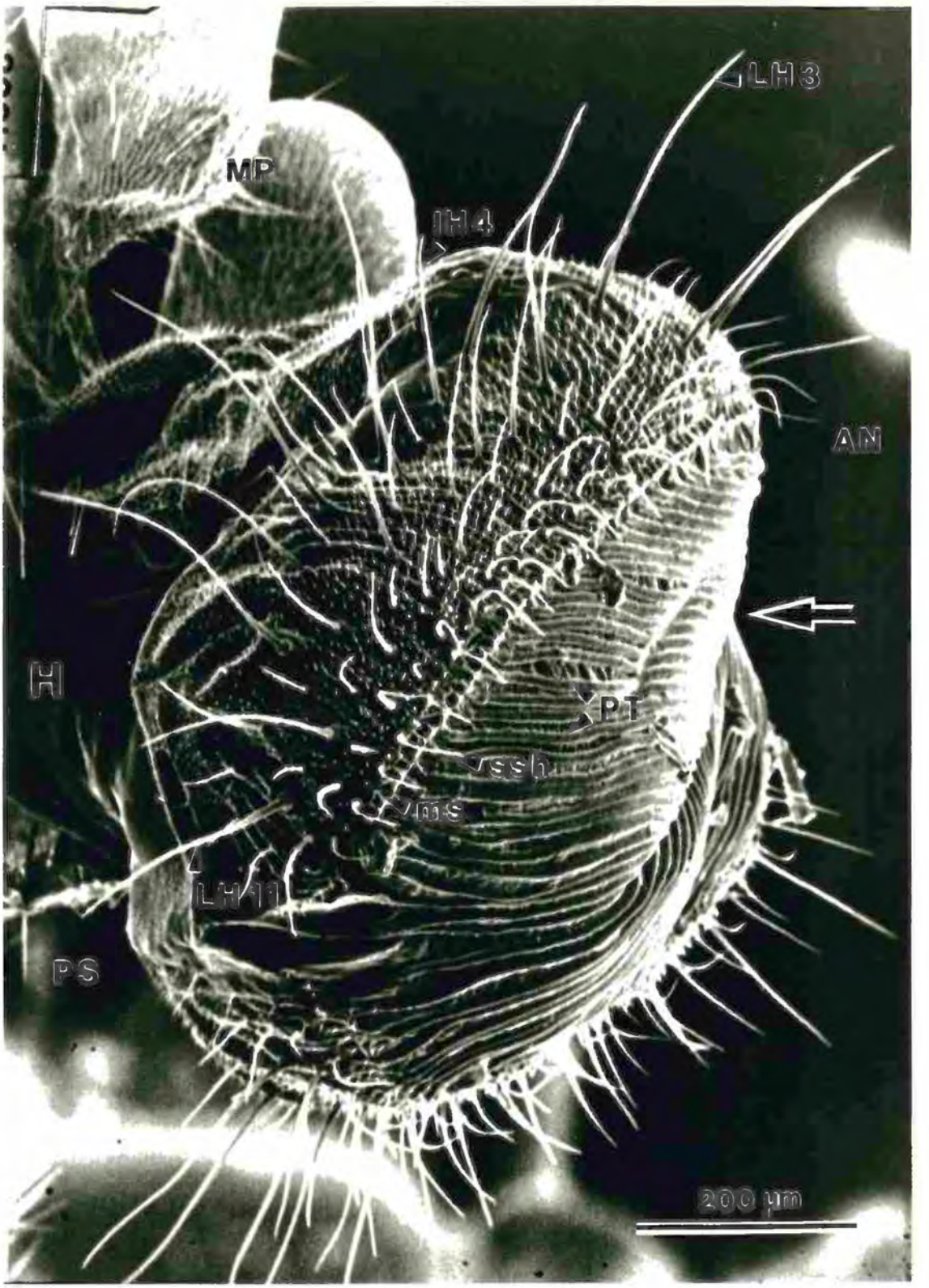
Scanning electron micrograph of the haustellum (H) and labellar lobes of a male D. tryoni. Distribution of sensilla of different lengths on the right labellar lobe is shown. The pseudotrachea (PT) originating from the posterior end (PS) of the oral surface converge into the oral opening (arrow) on the anterior (AN). Some numbered examples of sensilla are shown: longest sensilla (LH); short straight sensilla (ssh), with their tips curved towards the oral surface all along the marginal strip (ms),and a long sensillum (IH). MP maxillary palps.

length and total number of labellar sensilla.

The function of the attraction to and consumption of lures by males of some tephritid species is not certain. However, there are proposals concerning the co-evolution of these species with plants that produce some lure components and concerning the possible role of lures in intraspecific communication (summary by Brieze-Stegeman et al. 1978; Metcalf et al. 1979; Fitt 1981b). A deeper understanding of the neurophysiological basis of lure tasting may help to elucidate the significance of lures in the biology of these species and shed light on the evolution of lure tasting in male tephritids (Metcalf et al. 1979).

This chapter of the thesis presents the results of light microscopic mapping and morphological characterization of the fringe labellar gustatory sensilla of males of D. tryoni. Sensilla were classified into six types on the basis of their lengths. The numbers and pattern of distribution of the sensilla types on D. tryoni are compared with the patterns of sensilla on labella of other species.

Classification of labellar gustatory sensilla into morphological types, plus maps of their distribution, are useful in two ways: firstly, in enabling electrophysiological responses to be associated with specific sensilla; secondly in enabling comparative studies between species to support arguments regarding adaptation and speciation. More detail on the morphology of the labellar sensilla by scanning electron microscopy and a study on their ultrastructure by transmission electron microscopy (TEM) are presented in chapter 5 of this thesis.


LH3
MP
IH4
AN
H
PT
vssh
vms
LH1
PS
200 µm

where sensilla of another type were generally found.

This type of sensillum was one of the four whose mean number of sensilla was less than 20 on each labellar lobe (Table 3.1).

3.3.1.3. Short straight sensilla (ssh): $30 < l \leq 65$ ($\pm$ 5.0) µm

These sensilla form an upper row above the upper row of longest sensilla which is continous on the anterior part of the labellum and interrupted by the termination of the lateral process of the furca in the middle region (figs. 3.1A, B and 3.2). On the posterior half, a few of these short straight sensilla together with bristle sensilla form a row with relatively wide separations between the sensilla.

Sensilla of "ssh" type were also observed to form a large proportion (about 32%) of the sensilla in the marginal row, most of them on the anterior of the labellum. However, on the posterior half of the labellum, their number seems to have been lessened by the presence of the medium long sensilla in the marginal row in addition to these three types (sb, b,and ssh).

A number of these sensilla are also present in the middle region between the marginal row and the lower row of longest sensilla on the area of the labellum between LH2 and LH8. These form a sort of row with the short straight sensilla at positions above the spaces between sensilla in the marginal row.

The "ssh" sensilla had the highest number on each of the labella studied (Table 3.1). Numbers of sensilla on the labellar lobes of each haustellum were almost equal (equal on two specimens, differing with one sensillum on the labella of two flies, and a difference of three for the remaining one).

FIGURE 3.1A A map of the distribution of different morphological types of labellar taste sensilla on the left labellar lobe of a male D. tryoni. Sensilla were divided into six types with regard to the length of the sensillum shaft as represented with symbols denoting the basal sockets:

- • very short bristle sensilla (sb); $2.0 < l \leq 10$ µm
- o bristle sensilla (b); $10.0 < l \leq 30.0$ µm
- ◐ short straight sensilla (ssh); $30.0 < l \leq 65.0$ µm
- ⊗ medium long sensilla (MH); $65.0 < l \leq 90.0$ µm
- ◉ long sensilla (lH); $90.0 < l < 200$ µm
- ◎ longest sensilla (L); $200 \leq l \leq 290$ µm

Medium long and long sensilla are sub-types of intermediate (iH) sensilla. LF lateral process of furca, LS lateral sclerite, M mentum.

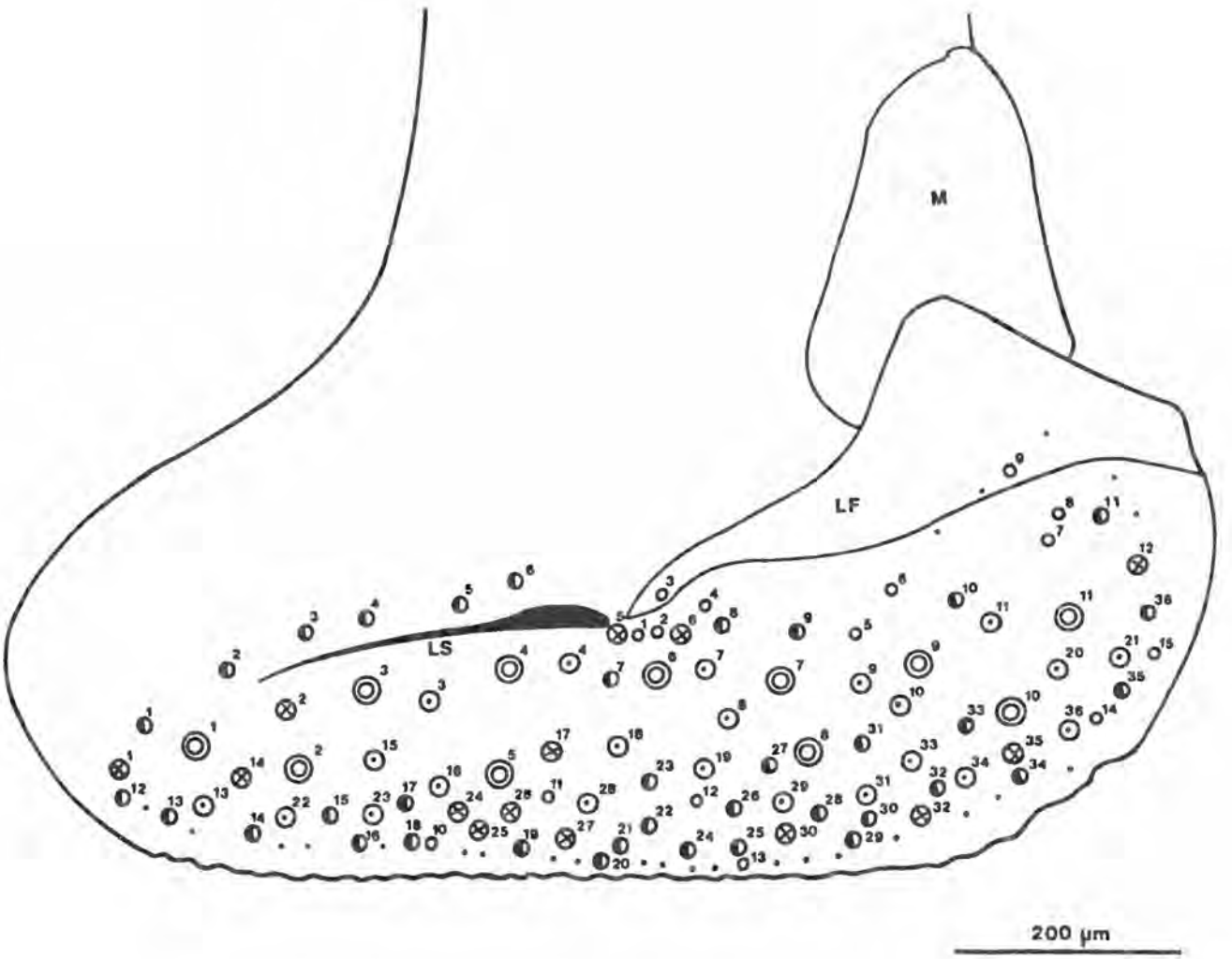


FIGURE 3.1A

FIGURE 3.1B A map of distribution of different types of labellar taste sensilla on the right lobe from the same male D. tryoni as for fig. 3.1A . The same notation applies.

★ Indicates an extra longest sensillum not observed on other labellar.

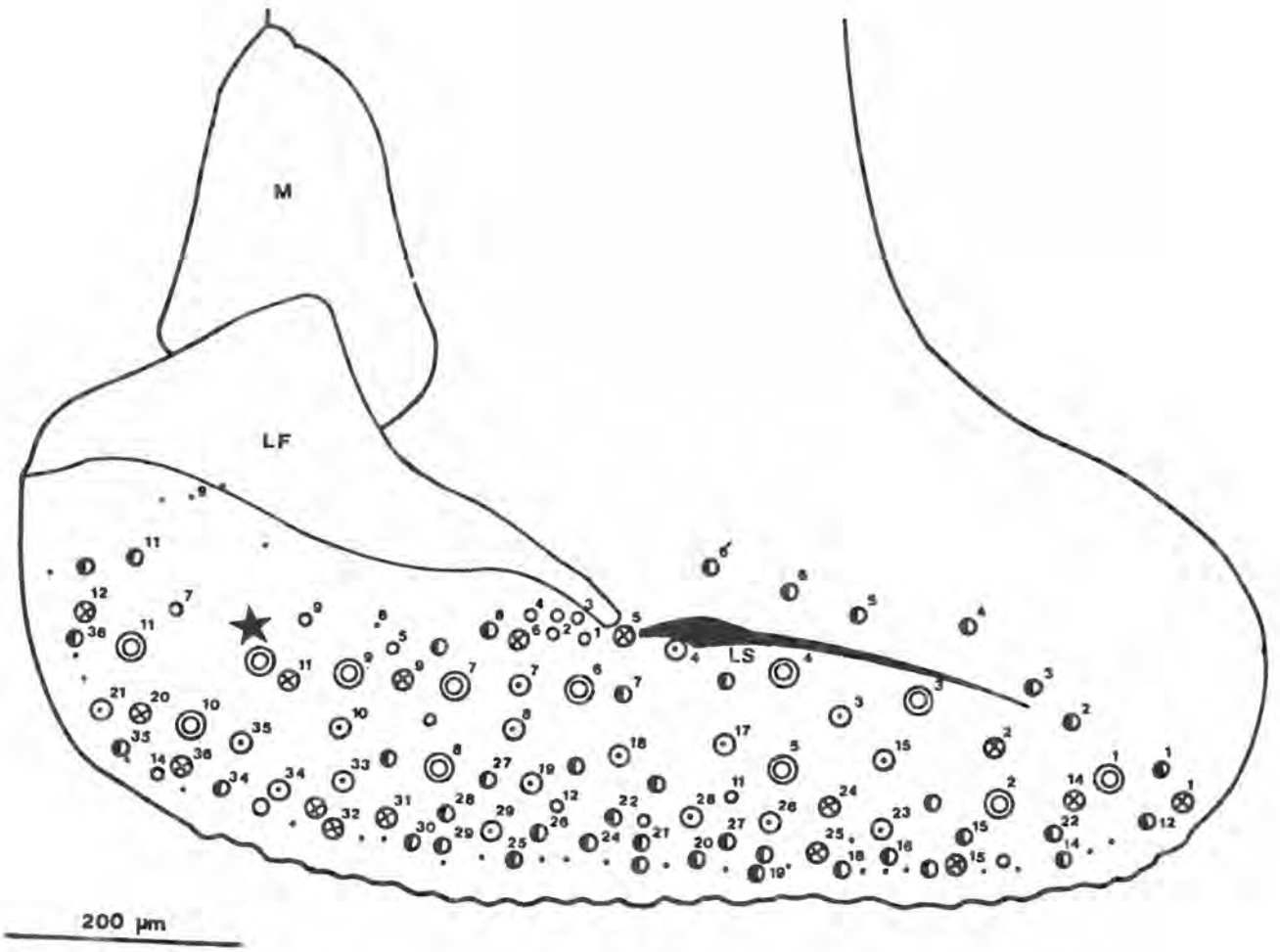


FIGURE 3.1B

FIGURE 3.2 A diagram of a left labellar lobe of a male D. tryoni representing relative dimensions and positions of different morphological types of fringe labellar taste sensilla. Longest sensilla (LH) are denoted by numbers as on the maps above, for ease of location. Scale bar = 172.0 μm .

FIGURE 3.2

However, the range of variation between different specimens was within that observed for the other types of sensilla discussed above.

3.3.1.4. Intermediate sensilla (iH): 65.0 < 1 < 200 (± 5.0) µm

Two types of sensilla were included in this one major group as sub-types, because of the large overlap in dimensions of the two types. Such overlap contributes to the variability in position of these two types of sensilla on the labellar lobes, however, more consistency in location is achieved when they are considered as one group.

(A) Medium long sensilla (MH): 65.0 < 1 ≤ 90.0 (± 5.0) µm

A sensillum of this sub-type seems to be associated with each longest sensillum, in the upper row of the latter. A medium long sensillum is located at the start (anterior of the labellum) and end of the row, one between each pair of longest sensilla LH1 and LH3, LH1 and LH2, LH2 and LH5, and between each of the consecutive longest sensilla LH7 to LH11 on the posterior of each labellum (figs. 3.1A, B; 3.2).

Alongside the marginal strip these sensilla are present on the part of the labellar lobe below the line joining LH1 and LH5 while on the posterior part they occur in the region below LH8.

In the middle region of the labellar lobe (below a line joining LH5 and LH8), a number of these sensilla seem to form a row above the marginal row, amongst the short straight sensilla and the long (lH) sensilla. Two sensilla of this group occur above LH6, near the lateral process of the furca (figs. 3.1A,B and 3.2).

The mean numbers of these sensilla are given in Table 3.1. Variability in numbers was largely because of the overlap of the dimensions of the sensilla in the two sub-types.

(B) Long sensilla (lH): 90.0 < l < 200 (± 5.0) μm

Like the first sub-type, these sensilla are associated with the longest sensilla. Each longest sensillum has two to three long sensilla in its proximity. Some of these sensilla are also located between two longest sensilla in the same row. This arrangement is most evident on the posterior half of the labellar lobe (figs. 3.1A, B).

The distribution of long sensilla appears to be restricted to the upper and lower rows of longest sensilla and, in the middle region of the labellar lobe, between these two rows, below LH6. Long sensilla in the lower row of longest sensilla are positioned directly below longest sensilla in the upper row (RW IV in figs. 3.2A, B). There is more consistency in the bilateral symmetry of this distribution, in a pair of labellar lobes of a given specimen, than those of the other sensilla described above.

The interlabella variation in the number of long sensilla on each labellum was of the same magnitude as for the other sensilla types. However, these differences between labella were of relatively few sensilla reflecting on those of their number on right and left labellar lobes (Table 3.1).

3.3.1.5. Longest sensilla (LH): 200 ≤ l < 290 (± 5.0) μm

These are long, stout sensilla, some of which appear heavily tanned when viewed with the light microscope. There were

eleven longest sensilla on eight labellar lobes out of the ten for five specimens. For the remaining two, 10 and 12 longest sensilla were recorded on the left lobe of specimen (4) and the right lobe of (5), respectively (Table 3.1).

Variability in position of the longest sensilla on the labellar lobes was also minimal except in specimen (5), where an extra sensillum was observed on a position normally occupied by a short straight sensillum, between LH9 and LH11 (labelled with a star in fig. 3.1B).

The pattern of distribution is in two, almost parallel, rows (rows III and IV in figs. 3.2A, 3.2B) with sensilla LH3, LH4, LH6, LH7, LH9, and LH11 in the upper row (IV) and the rest of the longest sensilla (LH1, LH2, LH5, LH8, and LH10) in the lower row (IIIB). Other sensilla alternate with the longest sensilla along the rows. The distribution is such that, between the foremost sensillum LH1 and the middle longest one (LH6), there is a pair of longest sensilla (LH3 and 4) which are directly above the other two (LH2 and LH5) in the lower row. Similarly, between LH6 and the posterior-most longest sensillum (LH11), LH7 and LH9 are above LH8 and LH10 respectively. Thus, on the anterior half of the labellar lobe, there are three longest sensilla in the lower row and two in the upper one while it is <u>vice versa</u> on the posterior half. The arrangement will vary slightly in cases where extra sensilla occur (fig. 3.1B).

All the longest sensilla in the upper row (IV), except LH6, are on the average longer than those of the same type in row IIIB. Longest sensilla LH7, LH9, and LH11 on the posterior part of the labellum are generally the longest of this

type, setting the upper limit of the length measured (Table 1 in appendix I). Similarly, the diameter of basal sockets and sensillar shafts of these sensilla were the largest. Because of their relatively greater length (over 200 um), it is anticipated that these sensilla would be the first to contact food substances when a fly extends its unopened proboscis (fig. 3.2 and 3.4). Also, these provide the widest circle of sensory input from the opened labellum.

3.3.2 Zonal arrangement of fringe labellar sensilla

From the above description of the labellar sensilla and the corresponding maps, different morphological types of sensilla are seen to be abundant on certain parts of the lateral fold. With respect to zonal distribution, three major zones were recognised in this work, each zone having one, two, or more rows of sensilla arranged in contour-like formation, approximately parallel to the marginal strip of the oral disc (see figs. 3.2A, B).

Zone A

Is defined as the upper part of the lateral fold above the upper row of the longest sensilla (figs. 3.2A, B). Only one row of sensilla was included in this zone, which is the anteriorly arched row (RW V) of six or more short straight sensilla, which is interrupted by the lateral process of the furca. A group of other sensilla (intermediate and bristles) are present at this point but the row continues posteriorly with a small number of the short straight sensilla and some bristles.

Halfway along the posterior upper part of the labellar lobe in zone A, is a short branch of row V with sparsely distributed short bristle sensilla (sb) and bristle

FIGURE 3.2A

A diagram of a typical left labellar lobe of a male D. tryoni showing three zones A, B, and C each recognised to contain a large proportion of certain types of the taste sensilla. Zones A and C have a large proportion of short straight sensilla, bristle and very short bristle sensilla and most of the intermediate ones. Dashed lines (-----) indicate rows (RW) of sensilla, the major ones (RW I, IIIA, IIIB, IV, and V) run almost parallel to each other along the side of the labellar lobe. Stars along the marginal strip mark points where pairs of short straight sensilla in two adjacent rows (RW's I and cII) were observed to occur. Scale bar = 200.0 μm .

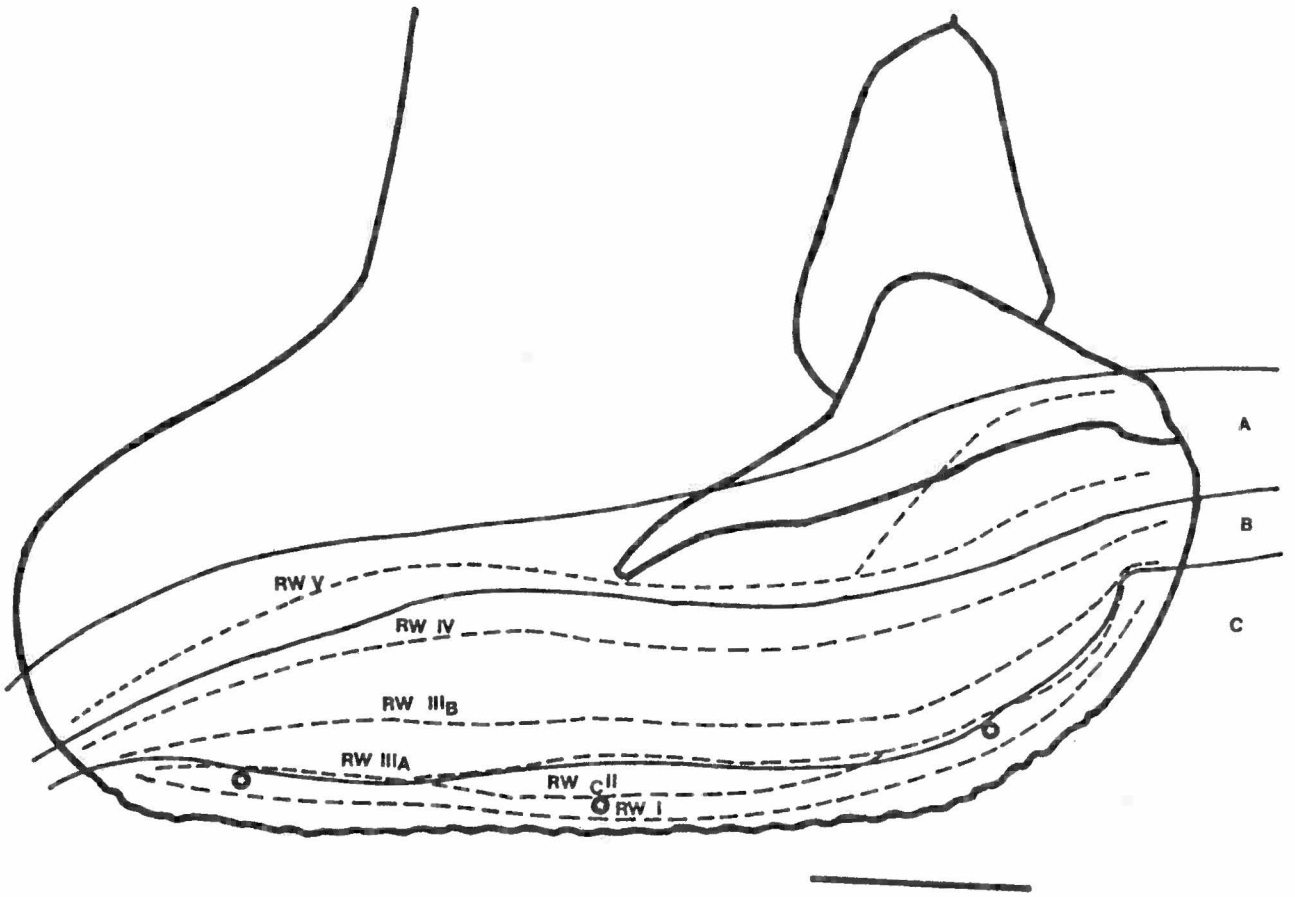


FIGURE 3.2A

FIGURE 3.2B A diagram of a typical right labellar lobe from the same male D. tryoni as for fig. 3.2A. The three zones and rows representing distribution of the labellar taste sensilla are similar to those of the left labellum above. Scale bar = 200.0 µm.

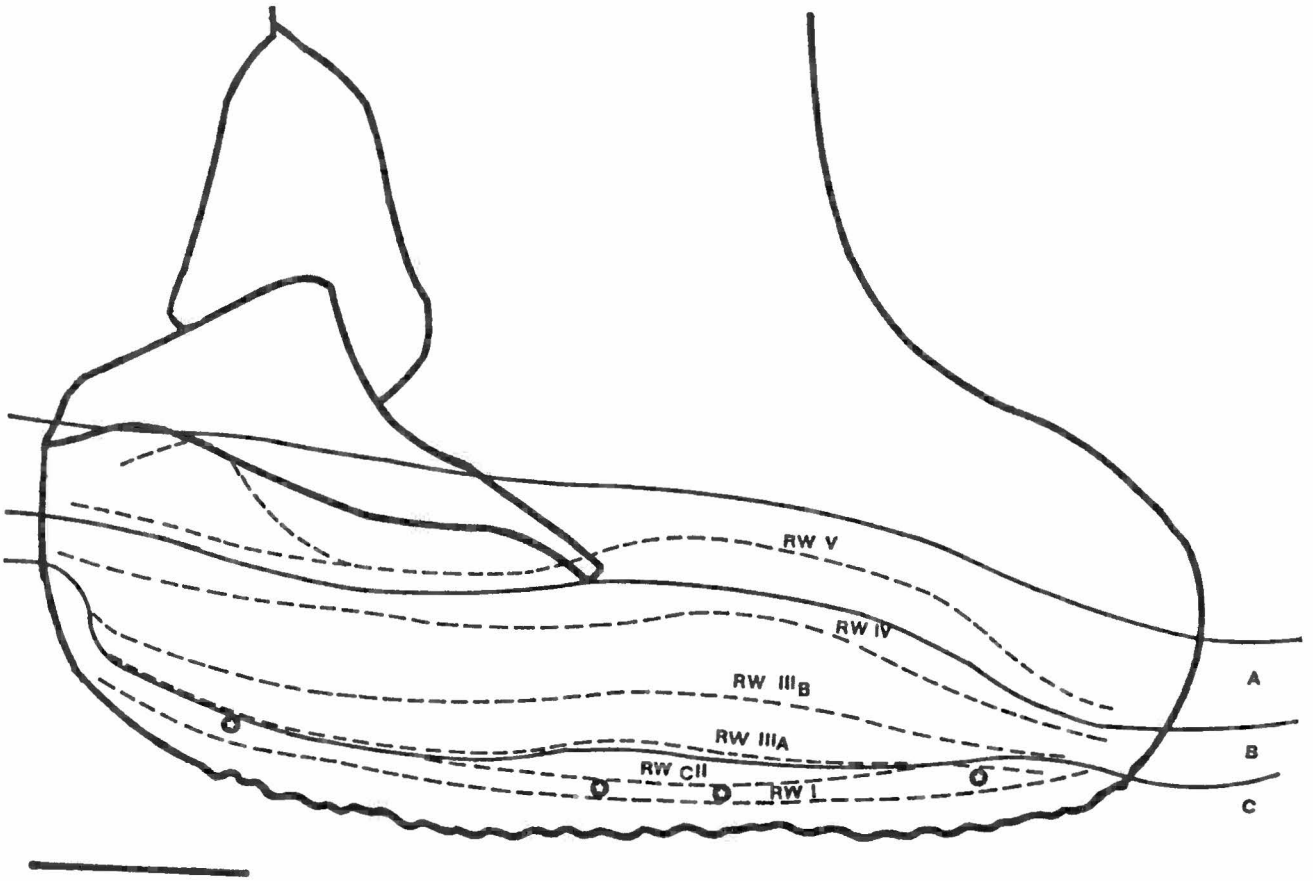


FIGURE 3.2B

sensilla (b).

Each of the short straight sensilla on the anterior part is located above each of the longest sensilla in row IV (in zone B), and one or two of the former are in the spaces above and between two longest sensilla. On the posterior half of the labellum the sensilla were situated directly above the longest sensilla.

Altogether, there were eleven to thirteen short straight sensilla in this zone (about 54% of the average number of sensilla in this row).

Zone B

This encompasses two major rows of sensilla (rows IV and IIIB) which are characterized by the presence of the longest sensilla in different proportions, as outlined previously. A third row, on the lower boundary of this zone, is also included. Row IV is of the longest sensilla alternating with the intermediate sensilla in approximately equal numbers. Three to four long sensilla are present between the two rows on the posterior half of the labellum.

Row IV has 7 longest sensilla out of the 11 on each labellar lobe. The rest of them (mostly four in number) are in the lower row (IIIB) in this zone.

Row IIIB also has alternating longest and intermediate sensilla. However, the number of the longest sensilla is about half that of the long sensilla. Thus, a maximum of three intermediate sensilla are present between consecutive longest sensilla in this row.

Because these two rows are almost parallel, the

positional relationship of sensilla in the two rows is such that, either some of the longest sensilla or the intermediate sensilla in row IIIB are directly below sensilla of the same or the other type in row IV above.

Row IIIA falls on the boundary between zones B and C and may be considered as a transition zone along which a large number of sensilla are of the intermediate type, punctuated by the presence of short straight sensilla. The middle region of the labellum on this contour has no longest sensilla (on row IIIB), contrary to what would be expected from the pattern of arrangement of longest sensilla in row IV. This imparts an appearance of high density of the intermediate and short straight sensilla on this part (fig. 3.2). Several bristle sensilla (nos. 12 and 11) were consistently observed in this row below the long sensilla (1H)19 and (1H)17.

Zone C

On the anterior and posterior parts of the labellar lobe, this zone includes a single row (I) having sensilla of different dimensions with a preponderance of the shorter ones.

The dominant types are the short straight sensilla which occur alternately with 1-3 short bristle sensilla. At various points along the marginal strip, bristle sensilla occur very close to the basal socket of some short straight sensilla. On the anterior and posterior parts, sensilla in row IIIA alternate with those in the marginal row. Pairs of sensilla were observed at points where two sensilla in the two rows occurred simultaneously, one directly above the other (figs. 3.1A,B; 3.2A,B and 3.2).

In the middle region of each labellar lobe, row IIIA appears to have a short branch in this zone (denoted RW cII; c for the intercalary of IIIA). Row cII has short straight sensilla as well as a few intermediate ones (figs. 3.1A,B).

3.3.3 Variability in length of the sensilla

Measurements of lengths of the labellar sensilla are recorded in appendix I (Tables 1- 4). These show that, for the sensilla classified into different morphological types, a given sensillum at a given locality of the labellar lobes has variable length. Sensilla are either slightly shorter or longer than the mean value for each, but in some cases (bracketed values in tables), the length was outside the dimensional limits set for each type. The range for each type of sensillum may indicate the length that can be attained by a sensillum at a given location on the labellum during development. Further measurements of the length of longest gustatory sensilla on the labella of large (N) and small (S) flies showed that, eight of the eleven longest sensilla were significantly longer on large flies (Table 3.2). Thus gross size differences of flies might be a source of variation, in a sample of flies of different sizes. For the flies used for length measurements (appendix I), the longest and intermediate sensilla were the least variable in length.

3.3.4 Relationship between size of the labellum and total number of gustatory sensilla

Some parameters were measured for two groups of male D. tryoni: large (N) and small (S) flies (Table 3.3). Ratio of the number of males to females at eclosion was on average 1:1 for the "N" flies. About 8% of the males died just after

eclosion and an extra four male flies did not inflate their wings. For the "S" group, 60% of the flies that eclosed from pupae were males and only 2% of these died whereas 5% of the remaining ones did not extend their wings fully.

Table 3.2 Mean length (± s.d.) of longest labellar gustatory sensilla of "large" (N) and "small" (S) male flies of D. tryoni:

	Mean length (± s.d.) µm		
LH	"N" (n = 10)	"S" (n = 12)	t; df = 20
1	264.9 ± 4.6	234.6 ± 6.1	1.96 ns
2	217.6 ± 3.6	193.5 ± 5.0	4.02 *
3	272.2 ± 2.4	248.6 ± 8.6	2.65 *
4	259.4 ± 6.5	235.2 ± 7.2	2.64 *
5	201.5 ± 4.7	175.6 ± 10.6	2.26 *
6	223.1 ± 3.7	195.5 ± 7.2	3.45 *
7	257.6 ± 4.2	226.9 ± 5.4	4.61 *
8	213.0 ± 3.6	185.8 ± 5.3	4.34 *
9	265.3 ± 6.0	251.2 ± 6.8	1.61 ns
10	223.9 ± 5.9	203.8 ± 6.5	2.55 *
11	259.2 ± 5.9	246.7 ± 8.0	1.29 ns

ns not significant for means differing by ≤20 µm.

* significant, $p \leq 0.05$

The mean fresh (wet) weight of both pupae and emerging adult flies, the width (w) and breadth (B) (fig. 3.3D) of the labella were on average higher for "N" flies (Table 3.3). Mean total number of sensilla on both labellar lobes of each fly

Table 3.3 Means (± s.d.) of some parameters measured as indicators of body size of male D. tryoni and relationship between size of labella and total number of gustatory sensilla:-

Size category	Pupa wt.(mg)	tf wingspan (mm)	wingspan / bodylength	tFwt. at eclosion (mg)	Labellum size (µm) β	Labellum size (µm) ƒ	T/sensilla no.
Large (N)	15.2 ± 0.8 (140)	14.0 ± 0.5 (56)	2.0 ± 0.04 (56)	12.1 ± 1 (60)	934.1 ± 45 (60)	308.9 ± 39 (60)	282.4 ± 5.27* (5)
Small (S)	10.0 ± 0.8 (135)	13.0 ± 0.8 (69)	1.9 ± 0.1 (69)	8.2 ± 1.3 (69)	817.7 ± 53 (69)	260.0 ± 28 (69)	254.3 ± 25.9 (6)

n.b. Numbers are means (± s.d.) for the number of flies in parenthesis.
tf, teneral fly
Fwt, fresh wt.
β, breadth
ƒ, width
* Total number of sensilla significantly higher ($t = 2.48$, $p < .05$; $df = 9$) on labella of large flies.

FIGURE 3.3A-D

A. Regression of the total number of labellar gustatory sensilla of male D. tryoni on width (ψ) of the labellar lobes for the pooled data of large (Δ) and small ($\blacktriangle$) flies. The regression equation and the coefficient together with the width limits are shown.

B. Regression of total number of fringe labellar sensilla on the breadth (β) of the labellar lobes. Other symbols as in A.

C. The regression of the total number of labellar gustatory sensilla on the approximated total surface area ($= 2\psi\beta$) of the labellar lobes of each fly. This was taken to be a better representation of the two-dimensional (or planar) distribution of sensilla. Other symbols as in A and B.

D. Diagram of the left labellar lobe showing the breadth (β) and width (ψ) measured of the area shaded with lines) where gustatory sensilla occur.

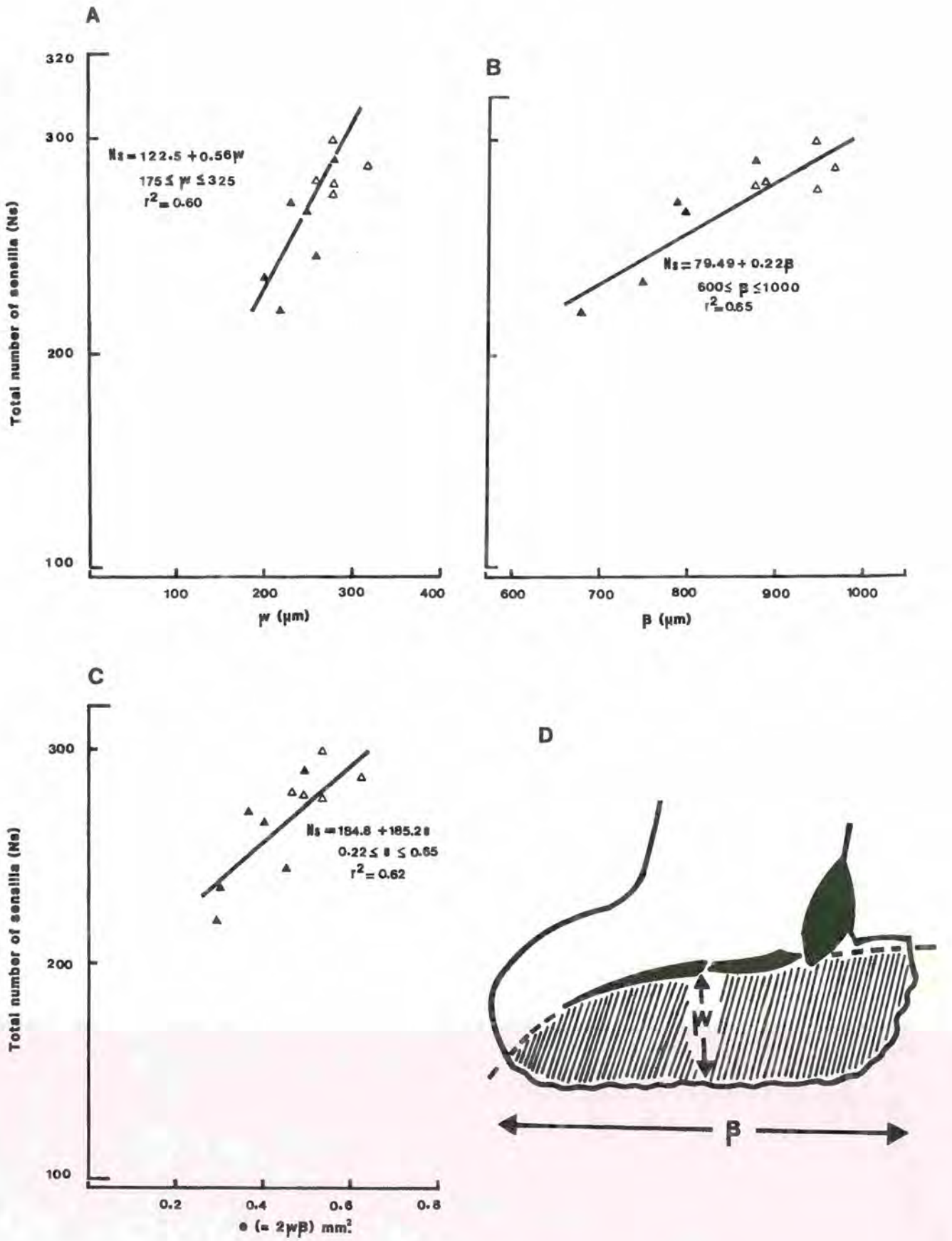

A
320
300
200
100
Ns = 122.5 + 0.56ψ
175 ≤ ψ ≤ 325
r² = 0.60
Total number of sensilla (Ns)
100
200
300
400
ψ (μm)
B
Ns = 79.49 + 0.22β
600 ≤ β ≤ 1000
r² = 0.65
600
700
800
900
1000
β (μm)
C
300
200
100
Ns = 184.8 + 185.2a
0.22 ≤ a ≤ 0.65
r² = 0.62
Total number of sensilla (Ns)
0.2
0.4
0.6
0.8
a (= 2ψβ) mm²
D
W
β

FIGURE 3.3

was significantly higher on labella of "N" flies than on those of small flies (Table 3.3).

From the regression lines in figs. 3.3A,B and C of the pooled data of five "N" and six "S" flies for which the sensilla were counted; the total number of sensilla was positively correlated to both the width ($r = 0.78$) and breadth ($r = 0.81$). The intermediate correlation coefficient ($r = 0.79$) between the total number of sensilla and the approximated total surface area ($= 2\pi B$) of the region of the labellar lobes bearing gustatory sensilla (figs. 3.3C,D) was considered to be more representative of the effect that size may have on the number of sensilla, depending on the compactness of epidermal cells in the epidermis during differentiation and morphogenesis of the sensilla. Longest sensilla did not increase in number so that the increase was in the shorter sensilla groups.

It is postulated that increase in the total number of labellar gustatory sensilla with increase in the surface area of the labellum is likely to occur within a range between a critical lower size limit and an upper one ($0.22 \leq e \leq 0.65$ sq. mm) about a modal size characteristic of individuals of the species. This implies that the total number of sensilla developed has also an upper and lower limit, i.e. $220 \leq Ns \leq 300$ for males of D. tryoni (fig. 3.3C). From these results, the average total number of sensilla in Table 3.1 seems to have been from flies of variable size, close to the average.

3.4 DISCUSSION ON TYPES, NUMBERS AND DISTRIBUTION OF FRINGE LABELLAR SENSILLA

The distribution of the labellar sensilla of the male D. tryoni has an arrangement that resembles that of C. vicina, especially on the anterior half of the labellum (fig. 1 of Maes and Vedder 1978). The arrangement of sensilla in almost parallel rows and the presence of a large proportion of sensilla of certain types in certain rows is also similar to that of Ph. regina (Wilczek 1967) and P. terraenovae (Wieczorek 1980). However, for the latter two species, the longest sensilla are in a single row whereas those of the males of D. tryoni are in two rows.

Numbers of sensilla in some of the types (especially the longest and the long sensilla) fall in the range of those observed for similar types (largest and large) in the other species: 9 longest sensilla in C. vicina (Maes and Vedder 1978), 11 for Ph. regina (Wilczek 1967), and a mean of 10 for P. terraenovae (Wieczorek 1980). Though homologous labellar sensilla on individuals of different species of flies have been described on the basis of relative lengths e.g. longest and long, the actual dimensions vary from one species to the other. Sensilla on flies of some of the species with relatively larger body size are longer than those on smaller flies of other species.

The average of the total number of sensilla on each labellar lobe and for both lobes of a fly for D. tryoni males are close to those reported for some other species (Table 3.4). Sturkow (1967) (cited in Maes and Vedder 1978) had observed a mean of 128.0 $\pm$ 1.94(s.e.) per labellar lobe of C. vicina flies

Table 3.4 Comparison of numbers of fringe labellar gustatory sensilla between males of D. tryoni and other dipteran species. R, L denote right and left labellar lobes respectively.

Insect	Mean number of sensilla per lobe				Mean of total number on R and L per fly		Reference
	Male		Female		Male	Female	
	R	L	R	L			
D. tryoni	125.0 ± 6.52	122.8 ± 3.96	-	-	248.2 ± 10.18	-	Present study.
P. regina	123.0	122.3	129.0	128.65	245.3	257.65	Wilczek (1967)
L. cuprina	-	-	104.2 ± 2.71	104.3 ± 1.51	-	208.5 ± 3.89	Sorby-Adams (1981)
C. vicina	-	-	131.8 ± 1.63*			263.7 ± 0.20	Maes & Vedder (1978, table. 1)
P. terraenovae	100*				200?	-	Wieczorek (1980)
D. melanogaster**	38.3 ± 2.6				79		Falk et al. (1976)
G. austeni	4	4	4	4	8	8	Rice et al. (1973)

* Labellar lobe not specified.

? Total number obtained by doubling the mean number of sensilla reported assuming it to be the same for right and left labellar lobes.

** Sex of flies not specified.

(sex of the flies not indicated). While there are differences in total number of labellar sensilla between species, variations also occur between sexes in some of the species e.g. Ph. regina (means for n = 20, Table 3.4). On the labella of female flies of the Australian sheep blowfly Lucilia cuprina Wiedmann, mean of the total number (208.5 ± 3.89(s.d.), n = 6), is significantly lower than that of D. tryoni males, Ph. regina, and C. vicina. This is because there are no short bristle sensilla on the marginal strip of the labella of L. cuprina (Sorby-Adams 1981), which numbered 17-29 on labellar lobes of male D. tryoni.

Zacharuk (1962) reported that, only slight differences in the number, distribution and structure of the cephalic sensilla were observed among larvae of seven species of Elateridae (Coleoptera). The advanced stages of these larvae had been found in very different habitats (soil, sand, and decaying wood). Organs on the antennal sensory appendices of two of them (Ampedus and Melanotus), which had more neurones and are envisaged to be of functional significance, are implied to have been the least variable. Because the species of flies cited above are different and have different feeding habits, close similarity in the number of fringe labellar sensilla as well as their pattern of distribution might reflect on their evolution from a common ancestral origin (all of the flies quoted belong to the Cyclorrhapha). However, there are also marked differences in number of labellar gustatory receptors that seem to be characteristic of the feeding habits of the species and size of insects (review by Chapman 1982). Those that feed on food from a variety of sources and therefore have to discriminate a wide range of chemicals have a large number of gustatory sensilla.

The more specialised feeders, some of which have been shown to rely on highly specific phagostimulants, have very few: G. austeni has only 8 labellar sensilla that have a mechanoreceptor and two chemosensitive neurones, analogous to the numerous fringe labellar sensilla of the other dipteran species and also only 8 equivalent to the interpseudotracheal papillae (Rice et al. 1973) (cf. Chapter 4 below). In D. melanogaster, size has been suggested to be the possible limiting factor rather than its range of food source (Chapman 1982). It has a total number of fringe labellar sensilla that are about one third those in D. tryoni, Ph. regina, and C. vicina (Falk et al. 1976).

Not all the labellar sensilla (particularly the short straight sensilla and bristle sensilla) in the upper rows contact food substances when the proboscis is extended or even when feeding is in progress. The arrangement of sensilla is such that, almost all or all the longest sensilla, a large number of the intermediate ones, and the short straight sensilla in the marginal row contact the substrate (fig.3.4 for hypothetical flat solid materials or very thin films of fluid food materials of high viscosity on a hard surface).

The superfamilies Tephritoidea (Trypetidea), Psiloidea and Sapromyzidea are considered to have evolved during the Paleogene era (65 million years ago) (Metcalf et al. 1975) from saprophagous (possibly feeding on decaying fruit) ancestors (Rohdendorf 1974). The short sensilla (bristle sensilla and short straight sensilla) in the upper rows on the labellum may not be utilized for the detection of food substances when initially contacted. However, this set of sensilla may provide

FIGURE 3.4 An idealised section through a labellum of a male D. tryoni at the region of longest sensillum 4, showing possible positions of different types labellar taste sensilla when the labellum is placed on a hypothetical, flat substrate in the filtering position. The substrate representing food material may be a thin film of liquid on a hard surface or fluid material of high viscosity. H haustellum, LAB labellar lobe, LS lateral sclerite, N neurones, PS pseudotrachea and "I" for intermediate sensillum (either long or medium long). Rest of notation as in the text. Bar ca. 100 µm.

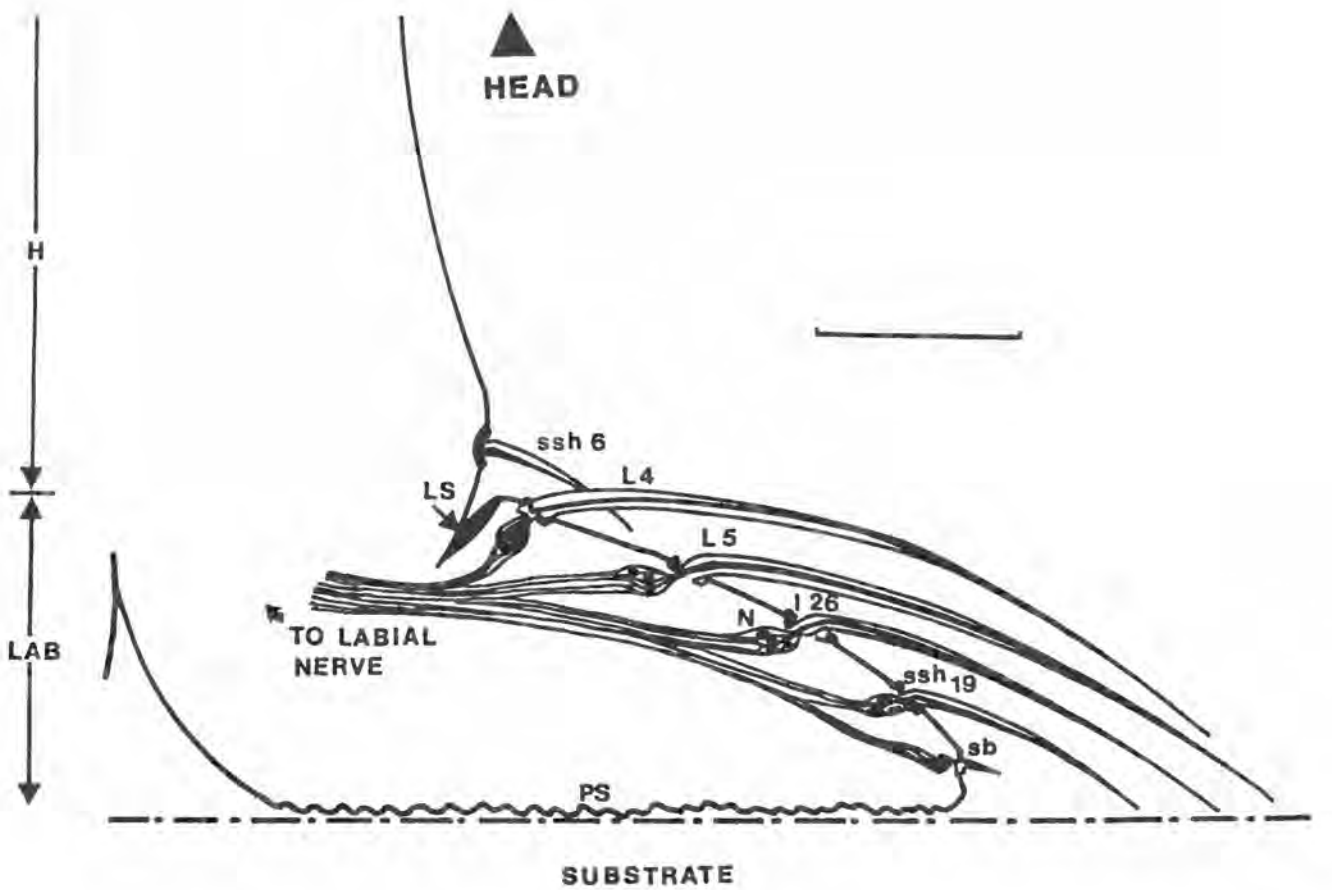

HEAD
H
LAB
ssh 6
L4
LS
L5
I 26
N
TO LABIAL
NERVE
ssh 19
sb
PS
SUBSTRATE

FIGURE 3.4

useful feedback to facilitate the proper positioning of the labella of flies feeding from cavities in ripe fruit. Dethier (1976) and Yetman and Pollack (1987) describe observations on blowflies localizing point sources on the labellar lobes by stimulation of labellar sensilla on different parts. They found that this elicited extension of the proboscis, with a rotation of the oral disc to the side bearing the stimulated sensilla. Thus, indicating the sensitivity of the sensilla and the functional significance of their central connections in locating the origin of the stimuli.

The sensilla discussed above are located over a slender lateral sclerite (LS in figs. 3.1A, B) which marks the upper fringe of the lateral fold on the anterior half of the labellum. Because most of the chemosensilla have been shown to have a mechanosensitive neurone (Chapter 5 of this thesis), these sensilla may perform a proprioceptive function when the labellar lobes are either in the cupping or filtering position (terminology of Graham-Smith 1930) during feeding, though this needs proof (fig. 3.4).

Labellar gustatory sensilla of different morphological types have been shown to have different patterns of neuronal activity. Maes and Vedder (1978) classified the labellar taste sensilla of C. vicina into three physiological types (A, B, and C) depending on the electrophysiological responses of their salt-sensitive neurones (P) to molar KCl. Type A response was mainly from largest sensilla with slightly less than 300 spikes per second at the onset of stimulation. The firing rate dropped to a tonic phase of about 150 per second after the first 50 milliseconds. Types B and C had 300-400 spikes per second in the

phasic phase of about 50 milliseconds, declining to a low tonic phase of about 50 impulses per second thereafter. Type B response in intermediate sensilla was slightly higher than type C response elicited in bristles, marginal and different sensilla. The sequence of response decreased: A > B $\geq$ C. Shiraishi and Tanabe (1974) obtained results showing that, for concentrations of sucrose between one and ten millimollar, the frequency of action potentials firing in the sucrose sensitive receptor neurones, of different types of labellar gustatory sensilla of Ph. regina, was highest in the large (L) hairs; less in largest (LL) hairs; and least in the intermediate (I), marginal (M), and different (d) hairs (the order of response being : L > LL > I = M = d).

One mode, from among those suggested for CNS integration of the sensory information input from a large number of neurones in the gustatory sensilla, is by "across-fibre patterning" (Erickson 1963; Maes and den Otter 1976; Maes and Vedder 1978). A threshold input from labellar gustatory sensilla of each morphological type may provide an abstraction of the chemical composition of food contacted. The axons of all labellar gustatory neurones in the labial nerve synapse with interneurones in the suboesophangeal ganglion (Dethier 1976). In sampling the sensory input from a large number of gustatory neurones generated by an appropriate stimulus at a given point in time, the CNS may perhaps function as either one of two electronic circuit equivalents suggested here. Both result from the parallel convergence of inputs of a large number of individual sensory neurones that synapse with relatively fewer interneurones in the CNS. Each of the models of operation has

some advantage in stimulus detection and representation. Firstly, the CNS may function as a "stagger-tuned amplifier" for which the receptor membrane capacitances are the tuning capacitors. Neurones in different sensilla types have been shown to have different spike frequencies for a given stimulus concentration (Maes and den Otter 1976; Maes and Vedder 1978), superimposition of their frequency characteristics may result in an overall response with a wide flat-topped frequency band. Such a band is representative of the stimulus from inputs of all neurones that respond. If any amplification occurs in the CNS, such a frequency response would permit amplification over a wide band of frequencies. However, the gain is lower than that of a single frequency circuit because the maximum frequency of each stage is different (Brophy 1977). This implies that, since single neurones are units that respond independently (equivalent of the single stages of a stagger-tuned amplifier) the gain in the CNS is lower than that of each neurone in the peripheral sensory system (cf. Dethier 1976). In cases where neurones are much fewer and "labelled lines" may be operative e.g. labellar gustatory neurones of G. austeni (Rice et al. 1973), the freguency response is narrow and sharp. Secondly, the CNS may in some way perform the function of a "signal averager". Sensory coding generated by chemical stimuli is electrically noisy. Post-synaptic superimposition of a large number of impulses of slightly different phase but the same amplitude (i.e. from homologous neurones) may result in cancellation of the background noise (Brophy 1977). Such a noise-free signal may enhance the discrimination ability of the system. Model making for this system is still at an elementary stage, much needs to

be done in future.

Dethier (1976) is of the opinion that, a large complement of gustatory sensilla, such as that on the labellum, provides a broad chemoreceptive field. A large proportion of the short straight sensilla are in the lower rows of the labellum. Thus, their contribution to the sensory input into the CNS arising from the group of all sensilla that contact food materials at any one time may be higher than from the other types of sensilla.

Variations in sensilla numbers

Considering total numbers of sensilla of all morphological types for a given labellum, these had consistently low variations which were not significant. This may partly be explained by the observation that, where a decrease in the number of sensilla in one type was recorded, there was an almost equal increase in the number of those in at least two of the other types (asterisks in Table 3.1). In Ph. regina, Wilczek (1967) observed that differences in numbers of sensilla of the different morphological types were largely due to the variability in the dimensions of sensilla rather than their actual numbers. It was suggested that possible differential growth may affect sizes of sensilla on various parts of labellar lobes. Thus, a sensillum at a particular position of a labellum, classified into one morphological type, may be classified into any of the other dimensionally close types on another labellum, depending on the dimensions attained during development.

Maes and Vedder (1978) did not observe any correlation between the length of the labella of C. vicina and the total

number of sensilla per fly. However, they speculated that the total number of sensilla might be positively correlated to size of the labellum, which in turn may be determined by the body size during development. For the male flies of D. tryoni, a positive correlation was obtained between the linear dimensions (and therefore the surface area) of the labellar lobes and the total number of the fringe gustatory sensilla. It was suggested that the increase in size led to increase in total number of sensilla, which can occur between upper and lower limits, characteristic of the species. Below a critical lower limit, pharate adult flies may not eclose or if they emerge they may be too weak to inflate their wings. Similarly, those of larger size than the upper limit may have tenerals that cannot inflate their wings. Therefore, the overall effect is selection against individuals outside the size limits of a species. Thirteen and eighteen percent of flies eclosing from the small and large pupae respectively died in puparia and in the process of eclosing. Other mortality factors have not been ruled out and there is also a need for determining the modal class size of D. tryoni pupae, under normal dietary and environmental conditions and the level of survival at eclosion for comparison.

In Holometabola, adult external and internal organs (except Malpighian tubules) develop from imaginal disks (Nothiger 1972). The number of sensilla and bristles developed may depend on an increase in number of the specialised epidermal cells of each disk during the last mitosis prior to imaginal differentiation, at the onset of metamorphosis. Heavy pupae have the potential of having a greater proportion of cells that are likely to develop

the respective cuticular structures. On the other hand, the ultimate size of an adult fly depends on several factors: its genetic composition; juvenile hormone (JH) and moulting hormone levels, whose presence at low and high levels respectively determine the length of the developmental period; the interaction between the internal metabolic clock that plays a role in regulating feeding behaviour; and the availability of nutrients during the larval stages. In the present work, availability of adequate nutrients was a factor that regulated the size of adult flies and indirectly the total number of labellar gustatory sensilla within the limit for survival.

There is also a possibility that large flies may have slightly larger epidermal cells. In addition to increase in the number of sensilla, effects of larger size might be expressed through increase in the size of the sensillum shaft and the diameter of the basal socket secreted by the trichogen and the tormogen cells respectively (Table 3.2).

Diameter of socket: length ratio of the sensilla

Lawrence (1966) suggested that the diameter of the basal socket is related to the length of a bristle (they are supposedly correlated: Wilczek 1967; Peters 1965 cited in Maes and Vedder 1978). The ratio may reflect characteristics of the secretion of the socket by the tormogen cell, after the formation of the bristle. The size of the socket may then be determined by the diameter of the existing outgrowth, rather than by the secreting cells. Measurements of socket diameters of labellar sensilla of male D. tryoni show a pattern which is suggestive of this method of size determination losing precision

for the very small bristle sensilla. Their socket diameters and those of bristle and short straight sensilla had appreciable overlap. It is possible that, for very small sensilla, physiological processes in the socket secreting cell regulate the size of the socket formed. Since the length of the sensillum shaft may be affected by the size of the fly, the basal socket would be expected to have relatively larger diameter in bigger flies.

The body size attained by an insect, length of sensillum shaft, and basal socket diameter are genetically coded, directly or indirectly. JH level and timing is involved in the determination and differentiation of specialised epidermal cells at metamorphosis. Some other factors may contribute to differences in size of labellar sensilla: amount of proteins available; rate of their conversion to cuticular processes; and the duration of secretion of the sensillum shaft. These may be such that all are higher at sites of development of longer sensilla. Peters and Richter (1965) observed that in the labellum of C. erythrocephala, the first primordia ("anlage of Binnenkanals") secreted by the trichogen cell 60 hours after commencement of pupation eventually became the largest sensilla.

Symmetry of sensilla distribution

On maps of the distribution of labellar sensilla of a fly, the major morphological types of sensilla occupy zones on each labellum that are symmetrical on both right and left sides of the labellum. Bilateral symmetry in the positions of most of the sensilla exists to a large extent, except in a few cases where there were extra sensilla on one side. In this situation,

as reported on Ph. regina (Wilczek 1967), positions of sensilla on one labellum are not an exact duplicate of the other, because differences in total number may affect spacing.

Held and Pham (1983) have suggested that spacing between the bristles on the basitarsi of D. melanogaster may be determined by the repulsive forces generated by the epidermal feet (cytoplasmic extensions of Locke and Huie 1981) that develop on bristle-forming cells. The spacing between bristles in a row was observed to depend on the number of bristles. Thus, it would be expected that, were such a mechanism operative among the sensilla-forming cells of the labella in male D. tryoni, there might be slight displacements of sensilla positions on labella of different size. However, the recognisable pattern of distribution is preserved. Most of the longest and intermediate sensilla, at particular localities on the labellum, had dimensions that were least variable. These serve as reference points relative to which other sensilla of the same type and those of other types can be mapped. In flies of other species which have similar numbers of labella sensilla to male D. tryoni e.g. C. vicina but have different body size, it would be expected that spacing between sensilla is also different.

Naming of the fringe labellar sensilla

The length of a sensillum (and therefore partly the diameter of its socket) was used as the main criterion for classification. As for other species, relative positions were also useful. This revealed that, most of the "marginal sensilla" and sensilla that were classified as "different hairs" by

Wilczek (1967) and Maes and Vedder (1978) may largely be homologous to a single morphological group: the short straight sensilla of male D. tryoni in the present work. Shiraishi and Tanabe (1974) showed that the dose-response curves of the two topographical types of sensilla in Ph. regina in response to sucrose, were almost equivalent and the sensilla were taken to be of the same "physiological" type.

Detailed descriptions of the labellar gustatory sensilla of Diptera (as with other sensilla in insects) require a synthesis of results from morphological, ultrastructure and electrophysiological studies. These may enable association of neuronal response patterns with specific morphological types of sensilla. Maes and Vedder (1978) observed that spikes from gustatory neurones in short sensilla in response to molar KCl had larger amplitude than those from long sensilla. Also, the mechanosensitive neurone was more sensitive in the short sensilla. Much work along these lines remains to be done.

APPENDIX I

Length (in µm.) of each sensillum recorded for five specimens. Each pair denotes the homologous sensilla on the left and right.

TABLE 1 Lengths of longest (LH) sensilla range, $200 \leq l \leq 290.0$ (± 5.0) µm. Bracketed values are less than the lower limit or higher than the upper limit set for each type of sensillum; (bs) for broken sensilla (not measured).

Specimen	5	4	3	2	1
Sensillum					
LH 1	235, 225	240, 235	225, 240	225, 215	245, 255
2	215, 225	210, 235	205, 220	225, 215	230, 222
3	225, 265	270, 220	265, 270	255, 260	260, 250
4	265, 265	255, 265	260, 265	(195), 250	265, 275
5	235, 200	215, 205	215, (175)	210, 215	210, 220
6	240, 235	(160), 205	225, 200	215, 220	220, 205
7	220, 230	265, 260	270, 270	275, 245	230, (bs)
8	225, 200	205, 205	220, 220	210, 215	280, 220
9	265, 260	265, 275	(bs), 230	270, 270	270, 275
10	205, 220	270, 220	215, 230	225, 240	220, 225
11	275, 250	275, 280	285, 245	280, 275	285, 275

TABLE 2 Lengths of intermediate sensilla (Long and medium long sensilla); range, 65.0 < l < 200.0 (± 5.0)μm.

IH										
1	65,	85	(50),	85	(55),	70	(55),	70	70,	75
2	80,	75	(55),(45)		95,	90	60,	65	(bs),	75
3	110,	100	80,	95	95,	160	85,	90	140,	80
4	105,	105	145,	175	170,	145	90,	95	65,	90
5	75,	70	60,	70	125,	100	70,	70	105,	85
6	90,	(40)	60,	75	85,	(45)	(50),(30)		80,	110
7	(20),110		125,	150	185,	165	(225),(220)		185,	bs
8	120,	110	90,	65	140,	110	140,	135	135,	90
9	100,	90	85,	80	bs,	145	120,	---	(50),	90
10	150,	170	150,	175	165,	165	155,	160	145,(205)	
11	110,	100	(40),	110	(40),100		125,	60	---,	130
12	75,	75	60,	bs	75,	75	60,	75	bs,	100
13	100,(20)		90,	105	100,	105	---,	105	90,	100
14	75,	90	(45),(50)		(30),	80	70,	70	(60),	40
15	110,	140	60,	80	(50),115		135,	135	90,	150
16	110,	---	130,	---	130,	---	65,	60	100,	80
17	90,	105	120,	135	90,	120	130,	130	95,	105
18	130,	140	65,	110	140,	100	---,(45)		90,	130
19	120,	125	150,	145	(50),120		140,	140	160,	140
20	85,	75	(55),125		130,(55)		85,	85	70,	110
21	120,	105	160,	60	(55),120		130,	130	125,(45)	
22	110,	65	95,	100	95,	80	100,	100	---,	60
23	100,	95	95,	100	125,	75	(55),120		110,	120
24	90,	75	(55),120		65,	115	80,	65	90,	70
25	75,	80	90,	125	85,	75	75,(50)		125,	100
26	70,	90	70,	70	105,	85	(20),(37.5)		90,(55)	
27	70,	---	90,	---	(45),	75	(25),(25)		(25),(25)	
28	95,	100	75,	105	115,	100	90,	90	115,(55)	
29	115,	80	115,	110	110,	115	60,	85	70,	95
30	85,(40)		65,	80	60,	85	100,	---	60,(10)	
31	95,	70	130,	65	90,	95	70,(35)		100,(50)	
32	70,	85	75,	85	100,	70	(50),67.5		140,(50)	
33	95,	110	(25),145		65,	140	95,(50)		150,	100
34	95,	95	110,	115	70,	115	110,	95	130,	70
35	70,	95	(40),(45)		(50),115		(30),(45)		(45),	75
36	100,	90	105,	110	130,	80	(55),	105	115,(40)	

TABLE 3 Lengths of short straight sensilla (ssh);

range, $30.0 < l \leq 65.0$ ($\pm$ 5.0)µm.

ssh 1	50, 50	40, 35	40, 40	50, 40	45, 55
2	55, 45	45, 40	50, 40	35, 35	55, 55
3	45, 50	50, 45	45, 40	30, 55	50, 55
4	50, 50	50, 65	50, 50	55, 55	65, 60
5	70, 60	bs, 50	45, 50	55, (75)	bs, bs
6	60, 65	65, (75)	65, 70	70, 45	60, 70
7	35, 55	(10), 35	50, 65	--, --	(115), --
8	35, 25	45, 45	(75), 55	25, (15)	(20), 60
9	55, 40	(15), 30	(15), 30	35, 45	60, (20)
10	25, 30	40, 35	35, 30	45, 65	45, 45
11	50, 35	45, 45	(85),(75)	65, (7.5)	(15), 60
12	35, 55	50, 40	60, (105)	70, 70	70, 65
13	55, --	25, 25	30, 40	(100), 50	55,(100)
14	45, 70	(90),(100)	45, (95)	50, --	60,(100)
15	65, bs	45, 55	55,(110)	(75), 55	--,(120)
16	60, 50	(15), 50	55, (15)	60, 30	--, 70
17	50, (10)	35, 45	--, 35		60, 70
18	50, 55	60, 50	40, 50	60, 60	50, 60
19	40, 45	50, (5)	60, 60	35, 70	(125),(75)
20	55, 65	55, (75)	35, 50	70, 50	60, 65
21	45, 50	45, 50	(80),(5)	40, 30	55, 60
22	65, 70	--, (75)	65, (10)	50, --	(20), 60
23	50, 50	(75), 40	30, 65	35, 45	(20), 60
24	65, (75)	(100), 45	60, 60	(75), 50	70, 50
25	55, 45	45, 55	70, 45	55, 55	60, 45
26	60, 50	(90), 65	40, (80)	(85),(85)	50, 30
27	35, 25	70, 25	70, 25	60, 60	(150), 60
28	40, 70	(20), 40	40, 35	--, 60	(120),37.5
29	50, 70	55, (80)	(85), 55	60, 35	(80),(100)
30	55, --	(85), --	--, (90)	(85), 55	55, 55
31	50, 70	(10), 40	55, 65	55, 60	(15),(95)
32	55, 50	40, 50	45, 70	(95), 50	--, 70
33	65, --	(110), 45	65, 65	(100),(95)	--,(110)
34	50, 45	60, 50	60, 40	55, 65	50, (15)
35	50, 50	(20), 50	(5), (120)	55, 55	65, 65
36	60, 50	70, 50	(15), (105)	(100),(75)	(155),(100)

TABLE 4 Lengths of bristle sensilla (b); range, $10.0 < l \leq 30.0$ ($\pm$ 2.5-5.0)µm.

(b) 1	15, (70)	15, (60)	--, (40)	(65),(85)	bs,(40)
2	30, (40)	(55), 35	--, (55)	(75),(75)	5,(40)
3	20, 25	10, --	15, (60)	30, (50)	20, 20
4	25, 20	15, 25	15, 15	25, (50)	20, 25
5	25, 20	(50),(85)	(70), 30	(60), --	(45),(45)
6	10, 10	15, (75)	10, 15	20, 20	15, 10
7	30, (85)	--, (45)	35, --	5, (85)	(65),(45)
8	30, --	bs, bs	(50), --	(45), 15	15, --
9	15, 15	--, 15	--, --	--, --	5, 25
10	15, --	--, 5	10, --	5, --	10, 10
11	25, 20	30, (50)	--, 10	35, 25	(85), --
12	20, 25	--, (50)	(85), 10	25, 25	30, --
13	25, --	15, (55)	--, 5	--, (50)	--, --
14	20, 15	(60), --	5, --	5, 10	15, 10
15	20, --	(75), 10	--, (65)	(50),7.5	10, 15

IV. MARGINAL AND INTERPSEUDOTRACHEAL PAPILLAE OF MALE *D. TRYONI*

4.1. INTRODUCTION

On the oral surface of labella is a series of pseudotracheae through which fluid food material is filtered and sucked into the hypopharynx and subsequently the foregut. In C. erythrocephala and Ph. regina, a single papilla occurs at the origin of each pseudotrachea, close to the marginal strip, whilst other papillae are located along the bases of the pseudotracheae between the latter and the interpseudotracheal plates (Graham-Smith 1930; Wilczek 1967).

Dethier (1955) observed two neurones innervating each papilla and suggested a third one. However, ultrastructural work (Larsen 1962b) and more refined methylene blue staining (Wilczek 1967) have shown four neurones. Similarly, for the papillae of C. erythrocephala (Peters 1963, figs. 25 and 26) and B. peregrina (Tominaga and Kabuta 1969).

The interpseudotracheal papillae were suggested (Lowne 1870 and Hewitt 1914 cited in Graham-Smith 1930) to be gustatory from observations on methylene blue staining of the dendritic canal. Although Graham-Smith (1930) suggested both chemo- and mechanosensitive functions for the neurones, it was Dethier (1955) and Dethier and Hanson (1964) who showed behaviourally and electrophysiologically respectively that some neurones are sensitive to electrolyte(s) (NaCl) and sugars, and one to mechanical displacements.

Observations were carried out on the sensory papillae on the oral surface of the labella of male D. tryoni: on their numbers, distribution and morphology; using histological staining and TEM to study their innervation. These parameters were compared between the peripheral (marginal) and

interpseudotracheal papillae.

4.2. MATERIALS AND METHODS

To map the distribution of the papillae, oral discs with intact marginal strips were dissected out under saline, from labella of one day old flies and transferred into Ponceau de xylidene stain in water with a few drops of acetic acid for three minutes to enhance visualization. Excess stain was rinsed off in saline followed by clearing in glycerine for 20-30 minutes. The tissue was mounted onto slides with Gater's medium and viewed through a phase-contrast microscope (Meopta).

The following procedure gave the best preparations for scanning electron microscopical observations on morphology: Diethyl ether was injected into the thoraces of male flies to distend their probosces, less than one hour after their eclosion. Pressure was exerted to evert and open the labellum further, prior to ligaturing a fly with a cotton thread in the region of the prothorax. Praparations were then kept in diethyl ether over night, cleared in two changes of amyl acetate, coated with gold and viewed on a Philips 500 SEM at 20 kV.

4.3. RESULTS

Sensory papillae all along the marginal strip at the origins of the pseudotracheae are referred to as marginal (or peripheral) papillae, whereas those amongst the pseudotracheae are the interpseudotracheal papillae sensu strictu.

4.3.1 Morphology of papillae

Papillae of male D. tryoni are 4-6 µm long. The

marginal papillae always have a stout peg and apparently appear to be of two classes: one has a blunt slitted tip whilst the other has a nipple-like tip with a pore proximal of the nipple (fig.4.1 C, D). The base of the peg is constricted and the basal socket (where observable) is a swollen membranous ring with an outside diameter of about 3.3 µm which seems to allow for lateral displacement of the peg (fig. 4.1 C). In contrast, the interpseudotracheal papillae are relatively slender and have a relatively sharp tapering tip. They sit in deep, sheath-like sockets with about one third the base length of the peg within the socket. The papilla peg is curved and lies closely apposed to the surface of the pseudotrachea with its tip pointing towards the longitudinal central fissure of the latter. Hence, the interpseudotracheal papillae seem to have limited scope for lateral displacement (fig. 4.1 E). The orientation of the tips of the interpseudotracheal papillae prevented viewing of the suspected terminal pore.

4.3.2 Numbers of papillae and their distribution

The mean of total number of peripheral and interpseudotracheal papillae per labellar lobe, and mean of total number of all the papillae per labellum for 6 flies are in Table 4.1.

There are 1-3 papillae along the whole length of a pseudotrachea and the distribution is such that if there is more than one, all the papillae may be on the same side of the pseudotrachea or they may alternate on either side (figs. 4.1 A, 4.2 A). The number of interpseudotracheal papillae decreases from the mid region (with longer pseudotracheae by *ca*.

100 µm) to the anterior and posterior of the oral surface of each lobe where pseudotracheae are shorter.

FIGURE 4.1

A. A scanning electron micrograph of the oral surface of everted labellum, showing the pseudotracheae (PT) converging on the periphery of the oral opening (PC). Bar = 20 µm.

B. At a higher magnification of the oral surface of the posterior right labellar lobe, some interpseudotracheal papillae (ip) along pseudotracheae and a marginal papilla (mp) close to the marginal strip which is covered by the marginal flap (mf) are conspicuous. Interpseudotracheal papillae 1 and 2 are on either side of a pseudotrachea and the longitudinal central fissure (fs and arrow heads) occurs along the whole length of the latter. Bar = 100 µm.

C. Fourth marginal papilla from the posterior on the left lobe of the oral surface with a slitted (st) tip. The peg has a constricted base (arrow) on a broad, ring-like socket (s) bordered by the terminal cuticular ridges (r) of a pseudotrachea. Bar = 1 µm.

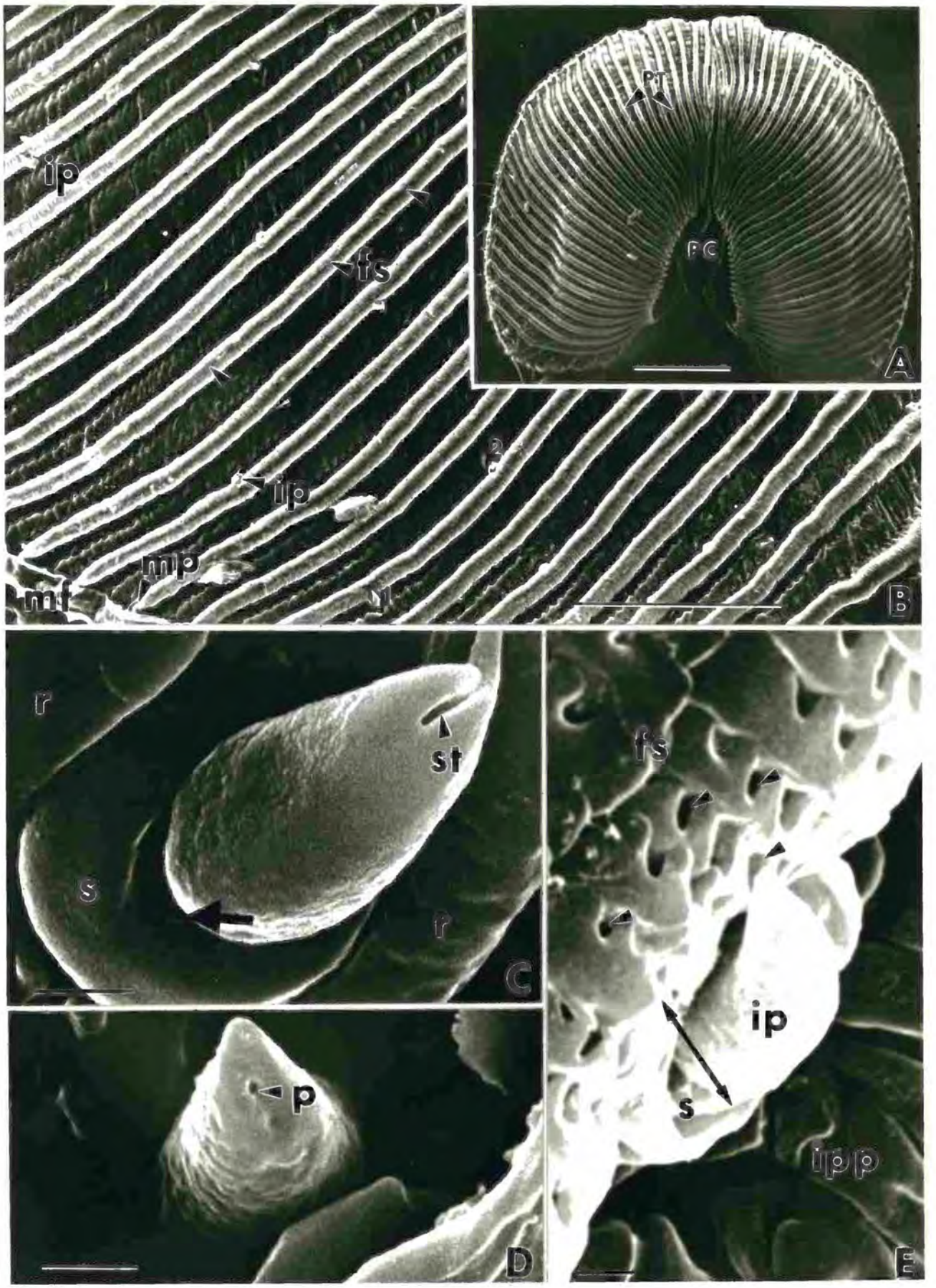
D. Third marginal papilla from the posterior of the oral surface of the right lobe, with a nipple-like tip above a pore (p). Bar = 1 µm.

E. An interpseudotracheal papilla (ip) closely apposed to the side of a pseudotrachea. The peg has a tapering tip and the base is in a deep sheath-like socket (s) bordering the interpseudotracheal plate (ipp). The tip faces the central fissure (fs), lying adjacent to the sieve-like processes of the pseudotrachea with openings (arrow heads) for fluid passage. Double-headed arrow

FIGURE 4.1 (cont.)

shows possible direction of mechanical displacement.

Bar = 1 μm.


ip
fs
ip
mp
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A
PT
PC
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s
r
C
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D
fs
ip
s
ipp
E

Table 4.1 Numbers of marginal (mp) and interpseudotracheal (ip) papillae on the oral surface of labellar lobes of 6 male *D. tryoni*. Means (± s.d.).

Sample	No. of papillae on oral lobes				total no.
	left		right		
	mp	ip	mp	ip	
1	32	30	32	30	124
2	34	32	32	32	130
3	34	30	34	29	127
4	34	29	32	25*	120
5	29	28	29	30	116
6	29	27	30	27	113
	31.8 ± 3	29.3 ± 2	31.5 ± 2	28.8 ± 3	122 ± 7
mp + ip	61.3 ± 4.0		60.3 ± 3.0		

Mean of total marginal papillae/labellum, 63.6 ± 4.0

Mean of total interpseudotracheal papillae/labellum, 58.2 ± 4.0

Mean numbers for mp, ip, and their total on right and left lobes not significantly different ($t = 2.2$, $p \leq 0.05$, $df = 10$).

* Lowest number probably due to obliteration of some papillae by folding of the oral surface during processing.

FIGURE 4.2

a. A map of the oral surface of the labellar lobes of a male D. tryoni showing distribution of marginal (O) and interpseudotracheal (●) papillae. Pseudotracheae of the left lobe are represented to illustrate occurrence of a marginal papilla at the origin of each pseudotrachea, close to the marginal strip (ms) and 1-3 papilla(e) in the interpseudotracheal spaces. The most anterior (AN) pseudotrachea is shaded to indicate its tubular form. mf: marginal flap; PS: posterior. Dots and circles are not to scale.

b. Diagram of the oral surface of the labella showing some of the variations in the anastomoses of the pseudotracheae in an area of occurrence of the papillae. Two pseudotracheae, one from each lobe may connect into one on the posterior as in (a) above or a separate connection may be present on each lobe (as in c).

c. Diagram of oral surface illustrating orientation of the tips of interpseudotracheal papillae (vertices of dark triangles) with respect to the pseudotracheae. Dotted median line represents the longitudinal central fissure.

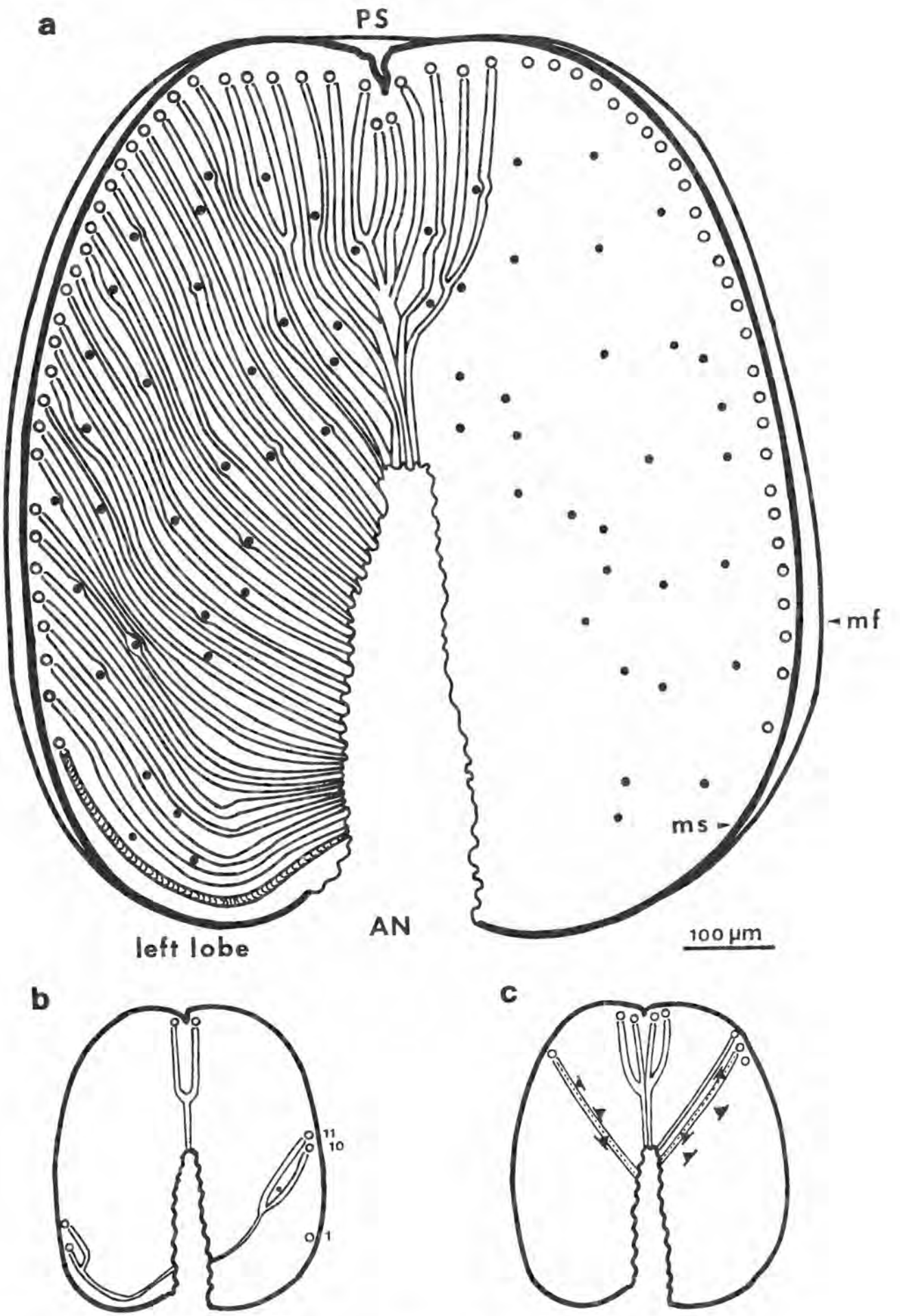


FIGURE 4.2

4.3.3 Innervation of papillae

4.3.3.1. Methylene blue staining of neurones

The procedure for injection of methylene blue is outlined in chapter 5 of this thesis.

Figure 4.3 A shows the marginal papillae are innervated by a group of at least three neurones with dendrites traversing an average of 13.$\pm$ 0.1 µm (range, 12-24 µm, n = 7) from the cell bodies to the base of the peg. Bundles of axons of each group run together with those from the neurones innervating the fringe labellar gustatory sensilla, in certain areas of the labella, to form confluent sub-branches of the labial nerve. For further determination of the exact number of neurones, ultrathin sections of the sensilla were examined on the TEM.

4.3.3.2 Ultrastructure of marginal and interpseudotracheal papillae

Cross-sections of the papillae just below the cuticle showed that they are innervated by four neurones (fig. 4.3B, C, and D). However, a cross-section of a peg close to the tip showed three dendrites in the dendritic lumen which are the putative gustatory receptors (fig. 4.3 E). At the lower level, the cuticular sheath invaginates between the dendrites almost enclosing one of them (fig. 4.3 B, C). This is likely to be the mechanoreceptor. Two of the dendrites apparently appear to have more microtubules than the others (fig. 4.3 C).

FIGURE 4.3

A. A photomicrograph of methylene blue stained bundles of neurones (n) innervating marginal papillae (mp and arrow heads) on the posterior portion of left labellar lobe of male D. tryoni. Dendrites (d) from the neurones to the pegs are 13 ± 0.1 µm long, and bundles of axons (an) extend posteriorly from neurones to join branches of labial nerve. LH 10: basal socket of longest sensillum 10. Bar = 10 µm (ca. x1.4K).

B. A transmission electron micrograph of a cross-section of a peg of a marginal papilla below the socket. There are four dendrites (d) three of which are almost enclosed in a separate cuticular sheath (cs) and all of them bathed by a uniform staining dendritic fluid inside the scolopale. Outside the scolopale (cs), there is the sensillar fluid with electron dense material with fibrillar inclusions (arrows) in the vacuole (v) of the trichogen cell within the thick external cuticle (c). Dense processes in the dendrites (*). Bar = 1 µm (ca. x13.3K).

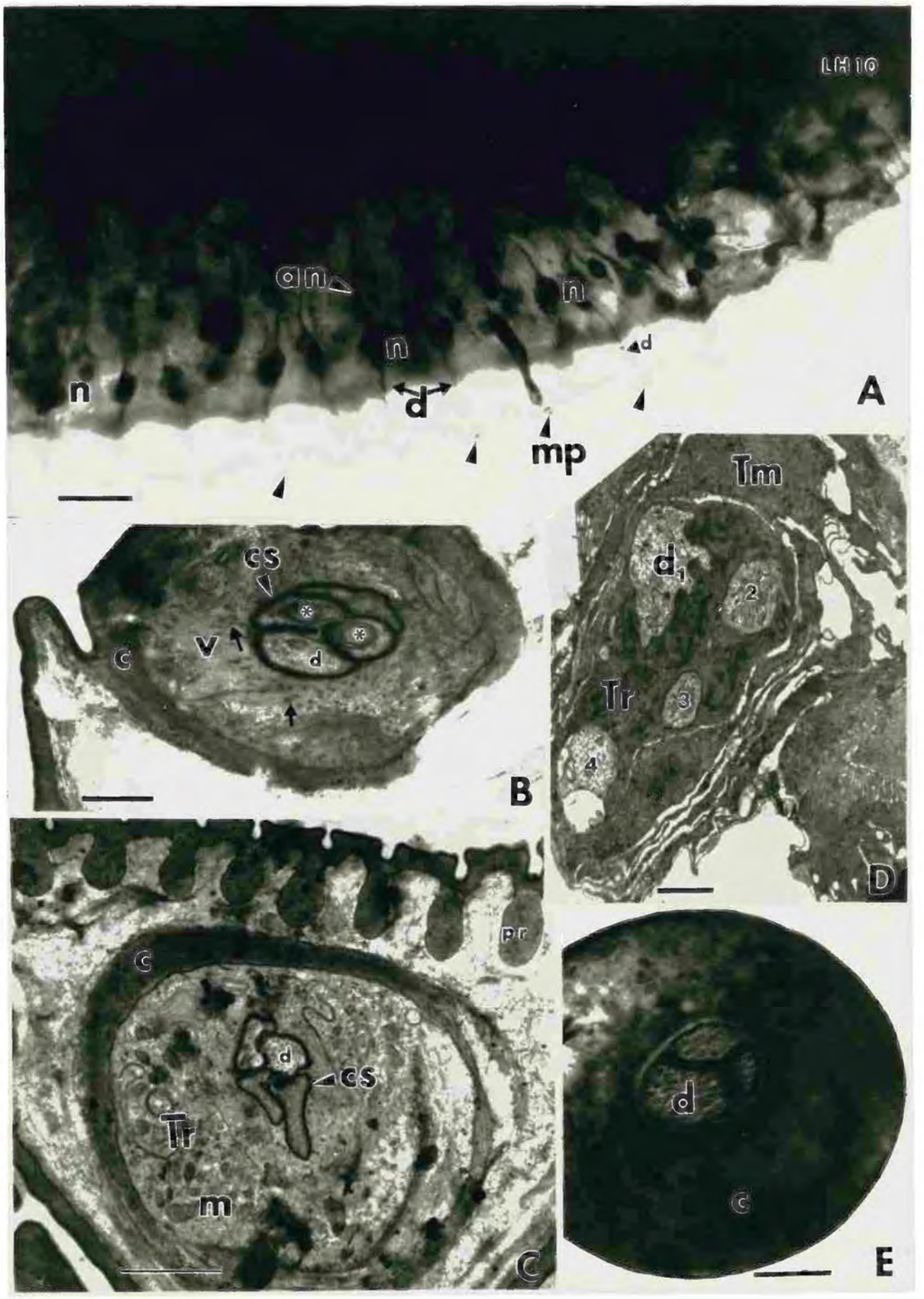
C. Cross-section of a papilla below the level of the cuticle but above the ciliary processes. Four dendrites are enclosed in a single cuticular sheath (cs) invaginating deep between the dendrites. m: mitochondria, pr: processes of pseudotracheal rings. Bar = 1 µm (ca. x19.3K).

D. Cross-section of an epidermal complex of a papilla below the ciliary region, showing four dendrites surrounded by trichogen (Tr) and tormogen (Tm) cells.

FIGURE 4.3 (cont.)

Bar = 1µm (ca. x10K).

E. Cross-section of a papilla peg near the tip with only three denrites (d) closely packed in the lumen within a thick cuticular wall (c). Bar = 0.2 µm (ca. x73K).


LH 10
an
n
n
n
d
d
mp
A
cs
v
c
d
B
Tm
d_1
2
Tr
3
4
D
pr
c
d
cs
Tr
m
C
d
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E

4.4. DISCUSSION

The total number of papillae on the labellar lobes of male *D. tryoni* (mean of about 122) is less than that cited for *Ph. regina* which has a mean of 132 (range 128-136) (Wilczek 1967); Dethier (1955) gave the range as 135-189.

The presence of four neurones below the level of the cuticle and only three of these extending their dendrites into the lumen of the peg, plus the presence of a slit or pore at the tip, indicate that the marginal and interpseudotracheal papillae have three gustatory neurones and a basal mechanoreceptor. The mechanosensitive dendrite supposedly terminates near the junction of the base of the peg and the basal socket, as has been shown for *B. peregrina* (Tominaga and Kabuta 1969); similarly for the papillae of *Ph. regina* (Larsen 1962b); and *C. erythrocephala* (Peters 1963). The interpseudotracheal pegs of *D. melanogaster* have only one gustatory neurone in addition to the mechanoreceptor (Falk *et al.* 1976). In *Ph. regina* and *B. peregrina* each dendrite is apparently enclosed in a separate cuticular sheath.

Arrangement of the papillae around the periphery of the oral surface and along the bases of the pseudotracheae is a highly specialised and adaptive pattern. When feeding on tiny droplets on a substrate, the marginal papillae are well situated to monitor their presense and chemical content, whilst the interpseudotracheal papillae are likewise suited to monitor food as it passes along in the pseudotracheae. When a droplet is depleted, suction may continue for a short time because papillae closer to the oral opening maintain the flow of sensory information to the central nervous system. In cases where food

material is marginally acceptable or, perhaps, in contact with unsuitable substances, there might be a tendency for the unsuitable substance to flow to the edges of the labellar lobes when a food droplet is depleted. Detection of the substance by the fringe labellar sensilla and/or the marginal papillae may elicit shifting of the labellum to another position or retraction of the proboscis.

About three quarters of all marginal and interpseudotracheal papillae are on the posterior half of the oral disc of the labellum. This is partly because a large number of the pseudotracheae originate from this part and the papillae are therefore strategically placed to monitor the bulk of the fluid materials imbibed. Further observations under a microscope, on a fly feeding on a smudge of cue-lure on the wall of a Petri dish, showed that even when the fly was dabbing the labellum on the surface, the posterior of the oral disc was not lifted off the substrate except for slight cupping of the lobes. Therefore a large number of the papillae remain in contact with the food.

The longitudinal central fissure of the pseudotracheae is formed by the overlapping of two edges of the cuticular processes forming the tube. Such an arrangement may facilitate slight adjustments of the diameter of the pseudotracheae and, together with slight linear extensions of the latter, enable suction of fluid material with particles of different sizes. They are probably opened up occasionally in the process of cleaning the pseudotracheae by flushing out any debris with saliva or regurgitated fluids.

The fringe labellar gustatory sensilla of male *D.*

tryoni and those of Ph. regina (Wilczek 1967), D. melanogaster (Falk et al. 1976) and the tarsal gustatory sensilla of Ph. regina (Adams et al. 1965; Matsumoto and Farley 1976) have a second lumen in the hair shaft that contains the receptor fluid. In contrast, marginal and interpseudotracheal papillae of these species (Larsen 1962b; Falk et al. 1976) and those of B. peregrina (Tominaga and Kabuta 1969) have one dendritic lumen only. However, continuation of the scolopale around the dendrites into the lumen of the papilla creates almost a similar arrangement to that in trichoid sensilla where two distinct lumina are present (Larsen 1962b). The receptor lymph has been suggested (Sturkow 1971, Broyles et al. 1976) to maintain ionic composition and concentration at a suitable level in the adjacent dendritic lumen for transmission of electrical impulses. It may also be a reservoir having a manometric function in maintaining the level of fluid bathing the dendrites without the need for instantaneous replacement by the trichogen cell. The very small size of the papillae (much shorter dendrites) and position on the oral surface where they are covered by fluids most of the time, may render maintenance of ionic gradients between dendrites and the dendritic fluid directly secreted by the thecogen cell sufficient.

Dethier and Hanson (1964) and Pietra et al. (1980) showed that the marginal and interpseuotracheal papillae of Ph. regina differ electrophysiologically from the fringe labellar gustatory sensilla. Unlike the latter, salt sensitive units in the papillae did not exhibit high frequency firing at initial contact, and subsequent adaptation, with monovalent chlorides (choline chloride, NaCl, KCl and LiCl). Firing of

neurones was continous at a level that varied randomly. Calcium ions, at concentrations greater than millimolar, reversibly inhibited firing of neurones sensitive to monovalent chlorides and sugars in fringe labellar gustatory sensilla of Calliphora vomitoria (Morita 1959), Ph. regina (Evans and Mellon 1962a; Rees and Hori 1968) and C. capitata (Gothilf et al. 1971; Angioy et al. 1978, 1978a). However, $CaCl_2$ in the range 0.1 - 2M was stimulatory to a NaCl-sensitive unit in the papillae, eliciting high frequency bursts of action potentials. Dethier and Hanson (1964) did not observe any electrical activity of a negatively salt-sensitive (water) neurone in the interpseudotracheal papillae.

Electrophysiological work still remains to be done on the marginal and interpseudotracheal papillae of D. tryoni.

V. EXTERNAL MORPHOLOGY OF FRINGE LABELLAR SENSILLA OF MALE *D. TRYONI* AND THE ULTRASTRUCTURE OF THEIR INNERVATION

5.1 INTRODUCTION

The labellum of male D. tryoni has a large number of setae on the labellar lobes and papillae on the oral surface (Ch. 3 and 4 of this thesis). It is therefore not only an organ for food uptake but also plays the role of a major sensory organ. For detection of chemical and tactile stimuli, the longest, long, medium long, and the short straight hairs are suspected to be innervated by several chemo- and mechanosensitive neurones, as has been shown for other dipteran species: Ph. regina (Larsen 1962b; Felt and Vande Berg 1976); Stomoxys calcitrans (Adams et al. 1965); and D. melanogaster (Falk et al. 1976). Bristles are also suspected to have several chemosensitive neurones though probably fewer than in the larger types and possibly a mechanoreceptor.

Different morphological types of sensilla have been shown to be innervated by different numbers of neurones. The "A" type (long) labellar sensilla of D. melanogaster have four chemosensitive neurones whereas the short bristles (B) have two (Falk et al. 1976). Each sensillum in both of these types also has a mechanoreceptor-type neurone. Larsen (1962b) reported 3-5 chemosensitive neurones in the labellar sensilla of Ph. regina. This variation in numbers of neurones innervating taste hairs may correspond to different morphological types of sensilla, though there are likely to be a range of contributing factors. However, Felt and Vande Berg (1976) reported 4 gustatory neurones and a mechanoreceptor in the largest labellar sensilla of this species. The labellar sensilla of the stable fly S. calcitrans are similary innervated (Adams et al. 1965).

In the face of the above similarity in numbers of

labellar gustatory neurones of the diverse species cited, there remains a question on their specialization for the detection of and discrimination of entirely different foods. This need can be met by identically placed neurones having different receptor properties, i.e. responding to different chemical groups. In addition, the central interpretation of sensory inputs may enable different behaviours to be generated from similar gustatory neuronal inputs.

In the electrophysiological characterization of the responses of chemoreceptors to test stimuli, it is clearly important to analyse the responses on the basis of the activities of separate neurones, responding to a range of concentrations. Each neurone's dose/response characteristics are then determined over a physiological range of concentrations of solutions of stimulating compounds. This is necessary because in some sensory systems, where several neurones in the same sensory receptor or organ respond simultaneously, two neurones may have electrophysiological responsiveness that operate in a push-pull manner (Rees 1970a; Davis and Sokolove 1975; Rice 1975; Yokohari and Tateda 1976; Hess and Loftus 1985). In such a case, the total spike response for all responding neurones from a sensillum, over a range of stimulus concentrations, may assume a constancy that does not represent the true functioning of the contained neurones. It is therefore important that an electrophysiological study be preceeded by an ultrastuctural investigation on the number of neurones innervating each morphological type of sensillum. This then helps in deciding on the number of neurones firing in response to a test chemical in

electrophysiological recordings.

In this chapter, the results of histological methylene blue staining (MBS) of labella whole-mounts, done initially to investigate the presence of neurones innervating the labellar setae, are outlined. Then, the enumeration of neurones innervating the sensilla of the six morphological types (Ch. 3) is done by means of transmission electron microscopy (TEM). For each type of sensillum, several (Nx $\leq$ 8) cross-sections of a sensillum were obtained and viewed at several levels: sensillum shaft, the socket, and the epidermal complex of neurones and sheath cells. A few longitudinal and oblique sections of the socket region generally revealed the tip of one dendrite pressed against the base of the sensillum shaft, adjoining the socket membrane.

Neurones whose dendrites were observed in the hair shaft have been referred to as gustatory or chemosensitive in this thesis. Neurones with distal dendritic tips terminating at the base of the sensillum shaft are referred to as mechanosensitive neurones or mechanoreceptors.

5.2 MATERIALS AND METHODS

5.2.1 MBS of labella whole-mounts for light microscopy

Male flies were taken from the culture 1-2 hours after eclosion before their cuticle was fully tanned. Individual flies were pinned onto paraffin wax in a dissecting dish and 1% methylene blue solution in insect saline injected into the haemocoele through a 24 gauge hypodermic needle inserted through the mesonotum. The MB-injected flies were then left pinned for 10-15 minutes during which time the stain was carried into the

head and the labella by the haemolymph. This process was speeded up by movements of the body parts of the flies which were still alive. The probosci were then excised and quickly rinsed in distilled water and passed though the following steps (adapted from Waladde 1979):

i. fixation was in three changes of 8% ammonium molybdate, after which the tissue was kept overnight in the same solution;

ii. thorough rinse in distilled water;

iii. dehydration was in tertiary butyl alcohol, three changes of 10 minutes each;

iv. the tissue was cleared in three changes of cedar wood oil (2 hours each) and then mounted onto slides with XAM, after settling for three days at room temperature, observations were done and photographs taken with a phase-contrast photomicroscope.

5.2.2 Tissue preparation for ultrastructure study with the TEM

Probosci of male *D. tryoni*, 1-3 days post-eclosion were excised under cold gluteraldehyde/paraformaldehyde fixative. These specimens were attached to the bottom of an excavated glass block with double-sided sticky tape and processed under a glass cover, using the following schedule:

i. primary fixation was in 2.5% gluteraldehyde/ 2% paraformaldehyde fixative in Sorensen's phosphate buffer for 2.5 hours at 4°C;

ii. the tissue was washed briefly in phosphate buffer of pH 7.24 and 450 mOsm/kg, each labellar lobe was dissected into two

halves to enhance penetration of the secondary fixative (1% OsO_4 in the phosphate buffer at 450 mOsm/kg), it also limited the number of sensilla to be sectioned each time but also ensured representation of all morphological types; post-fixation was for 2 hours;

iii. the fixed pieces of tissue were transferred into glass vials and osmium tetroxide rinsed off with 25% ethanol followed by dehydration in a series of increasing concentrations of ethanol for 10 minutes in each of 75%, 85%, and 90% ethanol at 4°C; further dehydration was in 95% ethanol for 7 minutes and two changes of 10 minutes each in 100% ethanol at room temperature, finally the tissue was passed though three changes in dried ethanol for 10, 20, and 30 minutes respectively;

iv. dehydrated tissue was cleared in two changes of propylene oxide for 30 and 45 minutes in stoppered vials;

v. the tissue was then impregnated with araldite in propylene oxide (propylene oxide to araldite 1:1 for 6 hours, 1:2 for 14 hours and in pure araldite for 8 hours); embedding was in fresh araldite in rubber moulds, which were left to polymerize at 60°C in an oven for 4 days;

vi. ultrathin sections were cut on an LKB V Ultramicrotome with glass knives and collected on 100 or 150-mesh coated copper grids and stained with 5% uranyl acetate and lead citrate (Reynolds 1963), these were viewed on a Corinth AE1 transmission electron microscope and appropriate electronmicrographs taken.

5.3 RESULTS

5.3.1 Observations on MB-stained neurones of fringe labellar sensilla

Whole-mounts of labella showed groups of stained neurones under the cuticle, innervating each hair (fig. 5.1A,B). The very small bristles on the upper part of the posterior of the labellar lobes are also innervated by one or two neurones (fig. 5.1A). For some sensilla in rows, close to the marginal strip, up to four stained neurone bodies with their distal processes extending over a distance of about 59 $\pm$ 4 µm (range, 50-75; Nx = 7), to the bases of large hairs, were conspicuous. However, the arrangement of neurones in close groups prevented counting of the actual numbers of neurones innervating most of the sensilla. The small bristles had smaller bundles of neurones, which either indicates the presence of up to two neurones or more neurones, with smaller perikarya. There are also multipolar neurones that stained and are suspected to be stretch receptors (fig. 5.1B).

These results reveal many of the details of the innervation of the labellar hairs but generally do not show the numbers of neurones innervating each hair. A further study to determine the actual number of putative gustatory and mechanoreceptor neurones was done using TEM.

5.3.2 Ultrastructure of innervation of fringe labellar sensilla

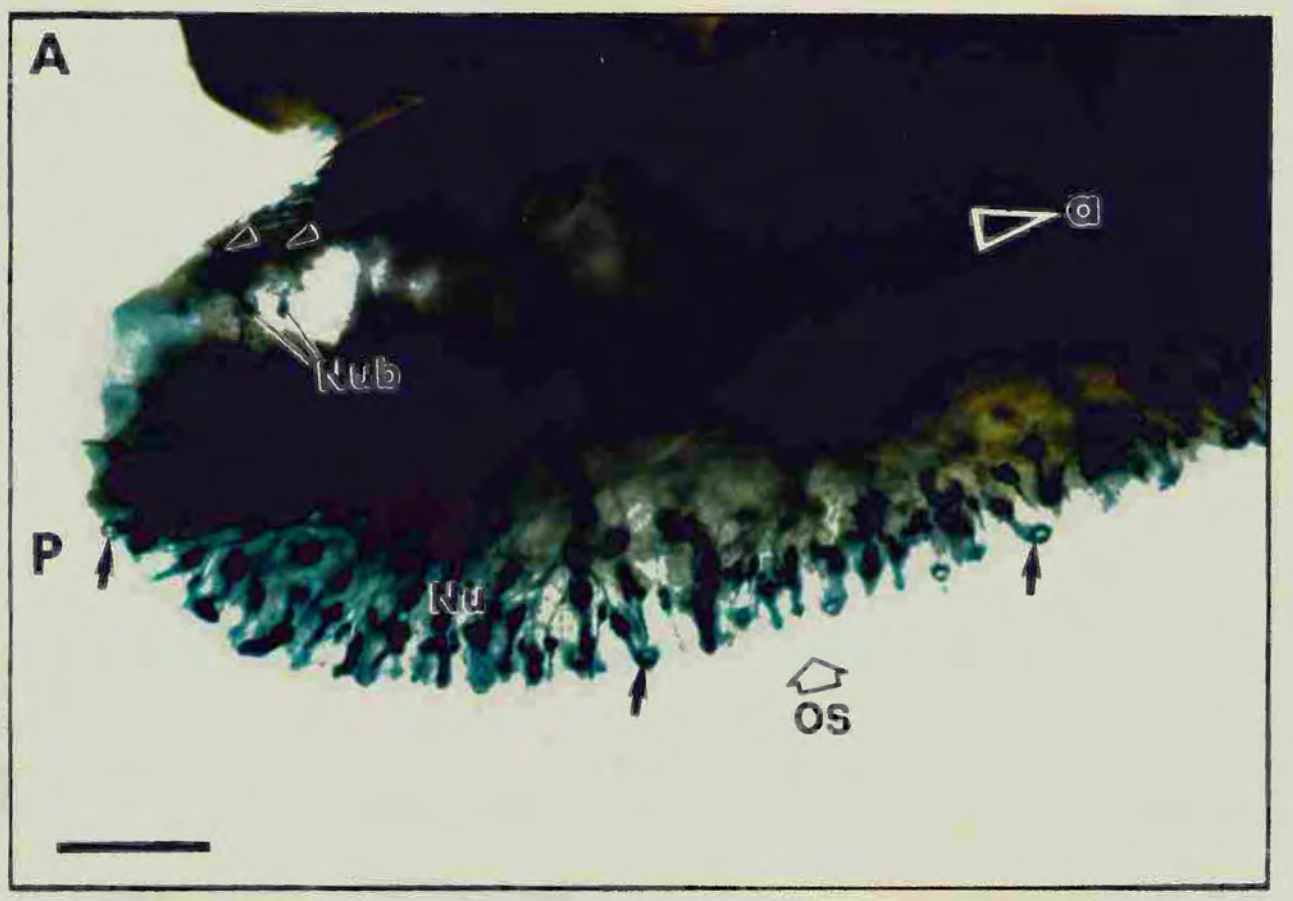
The results are presented in two parts: one for larger trichoid sensilla and the other for bristles and very short bristles.

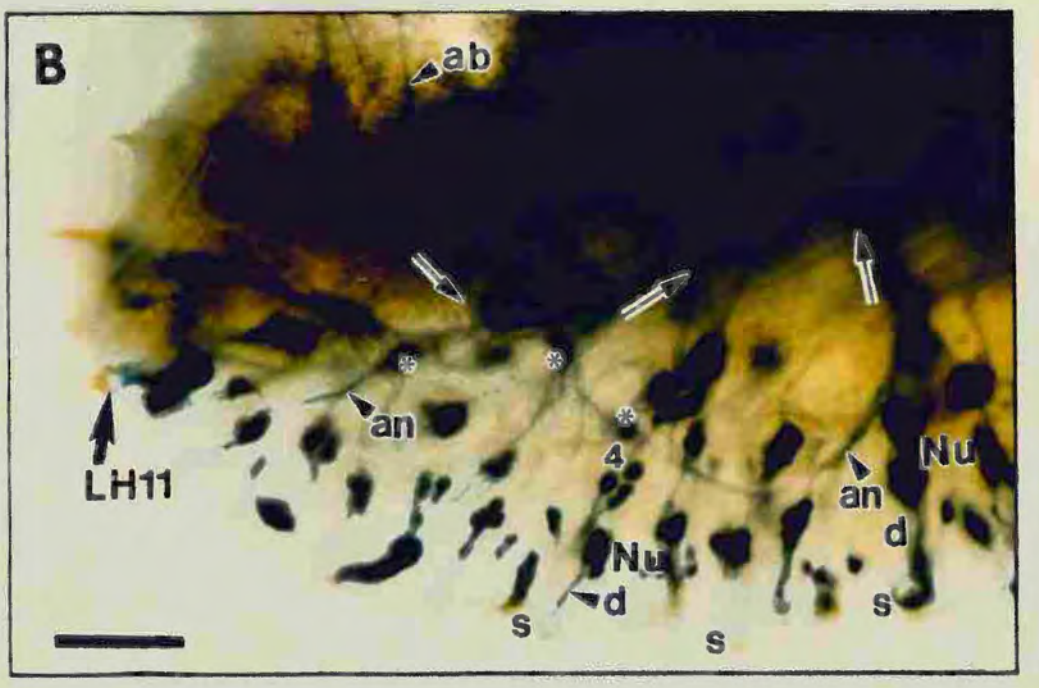
FIGURE 5.1

Photomicrographs of methylene blue stained whole-mount of the labellum of male D. tryoni showing:

A. Groups of neurones (Nu) innervating labellar hairs in the middle and posterior (P) parts. Dendrites extend from these neurones into the bases of hairs (arrows) and one or two neurones (Nub) innervate the short bristles on the upper part of the posterior of the labellar lobe (arrowheads). OS: oral surface, "a" indicates direction of anterior end of labellar lobes. Bar = 200 µm.

B. A higher magnification of the posterior portion of labellar lobe in A. showing stained groups of neurones (Nu) with dendrites (d) extending into bases of hairs (s). Their axons (an) join the posterior branch of the labial nerve to the CNS (arrows). ab: axons of the neurones innervating short bristles; "4": four neurones to a hair; *: multipolar neurones which are probably stretch receptors; LH11: basal socket of "longest" sensillum no. 11. Bar = 100 µm.


A
a
Nub
P
Nu
OS


B
ab
an
LH11
4
Nu
an
d
Nu
d
s
s
s

5.3.2.1 Longest (LH), Intermediate (iH), and short straight (ssh) sensilla

Observations with SEM showed longitudinal ridges running along the shaft from the base, of each sensillum, to the shoulder of the nipple-like tip. The longest sensilla have a basal socket of about 12 µm diameter with a cuticular rim rising about 7 µm above the general cuticle surface (figs. 5.2 A, B).

Cross sections of these sensilla, viewed with the TEM showed two lumina. The dendritic lumen has a variable diameter that seems to correspond to the size of different types of sensilla. Although the cross sections may not have been at corresponding levels, the diameter of the lumen seems to narrow down only close to the tip while elsewhere along the shaft it is almost uniform. Largest sensilla had the largest diameter and the short bristle sensilla had the smallest: LH > IH $\geq$ ssh >> b > sb (Table 5.1).

Longest, intermediate, and short straight sensilla each have four gustatory neurones whose dendrites were observed in cross-section in the dendritic lumen (fig. 5.2 B,C,E; Table 5.1). The epidermal complex of neurones and sheath cells, from socket level and below the cuticle extending beyond the basal level of the epidermis, comprises five neurones as shown for a short straight sensillum (fig. 5.2 F,G). However, from oblique and/or longitudinal sections of the socket area of ssh and LH, the dendritic terminal of a putative mechanoreceptor attaches to the cuticle of the sensillum shaft adjoining the resilin membrane of the socket (fig. 5.2D). The orientation of the distal, flat end of the tubular body in this dendritic

terminal is such that, any displacements of the sensillum shaft is most likely to cause stretching of the dendritic membrane (fig. 5.4).

Table 5.1 Ultrastructure of innervation of different morphological types of labellar sensilla of male D. tryoni: Diameter (Dl) of the dendritic lumen measured parallel to the crescent-shaped receptor lymph lumen of each sensillum; and a summary of the number of neurones innervating different morphological types of sensilla. G: gustatory neurone; M: mechanoreceptor; Nx: number of sections; (L,T,E) number of sections at three levels, L: longitudinal/oblique at the socket; T: transverse sections of the hair shaft; and E: transverse of epidermal complex below socket.

			Number of neurones		
Sensillum	Dl (±s.d.) μm.	Nx*	G	M	(L,T,E)
LH	1.1 ± 0.1	4	4	1	(1,8,?)
iH	1.0 ± 0.3	6	4	1	(0,6,?)
ssh	0.8 ± 0.2	4	4	1	(1,10,4)
b	0.53	1	4	1	(1,1,?)
sb	0.24	1	2	0	(0,2,5)
IP and PP	0.41	1	3	1	(0,1,5)

* Cross-sections of sensillar shaft only.

? All had 5 neurones at E level and some sections were difficult to assign to a particular type.

IP, PP Interpseudotracheal and peripheral papillae (Ch. 4).

FIGURE 5.2 A-G

Electron micrographs of the larger trichoid labellar sensilla of male D. tryoni:

A. A scanning electron micrograph showing examples of some sensilla on the anterior part of the labellar lobe: longest sensillum LH1; long sensillum IH 14; and a short straight sensillum ssh 1. All have longitudinal ridges (arrowheads) and a deep socket (s) with a rim. Bar = 20 µm.
Inset: Tip of IH 14 with longitudinal ridges and nipple-like tip (T). Bar = 2 µm.

B. TEM of a transverse section of a longest sensillum; D: gustatory dendrites in the dendritic lumen; c: cuticle of hair; rL: receptor lymph lumen with electronlucent substance. Bar = 1 µm.

C. Tranverse section of a long sensillum showing four gustatory dendrites (D). Bar = 1 µm.

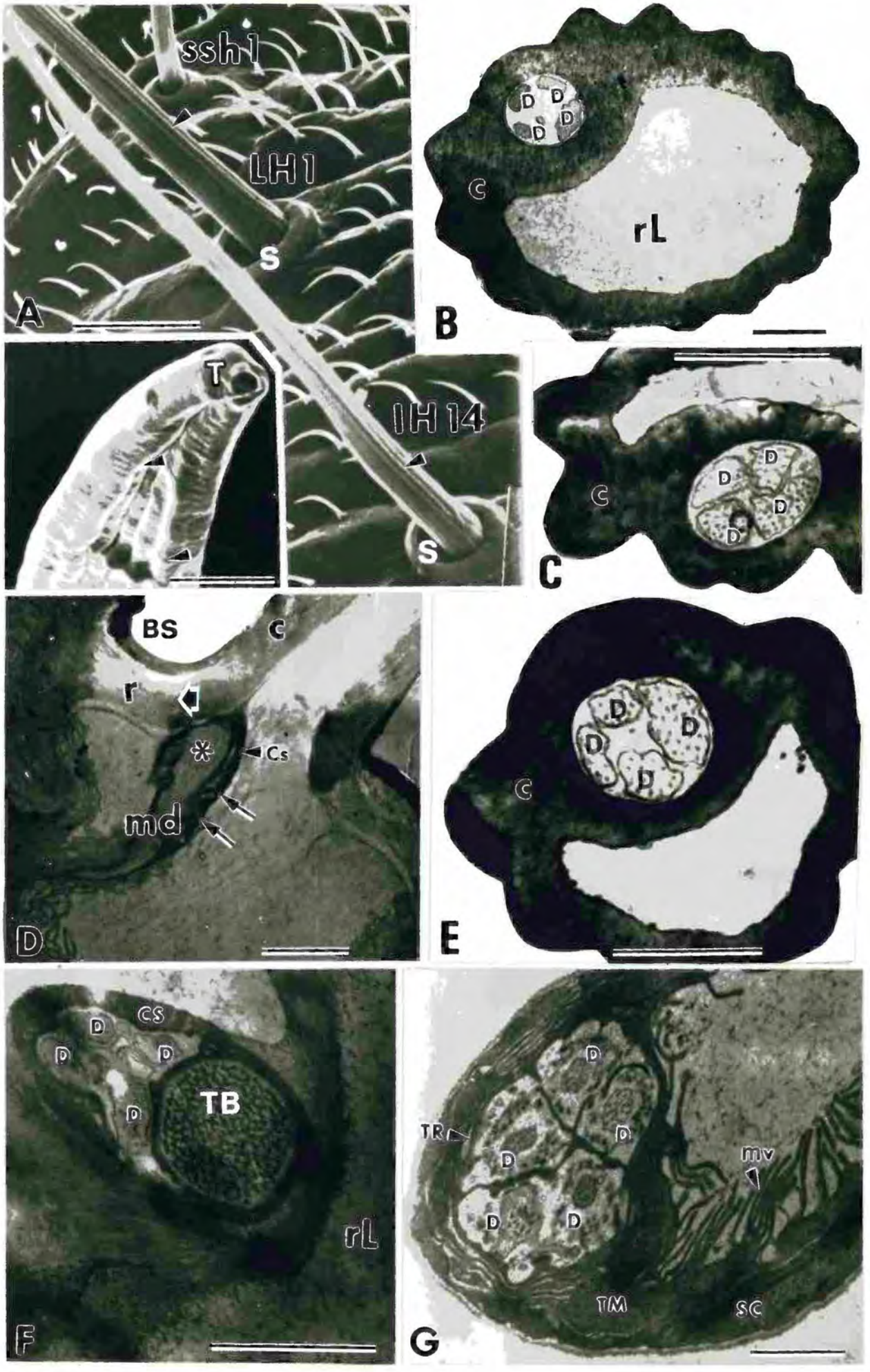
D. Oblique section of a longest sensillum at the socket. Cuticular sheath (cs) around the tubular body (*) of the mechanosensitive dendrite (md) attaches to the bent end of the cuticle (c) of the hair which is joined (broad arrow) to the resilin (r) membrane of the socket (BS). Note hollow spaces in cs on one side (arrows). Bar = 2 µm.

E. Transverse section of hair of short straight sensillum with four dendrites (D). Bar = 1 µm.

F. Transverse section of a marginal ssh just below the cuticle showing neurotubules in the tubular body of a very well developed putative mechanoreceptor (TB) and four gustatory dendrites. cs: cuticular sheath. Note dense substance between tubules. Bar = 1 µm.

FIGURE 5.2 A-G (cont.)

G. TEM section of ssh close to the ciliary region with five dendrites. mv: microvilli of tormogen cell (TM); TR: trichogen cell; SC: sheath cell. Bar = 1 µm.


sshl
LH1
S
A
T
IH14
S
B
D
D
D
D
C
rL
C
D
D
D
D
C
BS
C
r
Cs
md
D
C
D
D
D
D
E
CS
D
D
D
D
TB
rL
F
D
TR
D
D
D
mv
D
TM
SC
G

Marginal ssh sensilla which, are bent towards the oral surface, have a putative mechanosensitive dendrite with a tubular body of relatively large cross-sectional area (ca 0.57 sq. μm) and a large number of neurotubules compared to the other dendrites (fig. 5.2F).

The other lumen of the sensilla, which has larger cross-sectional area, contains an electronlucent substance only (figs. 5.2 B, C, E, F).

5.3.2.2 Bristle (b) and short bristle sensilla (sb)

Bristle sensilla have external morphological features that are similar to those of the longer sensilla except for the dimensions (fig. 5.3 A). Very short bristles are less than 10 μm long and can be put into three morphological sub-types depending on the morphology of the tip. Sub-type I has a nipple-like tip similar to other types of sensilla and is of variable length. Sub-types II and III have 2 and 3 projections of the tip respectively (figs. 5.3 B,A inset).

Observations with TEM showed four putative chemosensitive dendrites in the cross-section of a bristle sensillum. Although no other sections were obtained for this type of sensillum, it is suspected that these sensilla are also innervated by a mechanosensitive neurone.

Very short bristles of sub-type I have folds on the cuticle surface. Sub-types II and III have smooth walls and an expanded base in a wide socket (fig. 5.3A,B,E). They are gustatory sensilla, as indicated by the presence of a pore of about 100 nm at the base of one of the projections of a sub-type II short bristle (fig. 5.3 B). A cross-section of sensillum shaft

FIGURE 5.3 A-G

Electron micrographs of bristle labellar sensilla of male *D. tryoni*:

A. Scanning electron micrograph of part of a labellar lobe with sub-type I (sb) and sub-type II (arrow) short bristles and a marginal ssh sensillum. mf: marginal flap; ms: marginal strip. Bar = 20 µm.
Inset: Tip of type III sb with three projections (*). Bar = 2 µm.

B. SEM of type II sb with smooth wall and an expanded base in a wide socket (S). The bifurcated tip has a pore (p) at the base of one projection. Bar = 1 µm.

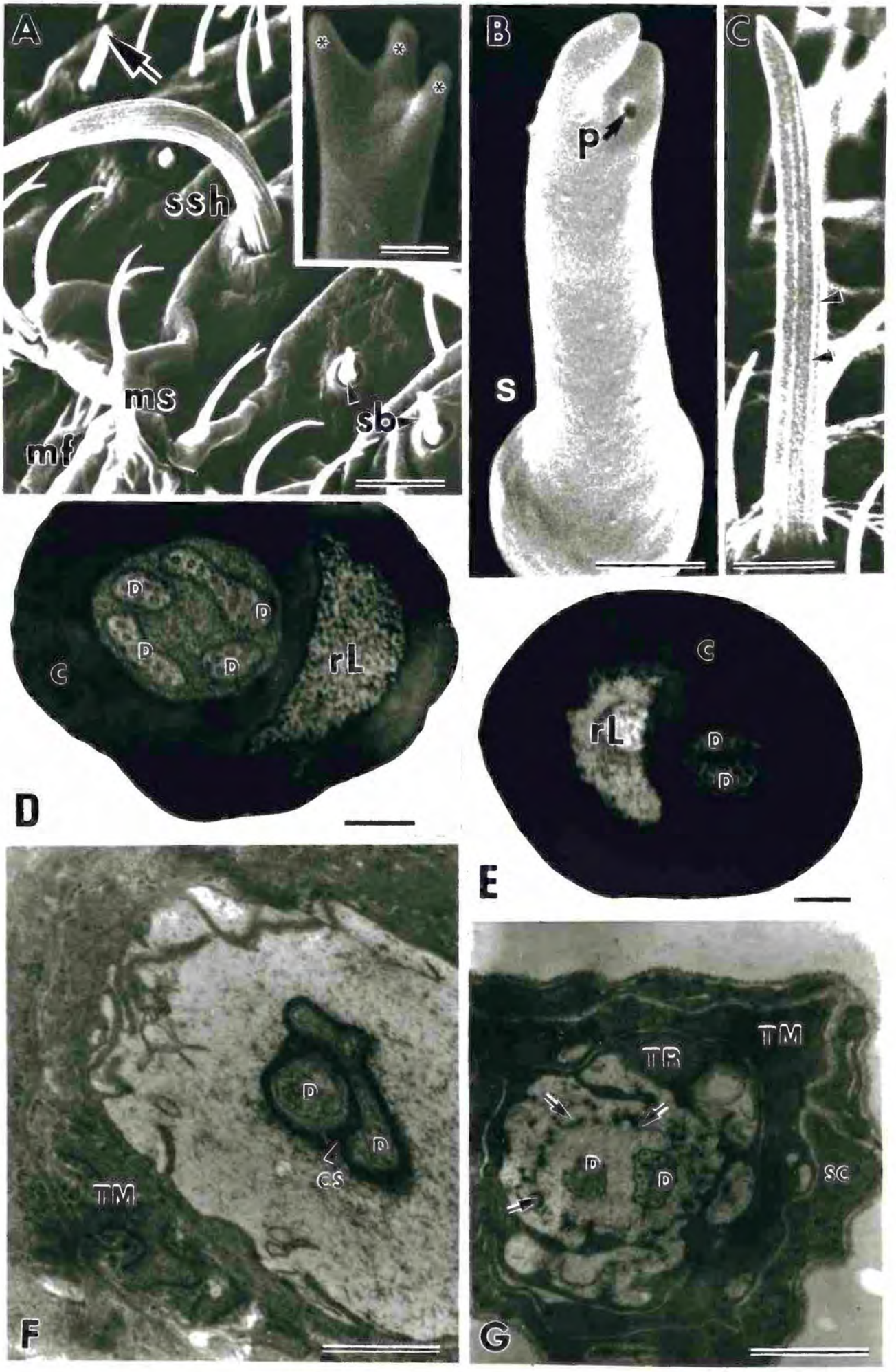
C. Bristle sensillum with longitudinal ridges and nipple-like tip. Bar = 10 µm.

D. Transverse section of a bristle (b); TEM showing 4 gustatory dendrites (D) and dense granular substance in the receptor lymph lumen (rL). c: cuticle of the hair shaft wall. Bar = 0.2 µm.

E. TEM of transverse section of a short bristle sensillum with two gustatory dendrites (D). Smoothness of external wall of the peg is conspicuous. Bar = 0.2 µm.

F. TEM of a transverse section of the epidermal complex of a short bristle just below the socket level. Each dendrite (D) is enclosed separately in a cuticular sheath (CS). TM: tormogen cell. Bar = 1 µm.

G. Transverse section of sb slightly above the ciliary region at the origin of the cuticular sheath (arrows). D: dendrites; TR: trichogen cell; TM: tormogen cell; and SC: sheath cell. Bar = 1 µm.


A
ssh
ms
mf
sb
B
p
s
C
D
C
rL
E
C
rL
F
TM
CS
G
TR
TM
SC

shows two dendrites filling the dendritic lumen (fig. 5.3 E). Further sections above and at the ciliary region show the two dendrites (fig. 5.3F, G), indicating that bristle sensilla are innervated by two gustatory neurones only and therefore may not play a role in mechanoreception. This is an interesting supposition because the broadened bases of these sensilla are inserted in relatively wide sockets that can allow for multidirectional displacement of the pegs. A broader study on the innervation of bristle sensilla may be necessary to determine whether the whole range of them lack mechanosensitive innervation.

The above results sample sensilla representative of the different morphological types and show that, except for the short bristle sensilla which have only two gustatory neurones, the fringe labellar sensilla perfom a dual function of chemo- and mechanoreception, having four putative gustatory neurones and one putative mechanoreceptor neurone each.

5.4 DISCUSSION

The innervation of the fringe sensilla of male *D. tryoni* is the same for five of the morphological types of sensilla. However, the pattern of firing in response to a chemical stimulus may differ from one type of sensillum to another, which may partly depend on the length and cable properties of their dendrites. The effect of variable lengths of dendrites of different sensilla types may be partly offset by their cable properties. Research remains to be done.

In these sensilla, the chemoreceptor to mechanoreceptor ratio is 4:1. This shows that chemical stimuli play a major role in food recognition by these flies. The mechano-

receptors are used for detection of texture of the food substrate. The marginal short straight sensilla have a particularly large mechanosensitive dendrite and are positioned along the marginal strip. They are also curved halfway along the shaft, so that when the labellum is in the feeding position, the tips are in contact with the food. The relatively large cross-sectional area of the tubular body of this dendrite and the high density of neurotubules indicate that these sensilla may play a major role in the mechanoreceptor field of the labellum. Rice (1975) suggested that mechanoreceptors with such densely organised cytoskeletons, filling the receptor terminals, are highly sensitive such that even the slightest deflection of the receptor terminal bends and stretches the receptor membrane. Thus, the short straight sensilla are well positioned and equipped for the detection of both chemical and fine tactile stimuli throughout the feeding process.

The structural attachment of the cell membrane of the mechanosensitive dendrite to the base of the sensillum shaft, in a longest sensillum and others in this study, seems to conform partly to the bend/stretch transduction mechanism in mechanoreceptors, hypothesized by Rice *et al*. (1973a). Leverage of the sensillum shaft on the tubular body of the mechanosensitive dendrite, functions as a depressible fulcrum and seems to lead to stretching of the membrane in addition to any slight bending of the cytoskeleton that may occur. The unattached end-circumference of the sensillum shaft acts as a fulcrum (F2), about which displacement of the hair may occur in any direction away from the point of attachment of the

mechanoreceptor (fig. 5.4 A). When the sensillum shaft is displaced in the direction D1, its leverage on the tubular body of the mechanosensitive dendrite (md) causes displacement of the end part of the shaft (*) in the direction X1. The pressure (p1) created, depresses the tubular body within the cuticular sheath (cs) in its direction; which thus plays the role of a depressible fulcrum (F1) (fig. 5.4 A). Since the cuticular sheath is attached to the base of sensillum shaft by ligament-like strands, displacement of the tubular body and the tilting of the sensillum shaft together with the cuticular sheath stretches the dendritic membrane (dm) as shown (ST1). This may facilitate movement of ions across the membrane and therefore, its depolarisation. Similarly, for a displacement in the direction D2, the component P.cosine θ of pressure P exerted on md along the axis of the neurotubules, depresses it (fig. 5.4 B). Together with the shifting of (*) in direction X2, a stretch in the direction ST2 of the membrane may occur, also leading to depolarisation. The magnitude of depolarisation would depend on the resultant stretch which in turn depends on the rigidity (density of neurotubules) of the tubular body of md and the initial displacement of the sensillum shaft.

Another structural observation worthy of comment is the innervation of the short bristle sensilla of male D. tryoni by only two chemosensitive neurones. These sensilla are located along the marginal strip of the labellar lobes and their length (less than 10 μm), is such that they do not seem to contact food substances during feeding. However, it seems likely that when a fly is feeding on large droplets of fluids, the marginal strip may be submerged and neurones in these sensilla

FIGURE 5.4

A. Diagram (not to scale) of a longitudinal section of the innervation of the base of a longest sensillum on the labellum of male D. tryoni. It shows attachment of the cuticular sheath (cs) over the tubular body of the mechanosensitive dendrite (md) to the base of the cutile (*) of a hair shaft as observed in electronmicrographs (e.g. fig. 5.2 D).

The mechanics of the pressure exerted on the tubular body by end of the hair when displaced in either direction are illustrated and are as explained in the text:

BS: basal socket of sensillum; C: cuticle; D1, D2: displacements by forces in the respective direction; dm: dendritic membrane; F1: tubular body of md functions as a depressible fulcrum for end of hair *; F2: fulcrum, point on unattached end of hair base; L: ligament-like strands attaching cs to hair shaft; p1, P: resultant pressures on tip of md due to D1 and D2; P.Cos θ: component of P along the neurotubules when P acts at an angle θ degrees; r: resilin; ST1, ST2: resultant stretch on the membrane; X1, X2: slight displacements of end of hair over tip of the dendrite.

B. Resolving of component of P along the axis of the neurotubules (m).

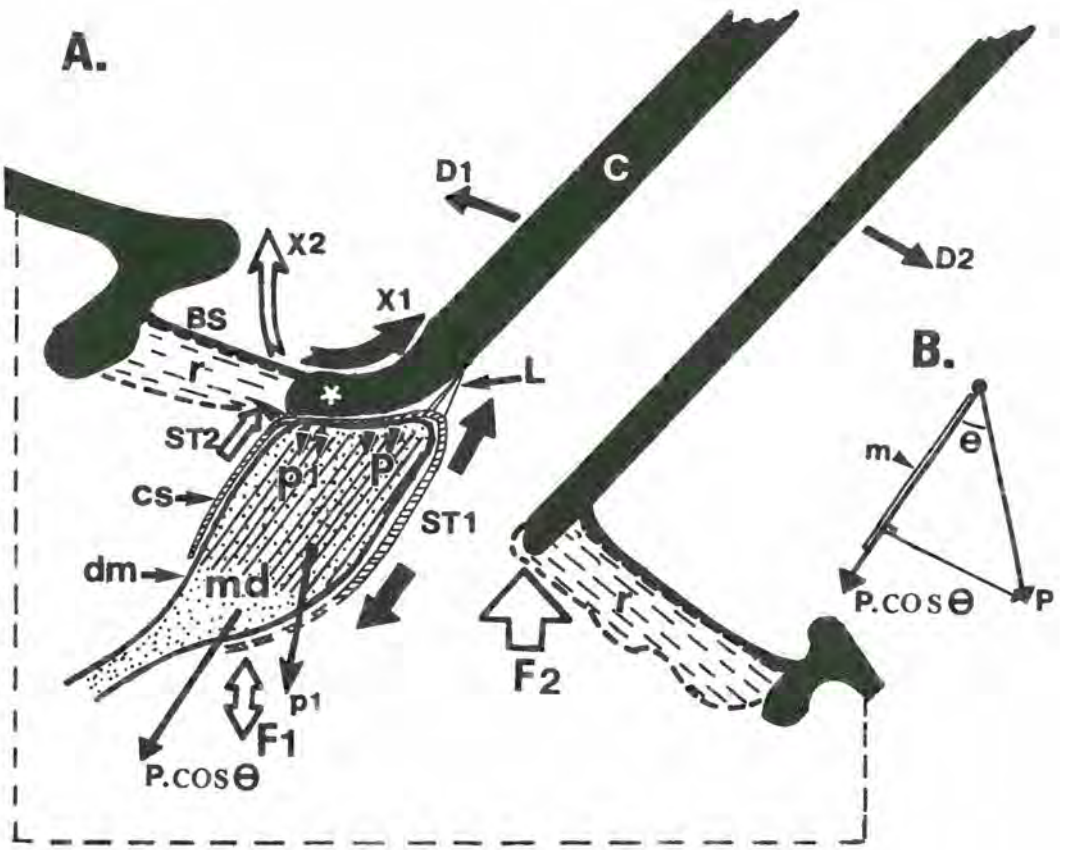


FIGURE 5.4

stimulated. On the other hand, there is a possibility of capillary rising of the liquid material on the cuticle of the labellar lobes. This may also lead to stimulation of these sensilla. Stimulation of mechanoreceptors in the other sensilla may not be adequate to indicate relative level of liquid food on the substrate with respect to the marginal strip. Therefore sensory impulses from neurones in the short bristle sensilla to the CNS may have the role of a gauge, indicating the level (quantity) of the food. In this way the motor feedback generated may play a part in the positioning of the oral surface on the food and the shaping of the labellar lobes for suction.

The innervation of most labellar sensilla of male D. tryoni by four chemosensory neurones is similar to that of some other dipterans: S. calcitrans (Adams et al. 1965); largest (IV) sensilla of Ph. regina (Felt and Vande Berg 1965); the marginal sensilla of C. erythrocephala (Peters and Richter 1965), and type A (long) sensilla of D. melanogaster (Falk et al. 1976). As suggested earlier for the total number of fringe labellar sensilla, similarity in the pattern of innervation of most of the groups of sensilla may indicate a common ancestor or a convergence in function. Thus, during speciation, food specialization and changes in feeding habits by some of the above species may have been accompanied by changes in sensitivity of gustatory neurones to certain chemicals rather than occurence of drastic changes in numbers or the ultrastructural organisation of neurones. Low numbers of sensilla are characteristic of relatively small flies e.g. D. melanogaster (Falk et al. 1976). In others, there have been

changes in both the total number of sensilla and the ratio of gustatory neurones to mechanoreceptors e.g. 2:9 in G. austeni (Rice et al. 1973). Because flies of this species are of similar size to the blowflies, narrowed food range and dependency on a very specific phagostimulant (ATP) seem to be the underlying factors leading to the above changes (Galun and Margalit 1969; Galun and Rice 1971; Rice et al. 1973; Chapman 1982). Reduction of the labella to smaller size on mouthparts adapted for piercing host tissue may also be a contributing factor.

The results described in this and in previous sections form a basis for electrophysiological investigation of the presence of a cue-lure sensitive neurone in the labellar gustatory sensilla of male D. tryoni. The presence of such a neurone make it necessary to carry out further electrophysiological tests for the characterisation of its responses.

VI. AN ELECTROPHYSIOLOGICAL STUDY OF SOME LABELLAR GUSTATORY SENSILLA OF MALE *DACUS TRYONI*

6.1. ELECTRICAL RESPONSES TO DILUTIONS OF LITHIUM CHLORIDE

6.1.1. INTRODUCTION

Males of D. tryoni are attracted to cue-lure which is the most potent attractant for this species and also for the melon fly D. cucurbitae, which does not occur in Australia. On locating the source of the lure, male flies have been observed and reported to feed "voraciously" on it for relatively long periods (Chambers et al. 1972; Drew 1982; personal observations).

Reports are available on the responses of tarsal and labellar gustatory receptors of some tephritids: D. oleae Gmelin and C. capitata to a number of sugars and salts (Gothilf et al. 1971; Angioy et al. 1978, 1978a). However, little is known of the nature of action potential signals that are generated in neurones of labellar sensilla by organic attractants that flies feed on. The prolonged duration of feeding and the nature of the chemical would be expected to affect the pattern of spike potentials generated and the overall adaptation characteristics of the receptors. Labellar gustatory receptors of Ph. regina respond differently to chemical components of different natural food substances and to pure chemicals (Dethier 1974).

Cue-lure [4-(p-acetoxyphenyl)-2-butanone] being a nonionic compound is electrically nonconducting. In order to carry out tip-recording, electrophysiological tests with nonelectrolytes on gustatory sensilla, it is necessary to choose an electrolyte of a concentration that least interferes with the electrical response of the test chemical. Conductivity of the solution should be such that it enables conduction of electrical signals, evoked by the test chemical from the

receptors, to the pre-amplifier.

Lithium chloride has been shown to be less stimulatory than other monovalent chloride salts to salt-sensitive neurones of some insects. Gillary (1966c) reported that, among the monovalent chloride salts of some alkaline metals: KCl, RbCl, CsCl, NaCl, and LiCl; LiCl was the least stimulatory to the type "I" labellar taste sensilla of Ph. regina. Stimulatory effectiveness was shown to decrease along the series KCl > NaCl > RbCl > CsCl > LiCl. Similar observations were made on the aboral and adoral labellar taste sensilla of C. vicina, (den Otter 1972a), where the sequence of stimulatory effectiveness evaluated statistically was slightly different: KCl > RbCl > CsCl $\geq$ NaCl > LiCl. An order of "increasing saltiness" determined by conditioning experiments on C. vicina has been reported to be NH_4Cl > KCl > NaCl > LiCl (Maes and Bijpost 1979). For this reason LiCl was chosen for use as the carrier electrolyte, the determination of the most suitable concentration being part of the experiment reported here. Getting (1971) and Fredman (1975) used 50mM LiCl to enhance conductivity of test solutions containing sucrose used on labellar gustatory sensilla of Ph. regina. This concentration of LiCl is said to have been non-stimulatory to both the cationic and anionic receptors while the conductivity enabled transmission of electrical signals generated by a water-sensitive neurone. In contrast, Zweypfenning and van der Molen (1980) reported that 50 mM LiCl significantly altered the course of electrical responses in sucrose-sensitive neurones, in different types of tarsal taste receptors of C. vicina.

Thresholds have been found to differ between tarsal and labellar taste sensilla of Ph. regina (Dethier 1976), and it is therefore probable that thresholds differ in homologous sensilla of individuals of labellate dipterans. It was therefore necessary to investigate the electrical responses of males of D. tryoni salt- and water-sensitive neurones to LiCl prior to the main investigation of lure stimulation.

An experiment was conducted with a series of concentrations of LiCl to determine:

1. the number of neurones responding at each of the LiCl concentrations;
2. the variations in responses of salt- and "water-sensitive" neurones in some longest and long labellar taste sensilla to a series of LiCl concentrations;
3. the concentration of LiCl that evokes minimum firing rate of salt-sensitive gustatory neurones whilst facilitating recording of electrical responses from other neurones.

6.1.2. MATERIALS AND METHODS

Male D. tryoni 7-24 days old, were taken singly from a laboratory culture kept at 26 ± 2 °C and 70% R.H. The head was crushed using a pair of fine forceps, to minimize nervous activity from brain and muscles, which interferes with electrical signals from the labellar sensilla. The applied

pressure also opened the labellar lobes giving easy access to the sensilla under study. An Ag-AgCl electrode (silver wire electrolytically coated with a thin layer of silver chloride) was inserted into the thorax through the intersegmental membrane between the metathorax and the first abdominal segment. The electrode was pushed through the neck so that its tip was positioned at the joint between the labellar lobes and the rest of the proboscis. To prevent evaporation of the test solution and drying up of the preparation, a cylindrical mesh cage (10 cm diameter, 9.0 cm high) lined with wet absorbent material (Wettex) enclosed the specimen thus maintaining high humidity around it. Two slits cut down the side of this humidity chamber allowed for the positioning of electrodes.

The conventional tip-recording technique (Hodgson, Lettvin, and Roeder 1955) was used. An Ag-AgCl recording electrode was inserted in a stimulating glass micropipette. Both electrodes were connected via a probe to a P16 D AC/DC differential amplifier (Grass Instruments) operating at a bandwidth of about 3.2 kHz (lower cut-off frequency = 0.1 kHz, upper cut-off frequency = 3.33 kHz) and x100 amplification. For visual display, a Tektronix 5103N oscilloscope with a 5A22N differential amplifier and a 5B12N dual time base was used while auditory monitoring was by means of a loudspeaker. Photographs of traces of responses for analysis were taken with a polaroid camera (Tektronix C-5A oscilloscope camera). Recording periods were less than three seconds and durations between consecutive stimulus applications to the same sensillum were at least five minutes to allow for the disadaptation of the neurones.

The three longest sensilla largely used for recording are LH2, LH5, and LH8 whilst for the long sensilla; 1H10, 1H15, 1H17, 1H33 and MH24 were used. Almost all of these sensilla are in rows IIIA and IIIB on the labellar lobes (Ch. 3).

The series of LiCl (of Laboratory grade) concentrations was made up in distilled water (containing 10 ppm. of "POW" detergent, U.M.I Chemicals, Brisbane) by stepwise dilutions. Number of spikes occurring in the one second after the initial, disregarded, 50 milliseconds of contact between the test solution and the tip of a sensillum were counted and dose/response curves plotted. Spikes were sorted into categories (largely three) depending on their peak to peak amplitude, the magnitude of the positive after-potential, and regularity of interspike periods for recordings in which the different spike types were present. Firing rates of spikes recorded from longest and long sensilla were averaged separately. The Student's t-test was used to test for significance between mean firing rates of corresponding neuronal categories in the two types of sensilla. The numbers of flies used for electrophysiological tests with all the chemicals are in Appendix VI.

6.1.3. RESULTS

6.1.3.1. Recognition of different categories of spikes

The spikes denoted by "n" were from a neurone whose response has a negative gradient with respect to increase in concentration of LiCl and small amplitude in the range 2.5-4.0 mV (figs. 6.1 - 6.5). The positive after potential at 5.0 and 10 mM LiCl was indistinct, presumably because of the low conductivity at these low concentrations leading to relatively

large fluctuations of the baseline. At these two concentrations, the spikes from this neurone were of high frequency (50 or more impulses in the first second). Where spikes from this neurone occurred at concentrations of LiCl greater than 50 mM, they were biphasic (positive after-potentials of up to 2 mV) and the amplitude of the depolarization phase was as at the lower concentrations. However, the firing rate was reduced to about 10 spikes per the first second (fig. 6.2d, Table 6.1).

Two other neurones were recruited into firing when the concentration of LiCl was increased to 50 mM and above. Spikes labelled "P1" were those from a neurone whose frequency of response was positively sensitive to increase in concentration of LiCl (figs. 6.1, 6.2, 6.4 and 6.5). These always occurred at 50 mM and generally at the higher concentrations but in some recordings, at 500 mM and molar LiCl, this neurone did not respond. Spikes were of amplitudes ranging between 4.0 and 6.5 mV, and positive after-potentials of between 1.9 and 2.5 mV. A second salt-sensitive neurone "P2" also had a firing activity that increased positively with increase in concentration of LiCl. Spikes from this neurone had higher amplitude than that of P1 spikes: 6.9 - 9.0 mV with positive after-potentials of 2.5 mV. Firing rate was 5-60 spikes in the first second at 100 mM, 500 mM, and molar LiCl (figs. 6.1, 6.2, 6.4 and Table 6.1). Reduction in amplitude of the spikes from this neurone was evident at 500 mM and one molar, in recordings in which the firing rate was greater than 30 spikes in the first second.

A fourth neurone denoted as Lp (large spike

FIGURE 6.1 Representative traces of electrophysiological recording from longest sensillum no. 8 on the right labellum of a male D. tryoni, 9 days old post-eclosion to LiCl: a. 5 mM; b. 10 mM; c. 50 mM; d. 100 mM; e. 500 mM; and f. one molar. "n" indicates neurone negatively sensitive to increase in LiCl concentration; P1, P2 denote those positively sensitive to increase in concentration of the salt. In this example, lowest overall firing rate was at 100 mM. The two traces presented for each concentration are the same but of different baseline scale; horizontal bar is 20 msec. for all upper traces (U.T.) and 0.1 second for the lower traces (L.T.).

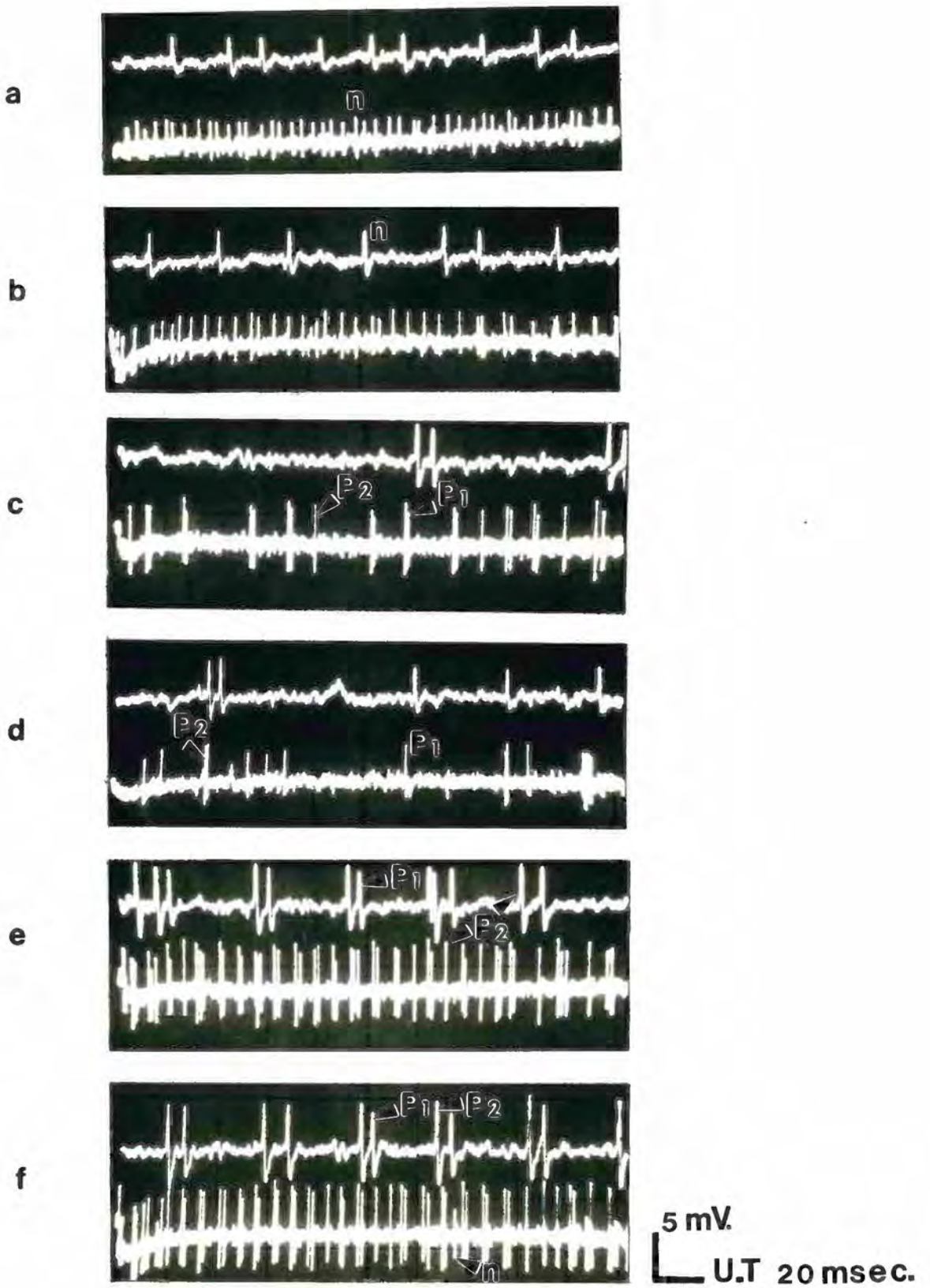


FIGURE 6.1

FIGURE 6.2 Representative traces of electrical responses from a long sensillum no. 33 on the right labellum of a 10 day post-eclosion male D. tryoni to LiCl: a. 5mM; b. 10mM; c. 50 mM; d. 100 mM; e. 500 mM; and f. one molar. Response of neurone "n" negatively sensitive to increase in concentration of LiCl was higher at low concentrations; positively salt-sensitive cells P1 and P2 had firing that increased above 50 mM. Unlike some longest sensilla responses (fig. 6.1); the overall firing rate in this long sensillum was lowest at 50 mM LiCl. "s+" are summations of spikes from two different neurones. Time base scale for upper traces (U.T.) and lower traces (L.T.) are as shown.

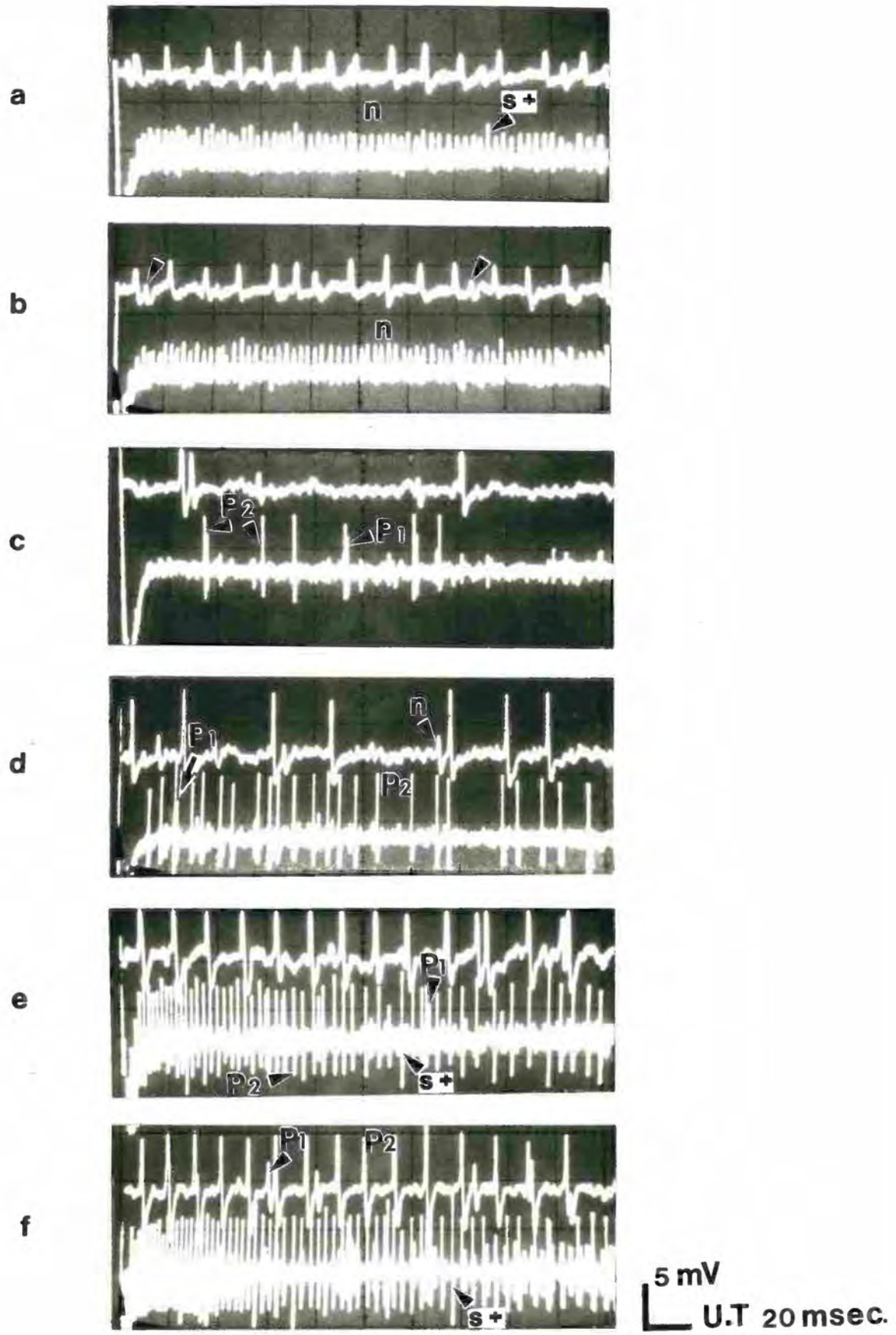


FIGURE 6.2

potential), fired randomly and was largely recognisable in traces where the other neurones n, P1, and P2 were also firing (fig. 6.3). The amplitude of spikes was up to ten millivolts. Its firing was observed at 100 mM and molar LiCl for some longest sensilla and only at the latter concentration from one long sensillum. When active, the neurone fired one or two spikes in the first second of recording for the above concentrations. The low mean firing rate of Lp when present, only at one or two concentrations of LiCl, made it impossible to include a meaningful dose-response graph in figs. 6.4A,B.

6.1.3.2. Probit vs. log (mM. concentration of LiCl) curves

For each of the neurones categorised above, the relative percent response (Ri) of the mean was calculated at each of the LiCl concentrations as: Ri = fi/fm x 100 %; where fi is the mean firing rate of a given neurone at each of the concentrations (i = 1, 2,---,6) from Table 6.1; and fm is the maximum mean firing rate obtained for each neurone over the range of concentrations used. The Ri values were then converted to probits (Table I in Finney 1971) and the values plotted against log (mM concentration) of LiCl (figs. 6.5A,B). Neurones have been named with regard to the gradients of their probit vs. log (mM conc.) plots (Tables 6.2A,B; fig. 6.2A,B): n-neurone negatively sensitive to increase in LiCl concentration; P1, P2 - those with positive sensitivity, with spike amplitude of P2 greater than that of P1. Equations Y1, Y2, and Y3 were calculated for the linear probit plots of the three neurones n, P1, and P2 respectively in longest sensilla. Corresponding equations Y' for neurones in long sensilla were also calculated.

FIGURE 6.3

a. Electrophysiological recording trace from a longest sensillum no. 2 of a male D. tryoni to molar LiCl. P1, P2 indicate spikes from the two salt-sensitive neurones as in the above records and Lp a neurone which was sometimes present but of rare occurrence.

b. A representative trace of an electrophysiological recording from a longest sensillum, no. 8 on the right labellar lobe of another male D. tryoni, nine day post-eclosion. Only neurone P2 was firing at a moderately high frequency with a resultant reduction of amplitude of spikes.

Time base scale: 20 msec for upper traces (U.T.) and 0.1 second for the lower traces (L.T.).

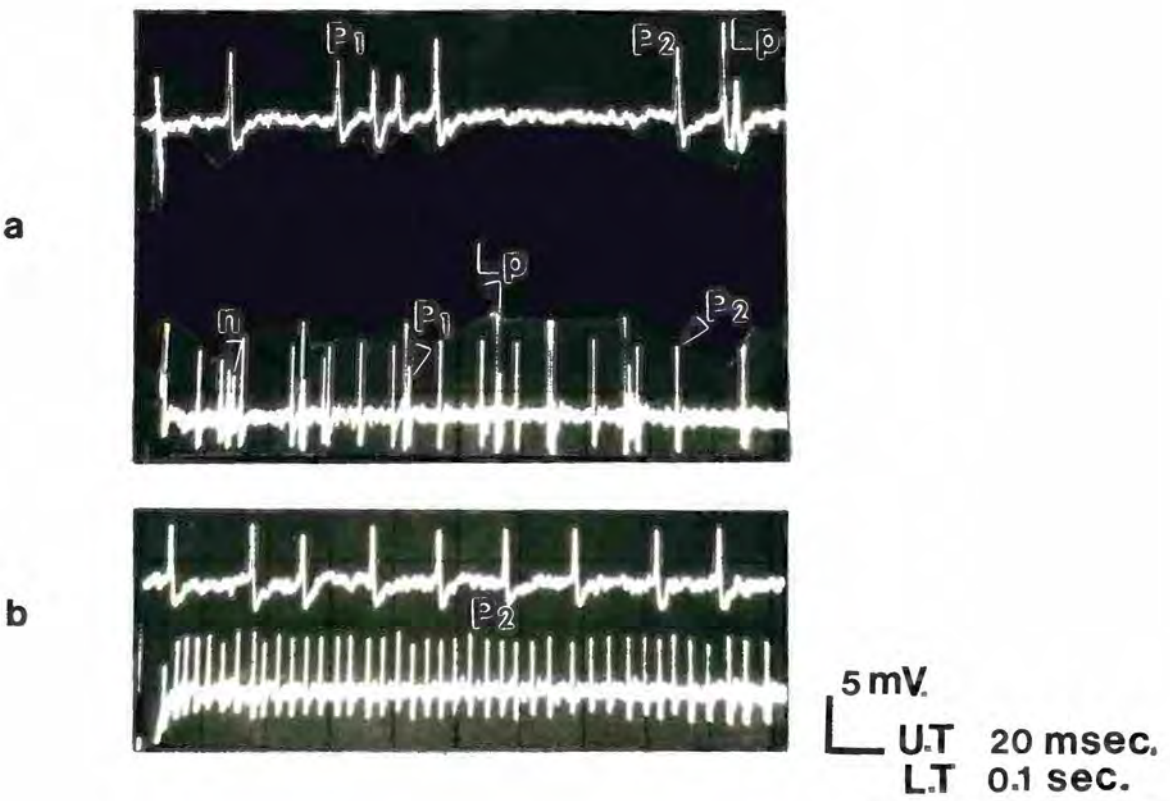


FIGURE 6.3

6.1.3.3. Dose-response curves

6.1.3.3.1 Neurone "n"

Table 6.1 shows the mean numbers of spikes from some longest and long labellar taste sensilla for six concentrations of LiCl. Neurone "n" had highest sensitivity at the lowest concentration (5 mM) tested and may be considered to be a "water receptor" (figs. 6.4A,B and 6.5A,B). For each of the six concentrations, the mean number of spikes recorded from this neurone was not significantly different in the two types of sensilla (Table 6.1).

Increase in LiCl concentration led to an initial steep decline in the firing frequency which then declined more gradually, eventually to zero at the highest concentration used, or much higher as estimated from the probit plots (figs. 6.4A,B and 6.5A,B). On the probit/log-dosage plots (figs. 6.5A,B), the response curves for this neurone clearly had negative gradients: -2.24 and -2.82 for longest and long sensilla respectively and was therefore denoted as negatively salt-sensitive. The respective regression equations of the lines were calculated as $Y1 = 8.75 - 2.24X$ and $Y1' = 9.23 - 2.82X$, where Yi is the probit units, X the log (mM conc.) units. Extrapolation of these linear plots to intercept the x-axis made it possible to estimate concentrations (Ca) of LiCl which would be expected to completely abolish firing of this neurone in the two types of sensilla (figs. 6.5A,B and Table 6.4). These concentrations are 5.0 and 1.23 molar for longest and long sensilla respectively; however, the estimate for longest sensilla appear to have been much higher than would be expected from the response curves (figs. 6.4A,B).

TABLE 6.1 Electrophysiology of longest (L) and long (l) labellar taste sensilla of male D. tryoni: mean number of spikes in first second (after 50 msec. recovery time) of neurones n, P1, and P2 for six concentrations of LiCl. Student's t-test was used on the data for comparison between electrophysiological responses of the three neurones for the two types of sensilla. Errors are ± s.e.

Neurone	n			P1			P2		
Sensilla type	L	l	p(t-test)	L	l	p(t-test)	L	l	p(t-test)
Conc.(mM)									
5	58.09 ± 6.01	58.63 ± 3.65	NS	0	0.25 ± 0.25	≤0.20	0	0	NS
10	55.0 ± 4.36	53.13 ± 6.43	NS	0	0	NS	0	0	NS
50	14.13 ± 2.50	19.43 ± 5.05	≤0.30	6.93 ± 1.57	8.57 ± 4.91	≤0.70	1.60 ± 1.04	1.29 ± 0.84	≤0.80
100	3.93 ± 1.43	6.71 ± 1.72	≤0.30	8.46 ± 2.16	10.0 ± 2.58	≤0.60	4.53 ± 2.01	10.85 ± 3.69	≤0.10
500	0.69 ± 0.61	1.14 ± 0.99	≤0.60	13.77 ± 3.13	11.28 ± 3.71	≤0.70	17.15 ± 3.0	49.14 ± 6.64	S
1000	1.71 ± 1.35	0	≤0.30	16.64 ± 3.63	14.71 ± 4.03	≤0.70	22.14 ± 3.69	52.0 ± 8.36	S

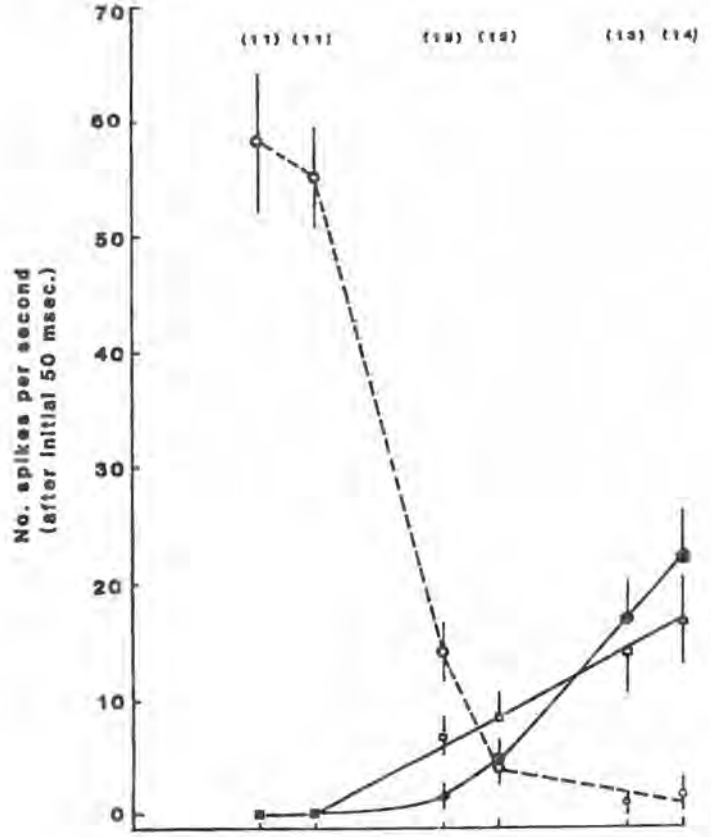
S: Differences of the means significant.

NS: " " " " not significant.

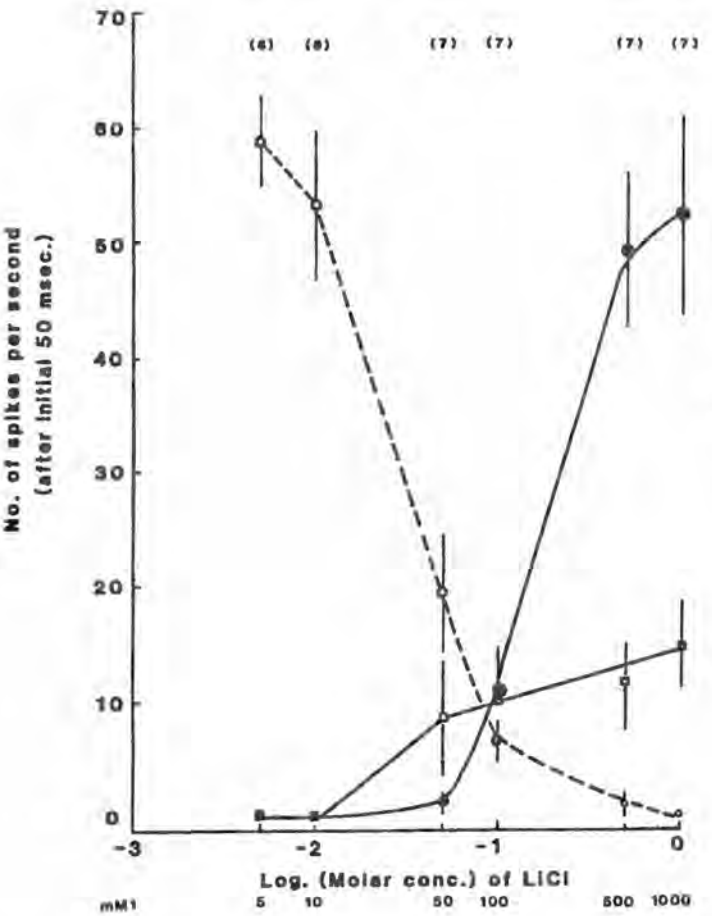
p-levels for nonsignificance are given.

FIGURE 6.4A Relation between concentration of LiCl and number of spikes generated in the first second (after 50 msec. recovery period in some longest sensilla of male D. tryoni. -o--o-- is the response curve of a neurone "n" whose firing decreased progressively with increase in concentration of LiCl; -□—□-, and —⊙——⊙— are response curves of neurones P1 and P2 respectively which fired at concentrations greater than 10 mM and were positively sensitive to increase in LiCl. Bracketted numbers at the top of the abscissa for each concentration are number of sensilla recorded from. Sensitivity of P2 to the salt exceeded that of P1 between 100 and 500 mM . Both figures 6.4A and B share the same scale on the x-axis. Error bars are ±1 s.e.

FIGURE 6.4B Relation between concentration of LiCl and number of spikes generated in the first second (after 50 msec. recovery period) in some long sensilla of male D. tryoni. Notation is as in 6.4A above. In these sensilla, P2 showed very high sensitivity which surpassed that of P1 at 100 mM and higher concentrations of LiCl. Error bars are ±1 s.e.



A. Longest sensilla



B. Long sensilla

FIGURE 6.4

Table 6.2 Analysis of spike firing rates from labellar gustatory sensilla of male D. tryoni stimulated with lithium chloride: Probits (Yi) equivalent of the relative percent response (Ri) calculated for neurones n, P1, and P2 at each of the LiCl concentrations from the mean firing rates in Table 6.1.

A. Longest sensilla

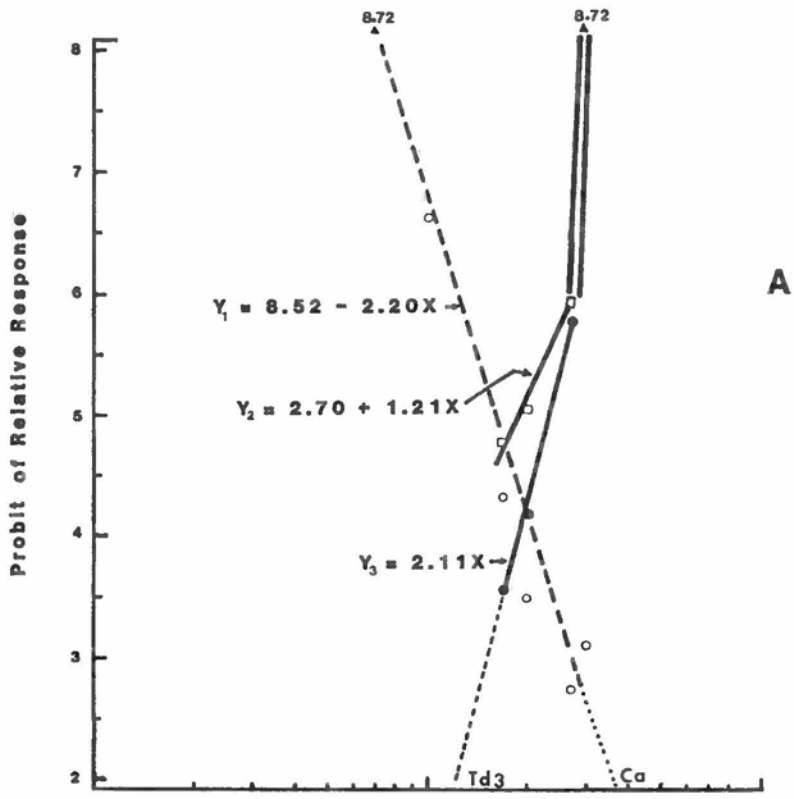
LiCl Conc. (mM)	Relative % Response (Probit equiv.); Ri(Yi) values		
	n-neurone	P1	P2
5	100.0 (8.72)	0 (-)	0 (-)
10	94.7 (6.62)	0 (-)	0 (-)
50	24.3 (4.30)	41.5 (4.79)	7.23 (3.54)
100	6.8 (3.50)	50.8 (5.02)	20.5 (4.18)
500	1.2 (2.70)	82.8 (5.94)	77.5 (5.76)
1000	2.9 (3.10)	100.0 (8.72)	100.0 (8.72)

B. Long sensilla

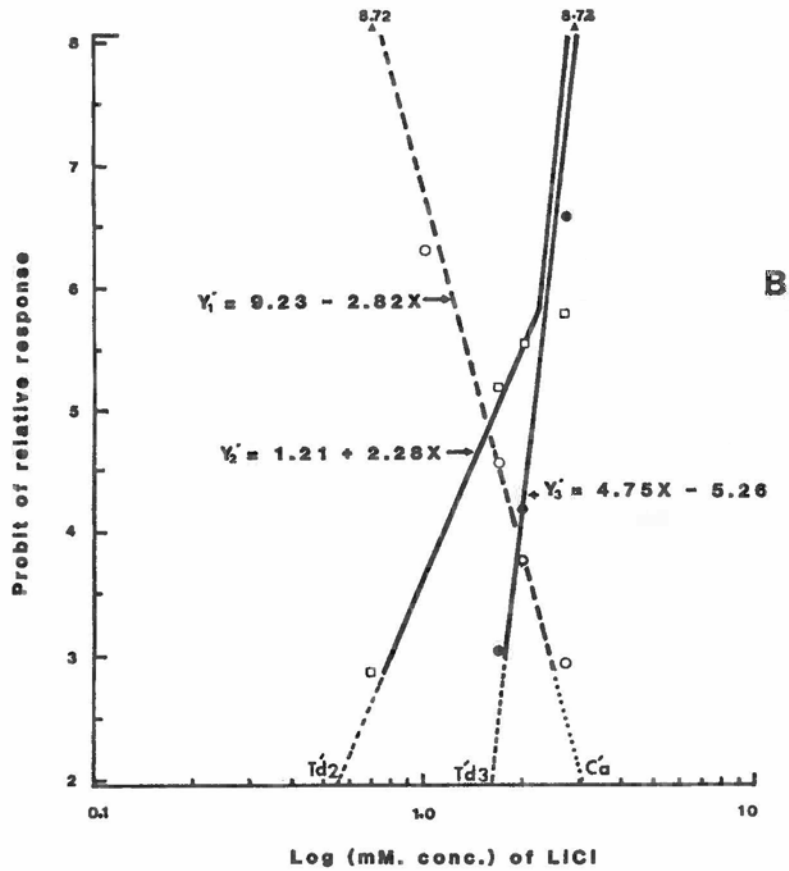
5	100.0 (8.72)	1.67 (2.86)	0 (-)
10	90.6 (6.32)	0 (-)	0 (-)
50	33.1 (4.56)	58.3 (5.21)	2.48 (3.04)
100	11.5 (3.74)	68.0 (5.47)	20.9 (4.19)
500	1.9 (2.93)	76.7 (5.73)	94.5 (6.60)
1000	0 (-)	100.0 (8.72)	100.0 (8.72)

FIGURE 6.5,A Relation between the probit of relative spike firing rate response and concentration of LiCl applied to some longest labellar sensilla of male D. tryoni. -o--o— is the linear plot of neurone "n" having a negative gradient with respect to increase in concentration of LiCl; -□—□— and -•—•- are the linear plots for positively salt-sensitive neurones P1 and P2. The Probit/log dosage equations estimated for the linear plots of each neurone are also included. Extrapolation of these lines to intercept the x-axis made it possible to estimate threshold concentrations (Td) for P1 and P2 and the highest concentration (Ca) of LiCl which would be expected to abolish the firing of neurone "n". Median concentration (M50) for each neurone was estimated from the point of intersection of the probit plots and the ordinate at 5 probit units.

FIGURE 6.5,B A similar probit vs. log dosage relation for some long sensilla of male D. tryoni. All notations are as in fig. 6.5,A above.



A. Longest sensilla



B. Long sensilla

FIGURE 6.5

6.1.3.3.2 Salt-sensitive neurones P1 and P2

Neurones P1 and P2 showed a low rate of firing (less than ten spikes in the first second) at 50 mM LiCl but with P1 having a mean firing rate exceeding that of P2 (about 7 impulses in the first second). Frequency of firing increased in both units with increase in concentration of LiCl. In some sensilla (one out of fourteen longest sensilla and two out of seven of the long ones), the spikes observed at 500 mM and molar LiCl were from P2 only (fig. 6.3,b). A small number of n spikes were present at 500 mM while P1 may have dropped out.

For the longest sensilla, the mean firing rate of P1 neurone at 50 and 100 mM LiCl were higher than those of P2 (significantly different; $t = 2.05$, $p \leq 0.05$; $df = 28$). At 500 mM and molar LiCl P2 had the higher rate (slightly significant; $t = 2.06$, $p \leq 0.05$, $df = 24$). However, the difference between variances of mean firing rates of P1 and P2 at these two concentrations were not significant ($F_{12,12} < 2.7$ and $F_{13,13} < 2.6$; $p = 0.05$) (figs. 6.4A; 6.5A). In long sensilla, P1 and P2 showed similar activity over the range 5 - 100 mM LiCl, the difference between the mean firing rate of the two neurones being slightly significant ($t < 2.2$, $p \leq 0.05$). At 500 mM and molar LiCl, the sensitivity of P2 was much higher than that of P1 (significant; $t = 2.18$, $p \leq 0.05$, $df = 12$) (fig. 6.4B).

Comparing firing rates of corresponding neurones n, P1, and P2 between longest and long sensilla (Table 6.1, figs. 6.4A,B, and 6.5A,B): n and P1 had mean firing rates which were almost the same (no significant difference; $t = 2.1$, $p \leq 0.01$). P2 had mean firing rates which were not significantly different between longest and long sensilla ($t = 2.1$, $p \leq 0.001$) at the

four concentrations; 5, 10, 50, and 100 mM LiCl. However, mean firing rate of P2 was much higher with 500 mM and molar LiCl in long than in longest sensilla (Table 6.1). Differences between firing activities of these neurones in the two types of sensilla are also reflected by the estimated values of the "median concentration" (M50) of LiCl and gradient (s) for each neurone. This is a concentration of LiCl at which a given neurone would be expected to fire at a frequency 50% of the mean of the maximum number of spikes recorded from it in the first second over the range of concentrations used (Table 6.3 from figs. 6.5A,B).

Table 6.3 Spike firing rates from labellar sensilla of male D. tryoni stimulated with lithium chloride: Estimated Median concentrations (M50) of LiCl for neurones n, P1, and P2 and gradients (s) of the probit vs. log (mM. conc.) plots for each of these neurones for longest and long sensilla.

Neurone	Longest sensilla		Long sensilla	
	M50 (mM)	s	M50 (mM)	s
n	46.77	-2.24	31.67	-2.82
P1	79.43	1.21 *	45.71	2.28 *
P2	234.4	2.11 *	144.6	4.75

* gradient (probits/unit log (conc.)) of the linear part of the plot crossing the 5.0 probit ordinate.

From Table 6.3 above, the M50's for P1 and P2 for the long sensilla are about 60% of the respective values for the longest sensilla. Gradients obtained for P2 in both types of sensilla were about twice those of P1 for each neurone.

Further, differences in the dose-response curves for P2 in the two types of sensilla led to the occurrence of the cross-over point of P1 and P2 curves at about 100 mM in long sensilla compared to between 100 and 500 mM in longest sensilla (figs. 6.4A,B). From the mean firing rate data and the statistical analysis (Table 6.1), 50 mM LiCl elicited the lowest firing rates in both P1 and P2. That of neurone "n" was in the range 0 - 36 spikes in the first second (mean: 14.13 $\pm$ 2.50 for longest sensilla and 19.43 $\pm$ 5.05 for the long sensilla). On the other hand, during electrophysiological recording from longest sensilla, about 40% of the recordings with 100 mM showed an overall minimum firing rate (sum of all spikes from the three neurones) which was less than ten spikes in the first second. Choice of 50 mM LiCl as the carrier electrolyte was because it elicited the minimum detectable firing in P1 and P2 irrespective of the rate in the n-neurone.

In addition to some of the parameters mentioned above, that can be estimated from the probits vs. log concentration plots (figs. 6.5A,B), another quantity can be estimated from extrapolation of the linear plots to intercept the x-axis: Td, the threshold concentration(s) of LiCl above which neurones P1 and P2 would be expected to start firing (or strictly respond at 0.1% of the maximum firing rate) (Table 6.4). Threshold values estimated for P1 in longest and long sensilla were almost equal while that of P2 in long sensilla was

2.5 times higher than that of the corresponding neurone in longest sensilla. For the two sensillar types, the estimated threshold for P2-neurone was higher than for P1 by a factor of 4 and 10 for longest and long sensilla respectively.

Table 6.4 Analysis of spike firing rates of labellar sensilla of male D. tryoni to lithium chloride stimulation: Estimated threshold (Td) concentration values for neurones P1 and P2 and the vanishing concentrations "Ca" of LiCl for activity of neurone "n"; by extrapolation of the linear probit plots in figs. 6.5A,B.

Neurone	Longest sensilla		Long sensilla	
	Td (mM)	Ca (mM)	Td (mM)	Ca (mM)
n	-	5,011.9	-	1,258.9
P1	4.17	-	3.63	-
P2	15.85	-	39.81	-

6.1.4. DISCUSSION OF DOSE-RESPONSE CURVES OF NEURONES n, P1, AND P2 TO DILUTIONS OF LITHIUM CHROLIDE

The results presented here show that, in both "longest" and "long" labellar gustatory sensilla of male D. tryoni, salt (LiCl) sensitive neurones (P1 and P2) had thresholds between 5 and 50 mM. However, the lower limit of this range appears to have been underestimated from the probit plots

for P1 in the longest sensilla since from Table 6.1 and figs. 6.4A,B, it would be expected to be greater than or equal to 10 mM. This may be attributed to the reduction of the number of points after conversion of percent-relative-response values to probits. Unavailability of points at both ends of the concentration range made fitting of the lines difficult and this may have contributed to overestimation or underestimation of some of the quantities presented above. For the negatively salt-sensitive (water-sensitive) neurone, firing activity was highest at the lowest concentration (5 mM.) of LiCl tested. Concentrations of LiCl less than 5 mM were not tested because it was envisaged that the resistance of the solution would be too high to be suitable for susbsequent lure studies. Threshold sensitivity may be characteristic of the particular receptor, the species of insect, and the physiological state of the individual (Dethier 1974a; Stoffolano et al. 1978; Angioy et al. 1983). Ambient conditions (humidity and temperature) and the pH of the test solution are other factors that may affect the threshold measurements (Gillary 1966a,b; Shiraishi and Morita 1969; Uehara and Morita 1972).

At 50 and 100 mM LiCl, the P1 neurone was equally or slightly more sensitive than P2. For concentrations of 500 mM and one molar, the reverse was observed. This difference is more evident for the long sensilla for which the gradient (probit units/unit log (mM conc.) for P2 was 2.5 times that of P1 for the section of the linear plots falling on the abscissa for 500 mM and that for molar LiCl (figs. 6.5A,B).

There are at least two possibilities in explaining

the observed differences in the sensitivities of the two P neurones. Firstly, the two neurones may be stimulated by the same ions in solution so that differences in firing would solely depend on the inherent electrical characteristics of each cell. Secondly, each of the two neurones in the same sensillum may be acted upon differentially by the cations or anions of the stimulating substance(s); Lithium or Chloride ions in the present case. Firing characteristics of the cells to the presence of these two ions may vary differently with increase in concentration of LiCl. The grading or stratification of sensitivity between neurone types could provide an efficient coverage of a wider range of stimulus strengths that the insects are most likely to encounter in their natural environment. Having these two neurones (P1 and P2) in the same sensillum could therefore be advantageous because, it implies maintenance of less sensilla, with 4-5 neurones, rather than a larger number of two physiological types each having one of the neurones. The second possibility may not necessarily mean coverage of a wider range of stimulus strength but entails assessment of the "quality" (composition) of the stimulus. This may facilitate a sharper discrimination between compounds with closely related molecular structure. The differences between P1 and P2 may stem from differences in receptor membrane molecules and/or from the dimensions of the sensilla and therefore the "effective dimensions" of the dendrites within them. The cable properties are presumably the same for dendritic membranes of the two neurones. Because these determine the nature of transmitted electrical signals, distance between the point of initiation of the receptor potential at the distal ends of dendrites and the

location of the "action potential initiation zone" may play an important role. This distance depends on the length of the dendrite and may affect the temporal characteristics of transmission of action potentials (Fujishiro et al. 1984).

The possible advantage of such grading in sensitivity of neurones is that those neurones which are more sensitive to a low stimulus strength may contribute to an intensification of the spikes generated. Processed at higher centres, this input may facilitate detection of minute quantities of substances. At relatively high concentrations (e.g. greater than 100 mM LiCl in the present study), high sensitivity of another set of neurones could ensure fast and proper screening of the "quality" (ionic composition and concentration) of the stimulus. This may in turn allow for the appropriate behavioural activity (e.g. rejection of high concentrations of salts) to be elicited within a few milliseconds of contact; in the process giving rise to avoidance of full adaptation and possible injury of the receptor membrane. High frequency spike discharges have been suggested to elicit rejection of concentrations of substances (salts, glycoalkaloids) that evoke such electrical activity (Dethier 1976; Blaney 1980; Mitchell and Sutcliffe 1984; Mitchell and Harrison 1985).

These results show that, in addition to morphological differences between longest and long sensilla, physiological differences in the electrical activity of their neurones are also implicated. Differences in responses of salt-sensitive neurones have been reported for various morphological types of labellar gustatory sensilla of C. vicina (den Otter 1971; Maes

and den Otter 1976; Maes and Vedder 1978). They showed that the frequency of spikes in response to molar KCl was in the order: Longest sensilla (L) > long (l) and Intermediate sensilla > bristle (b) and marginal sensilla (M). Response to a range of concentrations between one millimolar and molar sucrose reported for the labellar sensilla of C. vicina (Shiraishi and Tanabe 1974) are slightly different; the frequency of spikes was highest in marginal sensilla: M > L >> I = d (different). Probably the differences in responses to the two stimuli (salt and sugar) is that, the sensory input of the marginal sensilla in detection of sugar(s) (which elicit feeding behaviour) is more important in the final decision-making, as opposed to salts where detection by longest and perhaps some long sensilla (which are first to contact substrates) may be adequate to elicit rejection behaviour. Thus, contamination (or "unnecessary" adaptation) of the shorter sensilla, which usually are more responsive to substances that elicit feeding, can be avoided.

Decrease in the firing rate of the negatively salt-sensitive neurones, as solute concentration increases, is a possible means of centrally enhancing the input from neurones sensitive either to individual solutes or to some ions in a given solute (Evans and Mellon 1962). Neurone "n" in both longest and long sensilla showed a 50% drop in firing rate (with respect to that at 5 mM) at concentrations between 10 and 50 mM estimated to be 46.8 and 31.6 mM for longest and long sensilla respectively. Here, the responses of P1 and P2 have frequencies which are barely five spikes in the first second. Therefore, the activity of neurones negatively sensitive to the increase in concentration of LiCl may enhance the central effects of the

positively salt-sensitive ones. Neurone "n" also maintains the continuity of sensory information flow into the CNS on changes in concentration being monitored over the whole range.

Values of Td and Ca estimated for P2 and n from the probit plots were in good agreement with those in Table 6.1. However, the Td value for the P1-neurone from the longest sensilla seems to have been underestimated, since from Table 6.1 it would be expected to be greater or equal to 10 mM. Despite such a large difference (of about 50% in this case), "probit analysis" seems to offer more parameters for characterization of neuronal responses to test substances than the other graphical methods. den Otter (1968, 1972a) and Maes and den Otter (1976) have used the terms "positive and negative correlation" to describe the activity of neurones depending on whether their rate of firing increased or decreased when a higher concentration (relative to a low one) was applied. However, because only two concentrations (0.25 and 0.5 M) of five alkali chloride salts were used (den Otter 1972a), no dose-response curves were plotted which would have enabled them to estimate or calculate the correlation coefficients and other parameters as has been done here.

CONCLUSIONS

The results obtained show that;

1. A neurone (n) that is negatively sensitive to increase in concentration of LiCl (i.e. is increasingly active with lowering of LiCl concentration) is present in the longest and long labellar taste sensilla of male D. tryoni. Electrical

activity of this type of neurone was virtually the only firing observed at concentrations of LiCl below 10 mM. The response plots for this neurone had gradients (on the probits/log conc. plots) of -2.24 and -2.82 in longest and long sensilla, quantifying inhibition of its firing by increase in LiCl concentration.

2. Two other categories of neurones found in both the longest and long labellar taste sensilla of male D. tryoni are characterised by larger (P2) and slightly shorter (P1) spikes. Both P1 and P2 are positively sensitive to concentrations of LiCl greater than 50 mM. In one out of fourteen longest sensilla and in two out of seven long ones tested, only neurone P2 responded to 500 mM and molar LiCl. With regard to frequency of spikes of these neurones; P2's activity was significantly higher in long sensilla than in longest ones. Although this may be suggestive of a functional difference in different morphological types of sensilla, a much larger sample would have to be tested on to confirm such a conclusion.

3. A fourth neurone denoted by Lp (because it had the largest spikes recorded and relatively long interspike periods: ca 100 msec. - fig. 6.3, for which the frequency of Lp was highest) occasionally fired, generally when molar LiCl was applied. It remains to be investigated whether concentrations of LiCl greater than one molar tested in this study would regularly elicit responses in this unit.

4. Mean firing rates of P1 and P2 were minimum at 50 mM LiCl with gradients of 1.21 and 2.11 respectively in longest sensilla, 2.82 and 4.75 for P1 and P2 in long sensilla. This shows that at higher concentrations of LiCl the number of P2 spikes had increased at a rate nearly twice that of P1.

5. Choice of an appropriate concentration of LiCl, as a carrier electrolyte for application of cue-lure, involved deciding on whether to have a high response in the negatively salt-sensitive neurone(s), with minimum firing in the positively salt-sensitive neurones; or alternatively, a low firing rate of the former and higher one from the latter; i.e. a choice between 50 and 100 mM LiCl. A decision was taken to use 50 mM LiCl because at this concentration all the units (n, P1, P2) had relatively low frequency spike activity, thus offering the opportunity of observing any excitatory and/or inhibitory effects of cue-lure. This concentration of aqueous LiCl was of high resistance but had adequate conductivity for passage of enough action current to allow recording of the spike potentials.

6.2 ELECTROPHYSIOLOGY OF LABELLAR SENSILLA WITH CUE-LURE AND LURE FEEDING BEHAVIOUR

6.2.1. INTRODUCTION

When a fly extends its proboscis to taste food substances, longest and long labellar gustatory sensilla extending beyond the periphery of the unopened labellar lobes are the first to make contact. Observations on males of D. tryoni, feeding on cue-lure under a microscope, showed that these sensilla generally remain in contact with food as the labellum opens and throughout the course of feeding.

Studies on attraction to, and feeding on, lures by some tephritid species have included observations on the behaviour of: D. dorsalis on methyl eugenol (Steiner 1952; Metcalf et al. 1975, 1979, 1981); C. capitata on trimedlure (Nadel and Peleg 1965); and D. tryoni on cue-lure and some other derivatives of 4-phenyl-2-butanone (Monro and Richardson 1969). Some electrophysiological studies have shown that "largest" labellar gustatory sensilla of two species have neurones that are sensitive to sugars and salts: C. capitata (Gothilf et al. 1971; Angioy et al. 1978, 1978a) and D. oleae (Angioy et al. 1978, 1978b).

The initiation of ingestion of lures results from contact with labellar sensilla, one or more neurones in each sensillum generate the necessary sensory input to the CNS. The CNS generates motor output for the extension of the proboscis and opening of labellar lobes. Therefore, longest and long labellar gustatory sensilla of male D. tryoni were used in electrophysiological tests, to characterize responses of any

neurones sensitive to cue-lure. This is intended to form a base for further studies with cue-lure-like compounds to investigate features of the lure molecule that render it stimulatory to gustatory (and by extrapolation, olfactory) receptors. It was also hoped to obtain information on the molecular characteristics of the cue-lure receptor sites on the exposed dendritic membrane of the gustatory neurone.

Feeding behaviour preference tests were done with the same concentrations of cue-lure that were tested electrophysiologically, for comparison. Estimation of the behavioural threshold, from the numbers of flies feeding on the lure at the test concentrations, was another aim.

6.2.2. MATERIALS AND METHODS

6.2.2.1. Electrophysiology

The same procedure and electrophysiological recording apparatus used for recording with LiCl were used for testing a series of concentrations of cue-lure (suppliers: International Pheromones Ltd, Britain) (section 6.1.2). A series of different concentrations of the lure were made from an initial stock solution of 10 mM in 50 mM LiCl by decade dilutions. 10 mM was the highest concentration of cue-lure emulsifiable in the electrolyte without leaving traces of oily droplets. Mixing the two to form a uniform solution was by a whirl mixer or an ultrasonic bath.

Male flies used were 7-15 days post-eclosion. Stimulus duration was about two seconds. Time interval between consecutive stimulus applications on a single sensillum was kept at a minimum of four minutes, which was adequate for the

disadaptation of neurones. Concentrations of cue-lure were applied in an ascending order and the series bracketted with applications of 50 mM LiCl for the control. For the long-term adaptation applications, millimolar cue-lure in 50 mM LiCl was applied onto a sensillum for 16 seconds and responses recorded on strip film with a continous recording C4 "Kymograph" camera (Grass Instruments, Quincy, MA., USA.)

6.2.2.2. Behavioural preference tests

Tenfold concentrations of cue-lure between nanomolar and 10 mM in 50 mM LiCl were serially tested against one another in an ascending order. Solutions were made into 2% (volume by weight) agar (Davies Powdered Agar, grade J, David Gelatine Aust. Co.) gel which was dissolved by heating the mixture to near boiling point. About 15 ml of the hot gelatinous solution was poured into a plastic dish (6.7 cm diam. x 0.7 cm deep) and allowed to settle overnight. Tests were carried out early the next morning (7.00 - 9.00 hrs.). The semisolid lure/agar discs (in dishes) were presented in a simple Latin square, in a ventilated perspex cage (40 x 40 x 30 cm), into which an average of 40 adult male D. tryoni, 7-15 days old, were put through a port in the roof (fig. 6.6). Sugar and water were provided and appropriately positioned in a central part between the four discs. Mean room temperature during the period of tests was 22.7 $\pm$ 2.16° C (range, 17-24° C) and the relative humidity 57.8 $\pm$ 6.0% (range, 51-67%). Illumination around the discs by an overhead flood light and fluorescent tubes was at about 2,400 lux. The number of flies dabbing the lure/agar surface with their labella were recorded after the initial 10 minutes and

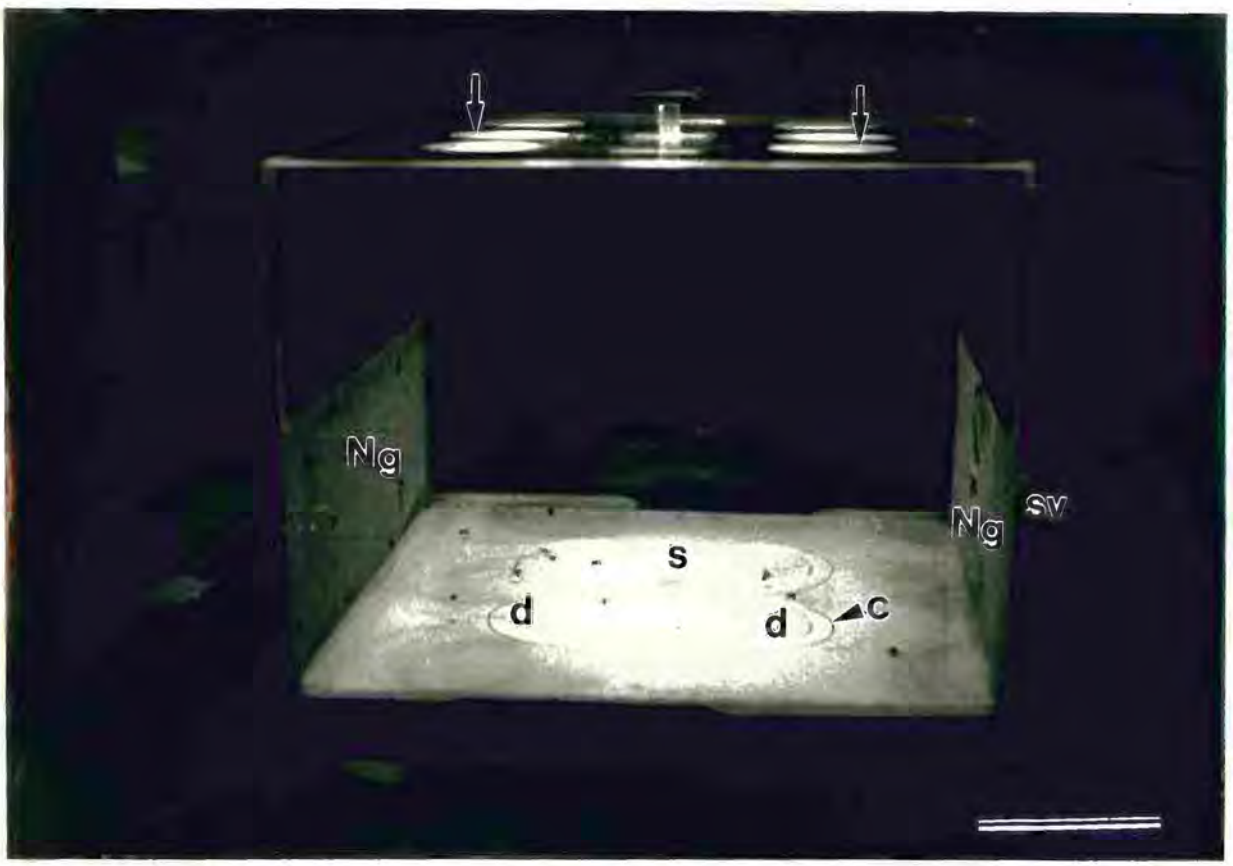


FIGURE 6.6 Photograph of the perspex cage used for preference tests on male D. tryoni with different concentrations of cue-lure. Half of two opposite sides and six ventilation ports on the roof (arrow) were covered with nylon gauze (Ng). d: plastic dishes with lure/agar medium; s: sugar cubes on a plastic top placed on an absorbent material (Wettex®) containing water; c: circle of 10 cm diameter around each dish; and sv: sleeve on front of the cage. Bar = 10 cm.

then at 5 minute intervals for an additional 25 minutes. New discs were prepared for each test and replicates had discs with specific concentrations of cue-lure rotated to different positions. The control was 2% agar in 50 mM LiCl in similar plastic dishes.

6.2.3. RESULTS

6.2.3.1. Recognition of different spike types

6.2.3.1.1. Neurone "n"

This had characteristics as described in section 6.1.3 above, with different concentrations of aqueous LiCl. This neurone fired in response to concentrations of cue-lure/50 mM LiCl, applied in a series, with an apparent reduction of the number of spikes at 0.1 and 1.0 mM cue-lure when the cue-lure sensitive neurone was firing maximally (figs. 6.7, 6.8).

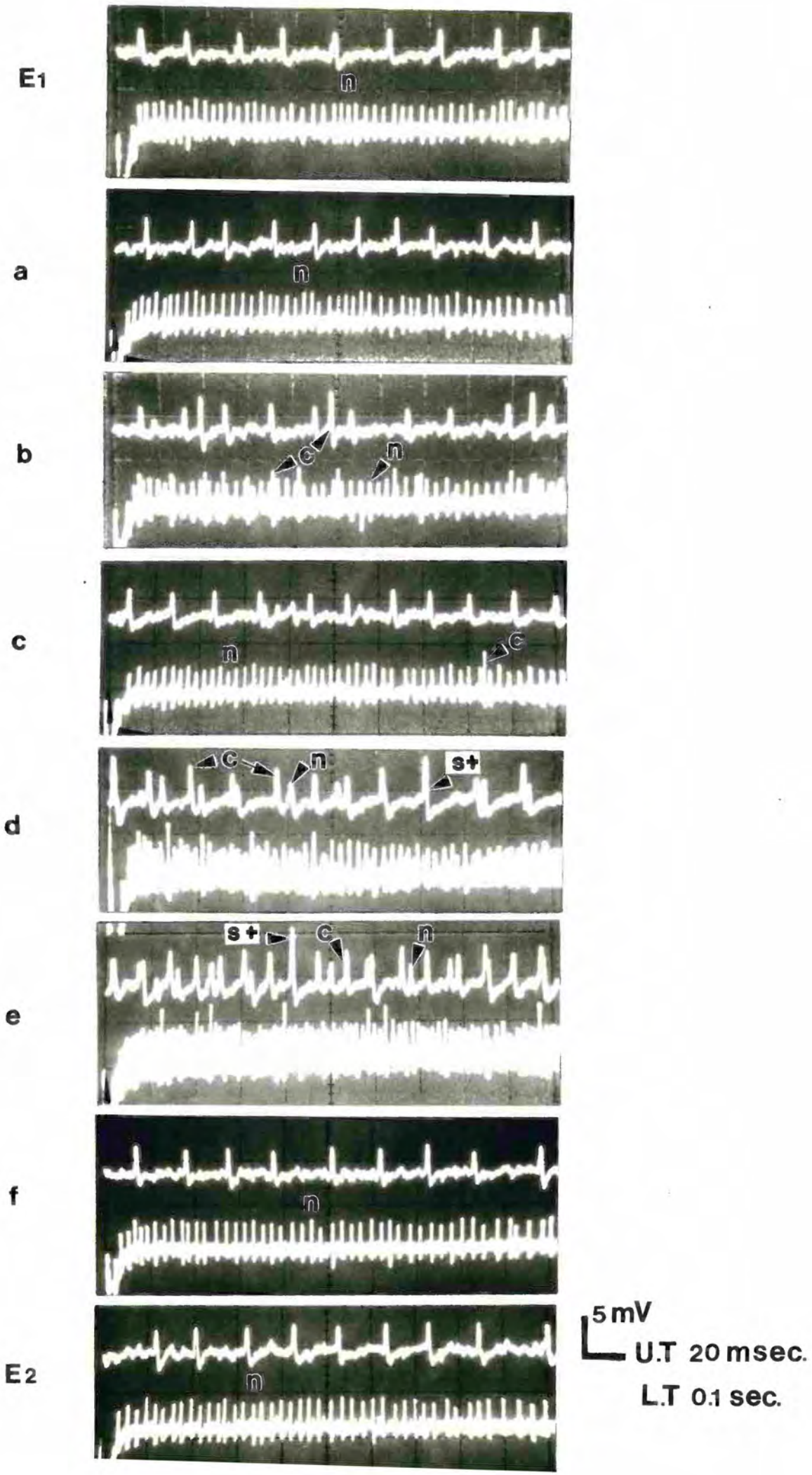
6.2.3.1.2. Neurone "c"

This neurone started firing (5-15 spikes in the first second) at a concentration of micromolar cue-lure but relapsed into inactivity at 10 µM. Peak to peak amplitude of spikes from this neurone was greater than 6.25 mV, falling in the range of that observed for P1, P2, and Lp in the experiment on dilutions of LiCl (figs. 6.7, 6.9).

6.2.3.2 Electrophysiology of some longest labellar gustatory sensilla with dilutions of cue-lure

For concentrations of cue-lure in the range 1-100 nM, only the "n" neurone fired. However, at micromolar concentration a small number of spikes were recorded from the "c" neurone (Table 6.5 A). For concentrations of cue-lure in the

FIGURE 6.7 Representative traces of a series of electrophysiological recordings from longest sensillum no. 2 on the right labellar lobe of a seven days old male D. tryoni with six concentrations of cue-lure in 50 mM LiCl: a. 0.1 µM; b. micromolar; c. 10 µM; d. 0.1 mM; e. millimolar; and f. 10 mM. E1, E2: bracketting control recordings with 50 mM LiCl. At 0.1 µM and 10 mM cue-lure the "n" neurone was predominant. Neurone "c", sensitive to cue-lure, started firing at micromolar concentration with a small number of spikes and almost none at 10 uM. The activity increased to a maximum at either 0.1 mM or millimolar (as in this example). There were few or no spikes from this neurone at 10 mM. S+ denotes summations due to overlap of "n" and "c" spikes. Time base scale: 20 msec. for the upper traces (U.T.) and 0.1 sec. for lower traces (L.T.).


E1
n
a
n
b
C
n
c
n
C
d
C
n
S+
e
S+
C
n
f
n
E2
n
5 mV
U.T 20 msec.
L.T 0.1 sec.

range micromolar to 10 mM, mean firing rate of neurone "n" to the carrier electrolyte is relatively constant except for fluctuations when the firing rate of neurone "c" peaked. For concentrations of lure less than 100 nM, firing was basically that from neurone "n" and despite the quiescence of the neurone sensitive to the lure at these concentrations (fig. 6.8B), firing of the "n" neurone still fluctuated about an average value of mean firing rate (spikes in first second after 50 msec. recovery period) typical of 50 mM LiCl (figs. 6.8A,B).

Response curves for neurone "c" in longest sensilla (fig. 6.8B) had a small peak at micromolar cue-lure, followed by a decline at 10 μM. The mean firing rate then increased, attaining maximum firing at 100 μM (Nx = 5) or millimolar (Nx = 9), at which the firing of "n" dropped sharply to about 50% of the mean level. No electrical activity of this neurone was recorded at 10 mM except the occasional one or two spikes. At peak firing activity of neurone "c", a large number of summations of spikes from the two neurones (n and c) occurred. Taking this into consideration, a response curve for neurone "n" over the range of concentrations tested, remains relatively constant with some fluctuations, at the mean firing rate obtained with 50 mM LiCl (Table 6.5). Firing of neurone "n" at 10 mM regained the level of the mean value and in some cases overshot it. Despite lack of any statistically significant changes ($t \leq 1.25$, $p \leq 0.05$; $df = 34$ at 0.1, 1.0 and 10 μM) in the level of firing of neurone "n" in the presence of cue-lure, there was an apparent increase in mean firing rate of this neurone at almost all the concentrations tested except those eliciting maximum firing in "c" (figs. 6.8 B). Applications of nanomolar and 10 nM

Table 6.5 Mean firing rates (± s.e.) of cue-lure sensitive neurone (c) and negatively LiCl-sensitive neurone (n) in longest and long labellar gustatory sensilla of male D. tryoni to various concentrations of cue-lure in 50 mM LiCl. Nx: number of sensilla tested.

Conc. of lure (mM)	(Nx)	Mean no. of spikes in first second after 50 msec. contact recovery		
				Summations
A) Longest sensilla:		n	c	(S± = n ± c)
50 mM LiCl	(6)	41.33 ± 3.05	0	0
10^{-6}	(6)	43.17 ± 3.70	0	0
10^{-5}	(6)	38.17 ± 2.73	0	0
50 mM LiCl	(21)	47.76 ± 3.66	0	0
0.0001	(15)	54.60 ± 4.40	1.20 ± 0.75	0.13 ± 0.13
0.001	(15)	47.13 ± 3.90	12.73 ± 3.01	1.73 ± 0.50
0.01	(15)	49.80 ± 1.30	5.0 ± 1.41	0.80 ± 0.38
0.10	(15)	30.93 ± 2.65	46.53 ± 3.72	9.87 ± 1.16
1.0	(14)	36.36 ± 4.23	45.93 ± 7.87	9.78 ± 1.76
10	(14)	51.21 ± 3.87	0.86 ± 0.59	0.14 ± 0.14
50 mM LiCl	(14)	43.36 ± 4.48	0	0
B) Long sensilla:				
50 mM LiCl	(11)	41.4 ± 3.44	0	0
0.0001	(11)	43.1 ± 2.90	0.90 ± 0.10	0.18 ± 0.10
0.001	(11)	39.2 ± 1.74	6.73 ± 2.24	0.18 ± 0.10
0.01	(11)	40.5 ± 3.0	3.0 ± 1.10	0.55 ± 0.40
0.10	(11)	31.9 ± 3.38	40.6 ± 4.60	8.09 ± 1.90
1.0	(11)	61.6 ± 7.18	10.1 ± 5.10	2.09 ± 1.20
10	(11)	31.5 ± 6.45	0	0.18 ± 0.20
50 mM LiCl	(11)	29.2 ± 3.92	0.73 ± 0.70	0

FIGURE 6.8

A. Dose-response relationship of spike activity of neurones "n" (--o--o--) and "c" (—•—•-) in long labellar gustatory sensilla (Nx = 11) of male D. tryoni to six concentrations of cue-lure (range, 0.1 µM - 10 mM). Neurone "c" had two peaks, one at micromolar and the other at 0.1 mM (as in this fig.) or millimolar. Mean firing rate of neurone "n" remained at a level almost equal to that of 50 mM LiCl (points ◉ , E1 and E2) at all concentrations except millimolar of the lure at which there was a sharp increase. Vertical bars are ±1 s.e.

B. A similar relationship as above for spike activity of the corresponding two neurones in longest labellar sensilla of male D. tryoni, in response to serial application of eight concentrations of cue-lure (1 nM - 10 mM) in two stages. Peak mean firing rate of neurone "c" was either at 0.1 mM (Nx = 5) or millimolar (Nx = 9). Mean firing rate of "n" fluctuated about the mean level of firing in response to 50 mM LiCl (point ●) except at 0.1 mM when spike activity dropped appreciably. Numbers above the points are for the sensilla tested and bars are ±1 s.e.

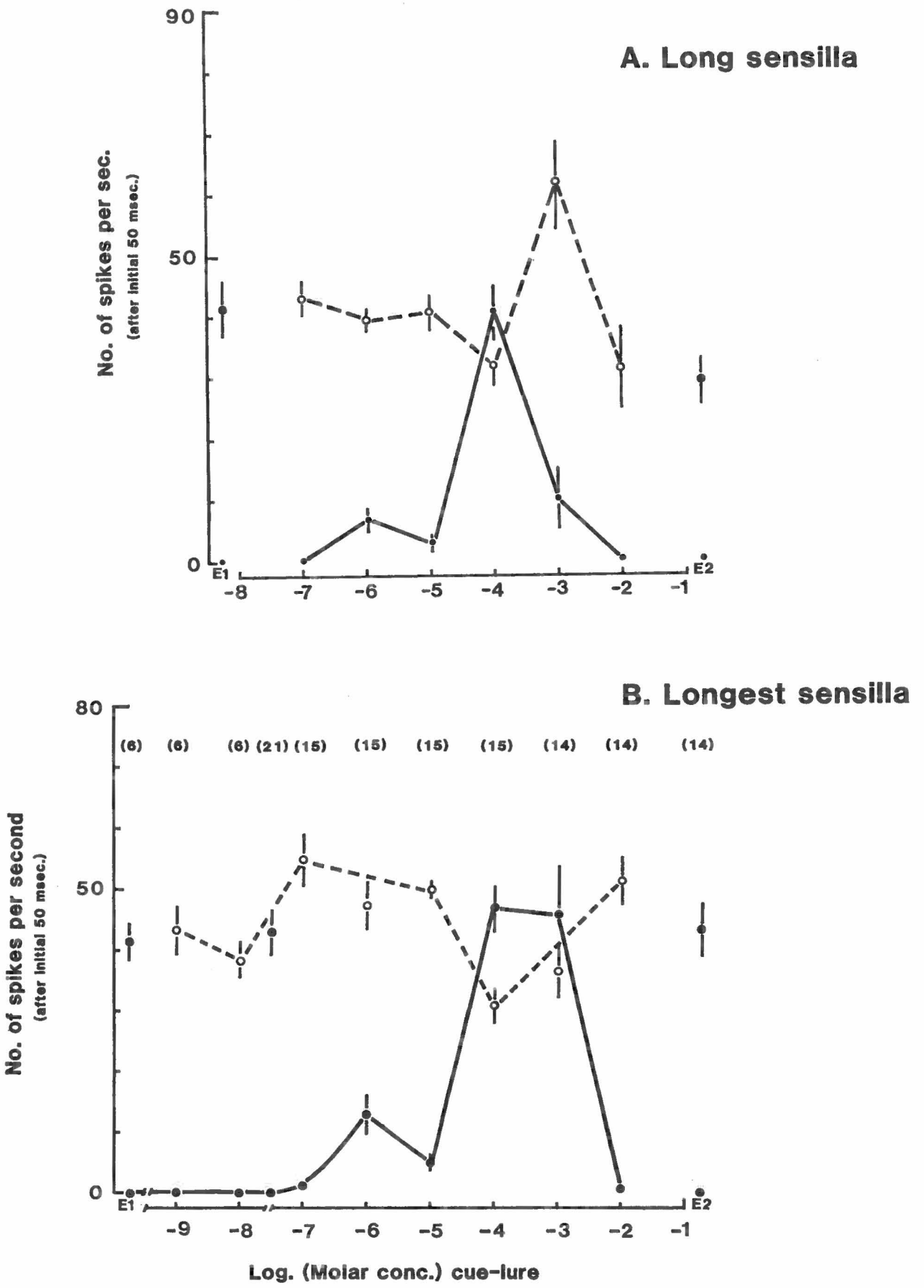

A. Long sensilla
No. of spikes per sec.
(after initial 50 msec.)
90
50
0
E1
E2
-8
-7
-6
-5
-4
-3
-2
-1
B. Longest sensilla
No. of spikes per second
(after initial 50 msec.)
80
50
0
(6) (6) (6) (21) (15) (15) (15) (15) (14) (14) (14)
E1
E2
-9
-8
-7
-6
-5
-4
-3
-2
-1
Log. (Molar conc.) cue-lure

FIGURE 6.8

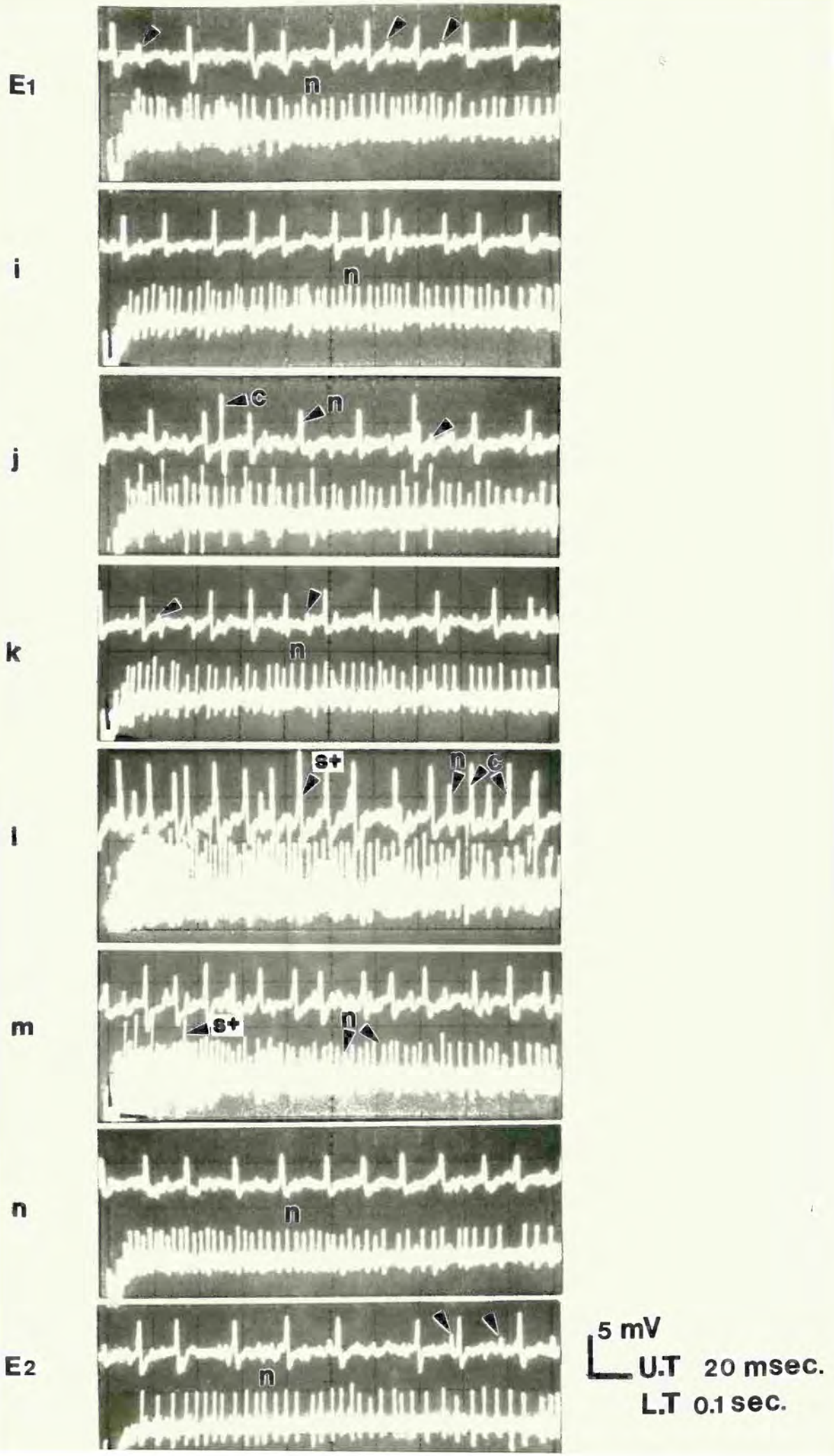
(10^{-6} and 10^{-5} mM) cue-lure on longest sensilla (Nx = 6) gave mean firing rates which showed no significant difference to those elicited by 50 mM LiCl ($t = 2.10$, $p = 0.05$; $df = 18$). This extends the range of concentrations of cue-lure, for which there was no electrical activity of neurone "c", to concentrations lower than micromolar (Table 6.5 A).

6.2.3.3. Electrophysiology of some long gustatory sensilla with cue-lure

For the long labellar gustatory sensilla recorded from (Nx = 11), neurone "c" had peak sensitivity in only one sensillum at millimolar of the lure, while the rest peaked at 0.1 mM. Data for all sensilla were pooled for calculation of mean firing rates (Table 6.5B, fig. 6.8A). Variation of mean firing rates for the two neurones were similar to those observed for longest sensilla (fig. 6.8B). Peak of mean firing rate for these sensilla at 100 μM of the lure was not significantly different ($t = 1.01$, $p \leq 0.05$; $df = 24$) from that for the longest sensilla (Tables 6.5A,B; figs. 6.8A,B). However, mean firing rate of "c" was significantly higher ($t = 3.7$, $p \leq 0.05$; $df = 23$) in longest sensilla than long ones at millimolar, which flattened the peak of the response curve for the former.

Firing of neurone "n" recorded with the electrolyte at the end of each serial application of cue-lure concentrations was lower than that observed with initial application of the electrolyte (points E1 vs. E2 on fig. 6.8 A), in seven out of the eleven recordings. Fluctuations of firing of "n" recorded with concentrations of cue-lure compared to that with pure 50 mM LiCl were small with the exception of that at millimolar

FIGURE 6.9 Representative traces of electrophysiological recording from a long gustatory sensillum no. 33 of a seven days old (post-eclosion) male D. tryoni with six concentrations (range, 0.1 µM - 10 mM) of cue-lure as in figs. 6.7 and 6.8. i. 0.1 µM; k. micromolar; l. 10 µM; m. 0.1 mM; n. millimolar; o. 10 mM cue-lure. Another neurone with a small spike (arrowhead) was firing at 50 mM LiCl and some of the lure concentrations. E1, E2: control recordings with 50 mM LiCl.
Time base scale: 20 msec. for the upper traces (U.T.) and 0.1 sec. for lower traces (L.T.).


E1
n
i
n
j
c
n
k
n
l
s+
n
c
m
s+
n
n
n
E2
n
5 mV
U.T 20 msec.
L.T 0.1 sec.

6.2.3.4. Long-term adaptation of neurone "c" to millimolar cue-lure in 50 mM LiCl

The responses of neurone "c" had a rapidly adapting phase within the first three seconds of stimulus application (fig. 6.10). Mean firing rate (Nx = 4) in the third second declined to slightly less than 50% of that during the first second. There was further decline with time to 10.2% in the thirteenth second and 4.8% in the fifteenth. Decline in firing rate during the 4-5 seconds after the first four seconds was slow and an almost stable level was attained after 10 seconds. The exponential model $y = \exp.(a + bx)$ was a good fit to the adaptation curve of "c" ($R^2 = 0.92$, $1 \leq x \leq 15$) where; y is number of spikes in one second interval and x, time interval in seconds. The value for the mean firing rate during the first second, obtained experimentally, was higher than in the model. This may reflect a slowing down of the adaptation rate by other, non-linear, time-dependent, receptor membrane processes at the onset of stimulation. The firing rate might tend to be asymptotic (at about 4% of the initial rate) to the time axis, if the stimulus was applied for a longer period.

Although the firing rate of neurone "n" was lower than in previous records, decline in firing rate of this neurone was minimal. Over the fifteen seconds of continous stimulation, the decline was at the rate of 0.14 spike/second (negative slope of dotted line in fig. 6.10), resulting from a drop of about 22% of the initial firing rate after fifteen seconds. The actual pattern of firing of "n" seems to follow an oscillatory underdamped function. This may partly explain why the linear model $y = a + bx$ was not a good fit ($R^2 = 0.42$).

FIGURE 6.10 Long-term adaptation of neurone "c" (—●—●—) and "n" (—o--o--) in longest labellar gustatory sensilla (Nx = 4) of male D. tryoni to stimulation with millimolar cue-lure in 50 mM LiCl. The phasic part of the firing of neurone "c" was within the first three seconds, whilst an almost stable tonic phase was attained after the first nine seconds. Firing of neurone "n" fluctuated about an almost constant mean level. Vertical bars are ±1 s.e.

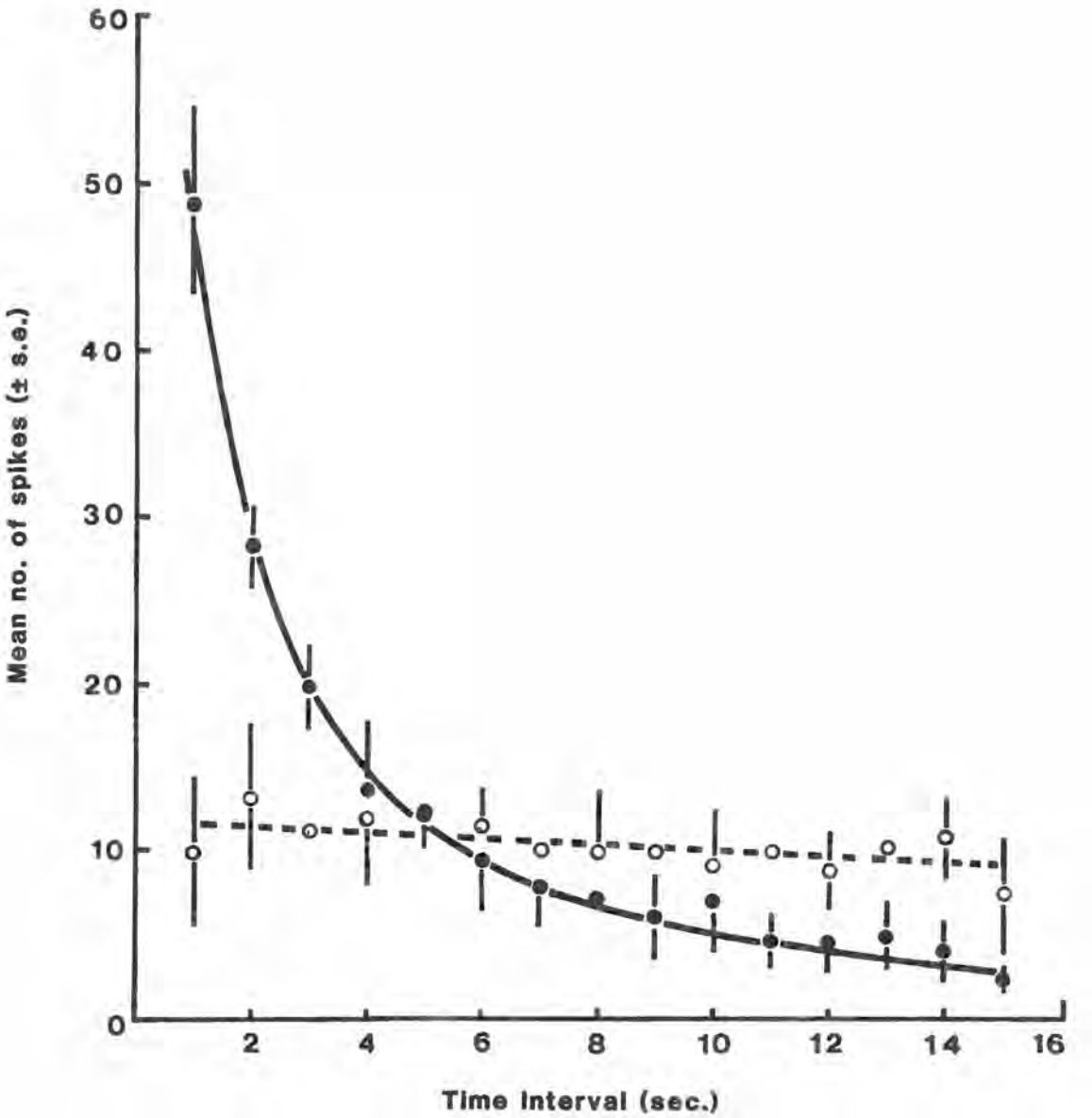

60
50
40
30
20
10
0
Mean no. of spikes (± s.e.)
2
4
6
8
10
12
14
16
Time Interval (sec.)

FIGURE 6.10

6.2.3.5. Feeding preference tests on different concentrations of cue-lure

A method of calculating the relative percent response between two concentrations of cue-lure to remove the effect of one on the other in choice tests was devised. If these concentrations are z and y, the percent relative feeding response (Fy) of flies on y in the presence of z is, Fy = {Yf/(N)(T)-Zf} x 100, where Yf and Zf are the cumulative numbers of flies feeding on y and those on z respectively over the total period of observation. N is the total number of flies in the cage and T the number of intervals at which counts were made.

Relative feeding responses on (and attraction to) concentrations of cue-lure between nanomolar and 10 mM had a similar pattern to the dose-response curves obtained for longest and long labellar gustatory sensilla in the electrophysiological study (fig. 6.11). There was no significant preference for nanomolar cue-lure to plain agar. For concentrations between nanomolar and micromolar, the number of flies responding was very low and flies did not show preference for tenfold higher concentrations in this range (Table 6.6, fig. 6.11). However, a significant increase (x6.5) in the number of flies feeding occured at 5 µM. For subsequent concentrations there was preference for the higher concentration in each test. This was maximum at millimolar cue-lure. Therefore, the behavioural threshold concentration for male D. tryoni was between micromolar and 5 µM cue-lure.

In experiment 9 (Table 6.6), 10 mM cue-lure had a

Table 6.6 Relative feeding responses of male D. tryoni between cue-lure concentrations in 2% agar made in 50 mM LiCl. Nx: replicates.

Exp.	Nx	Concs.	Mean % Response (± s.e.)	Fz/Fy	Student's t test	
					t	df
1.	3	2% agar	0.51 ± 0.51			
		vs.		2.96	0.87 ns	5
		1 nM lure	1.51 ± 1.04			
2.	4	1 nM lure	0.98 ± 0.52			
		vs.		0.57	0.69 ns	7
		10 nM lure	0.56 ± 0.33			
3.	4	10 nM lure	0.25 ± 0.25			
		vs.		0.52	0.43 ns	7
		0.1 µM lure	0.13 ± 0.13			
4.	4	0.1 µM lure	0.38 ± 0.24			
		vs.		0.68	0.42 ns	7
		1 µM lure	0.26 ± 0.15			
5.	5	1 µM lure	0.83 ± 0.67			
		vs.		6.48	3.24 **	9
		5 µM lure	5.38 ± 1.24			
6.	4	5 µM lure	2.07 ± 0.30			
		vs.		3.73	3.93 **	7
		10 µM lure	7.73 ± 1.41			
7.	4	10 µM lure	2.94 ± 1.02			
		vs.		10.8	3.28 **	7
		0.1 mM lure	31.6 ± 8.70			
8.	4	0.1 mM lure	9.75 ± 2.31			
		vs.		3.86	5.40 **	7
		1 mM lure	37.6 ± 4.62			
9.	4	1 mM lure	16.8 ± 5.89			
		vs.		1.65	1.36 **	7
		10 mM lure	27.6 ± 5.33			

ns: not significant; **: difference significant at p = 0.05.

FIGURE 6.11 Relationship between relative numbers of male D. tryoni feeding on concentrations of cue-lure in 2% agar made in 50 mM LiCl. Percent relative feeding response for each concentration was calculated with respect to a one-tenth dilution. C is the control which was 2% agar in 50 mM LiCl. Error bars are ±1 s.e.

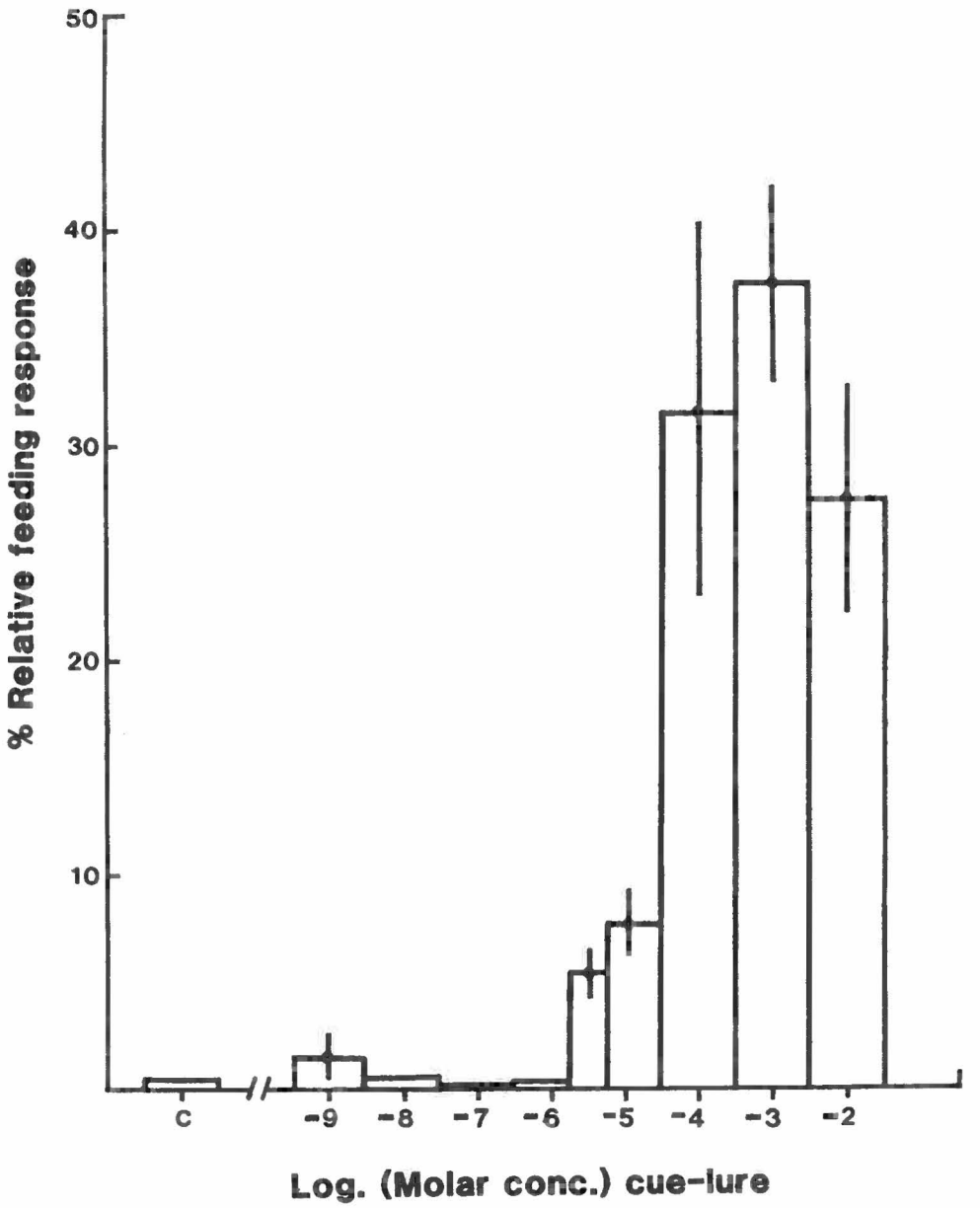

50
40
30
20
10
% Relative feeding response
C
-9
-8
-7
-6
-5
-4
-3
-2
Log. (Molar conc.) cue-lure

FIGURE 6.11

slightly higher but not significantly different ($t = 1.36$, $p = 0.05$; $df = 7$) relative feeding response; it was still lower than that recorded for millimolar in exp. 8. Even though no higher concentrations than 10 mM were tested, preference for feeding response of male D. tryoni on tenfold higher convvcentrations of cue-lure was maximum at millimolar and decreased slightly at 10 mM.

6.2.4. DISCUSSION

Results obtained from application of a series of concentrations of cue-lure onto longest and long labellar gustatory sensilla of male D. tryoni showed that, in addition to the neurone (n) responding to the carrier electrolyte, at least one other neurone (c) sensitive to cue-lure, fired at concentrations greater than 0.1 µM. The latter neurone had two peaks on the dose-response curves; a small peak at micromolar and a larger one at either 0.1 mM or millimolar of the lure. On the other hand, the dose-response curve of neurone "n" had small fluctuations about the mean firing rate recorded with 50 mM LiCl (control) except for an apparent decline, when the firing rate of "c" was maximum. There was a recovery in the firing rate of the n-neurone when "c" did not fire, at 10 mM cue-lure. This apparent "reciprocal firing" of the two neurones occured at concentrations of cue-lure greater than 0.10 µM. This relationship between the electrical activity of the two neurones differs from that reported for the peripheral interaction of neurones sensitive to water, sugars, and salts (Evans and Mellon 1962; Rees 1970a; Fredman 1975; section 6.1 of this thesis). Increase in concentration of sugars and/or salts in aqueous solutions

become inhibitory to firing of neurones sensitive to water (negatively salt-sensitive) after a certain critical concentration, characteristic of the solute, is exceeded. Full inhibition may occur at much higher concentrations. Increase in concentration of cue-lure does not seem to have had such an inhibitory effect on the neurone that responds to the very dilute electrolyte; rather, it appears to have exerted a facilitatory effect which resulted in a small increase in the firing rate of neurone "n". More experimental work on the effects of change in osmolarity of test solutions rather than the actual concentration of the solute, may provide a better insight into the dose/response relationships of these receptors (cf. section 6.4).

There are several processes that may give rise to the occurrence of two peaks on the dose/response curve of neurone "c" for cue-lure in longest and long labellar gustatory sensilla (fig. 6.8). There are three possibilities: two separate neurones each with a receptor site; a single neurone with two separate receptor sites; or a neurone having a single receptor site with two subunits. In all these one of the active sites would have a threshold and saturation level slightly different from the other. This would imply the presence of different receptor proteins with different binding capacities for the lure molecules. One such receptor site or subunit is activated at low concentrations (0.1 - 10 μM); the second site or subunit has a threshold close to 10 μM and binds more molecules at higher concentrations when the first site is saturated. If such is the situation, binding of lure molecules onto the second (higher threshold) receptor site or subunit, at concentrations lower

than 10 μM, cannot be ruled out. However, the inflection on the dose/response curve at 10 μM may be indicative of the first (lower threshold) receptor site/subunit saturating and perhaps having minimum contribution to the peaking of the response at 0.1 mM or millimolar of the lure. The second peak of the response curve could then arise from the progressive activation of a second receptor site or subunit. To explain the stimulatory effectiveness of glucose and fructose in the same solution or that of sucrose and glucose, Morita and Shiraishi (1968) suggested that the pyranose and furanose sites on the sugar receptor of B. peregrina can be considered as two subunits of a single receptor site. Their "classical complex" and "allosteric" models were based on the assumption that one molecule of a disaccharide (e.g. sucrose) can combine with each receptor site subunit at two different parts of its constituent monosaccharide molecules, giving rise to observation of different electrical responses with solutions of mixtures of sugars. The characteristic electrical activity evoked would depend on differences in the degree of activation of each subunit.

Neurone "c" did not fire in 13 out of 15 recordings with 10 mM cue-lure. In the remaining two recordings only 5 and 7 c-neurone spikes were counted. It is most likely that at this concentration either adaptation occurs instantaneously on contact, so that no spikes are recorded, or that the activity of this neurone is blocked. Neurone "c" is rapidly adapting, with a marked phasic effect of about three seconds at millimolar cue-lure. For the suggested logarithmic curve, a tenfold increase in concentration (10 mM) would have reduced the firing rate appreciably. However, lack of firing

from this neurone is suggestive of other electrobiological processes being in operation. Konishi (1967) reported a similar dose/response curve for gustatory chemoreceptors of a freshwater fish (carp) in response to dilute solutions of inorganic electrolytes. Maximum discharge for NaCl was at 1/512 M followed by a decline at higher concentrations. A possible screening effect arising from approach of cations to the inner layer of the (double layer) membrane of the receptor was suggested to be a possible cause for the decline. The decline and subsequent cessation of firing of neurone "c" responding to cue-lure, in the present work, may have been due to a different phenomenon. At relatively low concentrations of cue-lure (less or equal to millimolar), a large proportion of the lure molecules may bind with the gating mechanisms of the receptor membrane channels with only a few reacting with other parts of the membrane. This would give rise to an overall increase in permeability and flow of ions, thus generating action potentials. The limit of response coincided with the limit of effective emulsification of the lure in 50 mM LiCl at room temperature. At concentrations greater than millimolar cue-lure, in addition to normal binding of molecules as suggested above, a number of the weakly hydrophilic molecules may embed into the general matrix of the receptor membrane, increasing the compactness of the lipoid membrane components. Depending on the concentration of the lure molecules, there may be a reduction of Na^+ and K^+ conductance, leading to a decline in the firing activity or blockage at 10 mM and higher concentrations. For blockage to occur at 10 mM cue-lure, the receptor membrane must have a high affinity for lure molecules such that their

permeation into the receptor membrane is almost instantaneous at contact, to override the rapid changes in ionic conductances that would otherwise facilitate generation of action potentials. Probably this is facilitated by polar regions on the membrane, that provide parallel permeation paths, bypassing the membrane lipids as suggested by Diamond and Wright (1969). There are other possible explanations and further work is needed to investigate the biphasic electrophysiological response to increasing cue-lure concentrations.

In longest sensilla, the firing rate of neurone "n" showed a recovery at 10 mM cue-lure to the level obtained with concentrations less than 0.1 mM. However, no concentrations higher than this were tested because of saturation of the carrier electrolyte. When compared with firing of salt-sensitive neurones at concentrations of LiCl greater than 100 mM (section 6.1), which could elicit behavioural rejection, it is seen that for the salt, the firing rate of "n" was minimum or none at all. It is therefore possible that the steady firing of "n" contributes to the sensory information to the CNS, and together with the "c"-input, maintains the prolonged feeding of male D. tryoni on cue-lure. Although the behavioural effects of firing of "c" are not known, the low firing rate in the tonic phase may also be essential in maintaining feeding. The role of flushing with saliva or regurgitated gut fluids, on the disadaptation of labellar gustatory neurones, is also in need of investigation.

A range of organic compounds have been reported to affect the electrical activity of neurones sensitive to water, sugars, and salts. Hodgson and Steinhardt (1967) reported

that increase in concentration of amines and alcohols in salt and sugar solutions blocked the firing of neurones sensitive to these substances as well as those sensitive to water. Blocking was consequently suggested to be the mode of action of these substances, in eliciting behavioural rejection in feeding tests, rather than their stimulating specific neurones in gustatory sensilla. den Otter (1972b,c) theorized on the possible mechanism through which organic compounds (alcohols, amines, and fatty acid salts) stimulate gustatory receptors of insects. He suggested that molecules of the nonionic compounds (with particular reference to alcohols and amines) have hydrophobic interactions with molecules in the interior of the lipid bilayer of the receptor membrane. By either forming micelles or combining with charged groups of molecules, the resulting reduction in negative charge density leads to increased compactness of the receptor membrane. Hence, giving rise to a reduction of pores available for flow of ions and therefore the blocking of the electrical activity of neurones sensitive to water, sugars, and salts. However, no experimental evidence has been supplied to support this theory. Much remains to be done on the molecular dynamics of receptor membranes.

In the case of fatty acid salts, gustatory receptors of Ph. regina fired sporadically (injury effect) and subsequently ceased firing (Dethier and Hanson 1968). These compounds have been shown in vitro to irreversibly denature egg albumin proteins (Bull and Breese 1967). They might have a similar denaturation effect on receptor membrane proteins leading to an irreversible blockage of generation of electrical activity in gustatory neurones. Further support is provided by

Hodgson and Steinhardt's (1967) observation that, concentrations of glycerol that blocked the inhibitory action of the octylamines in the gustatory receptors were comparable to those used in stabilisation of proteins against denaturation.

Nonionic compounds have to be dissolved in an electrolyte when using the electrophysiological "tip-recording" technique. This may be similar to real life situations where flies regurgitate some fluid from the gut and/or pump out saliva, in which solid food materials dissolve or mix prior to ingestion. Hansen-Bay (1978) reported that saliva secreted by mature C. erythrocephala blowflies contains 75 mM chloride ions, while that from younger flies had up to 165 mM. Hence, the electrolyte-test substance combination may be the natural basis of stimulation of labellar gustastory sensilla especially in situations where soluble, nonionic substances are fed upon. Further work on the role of saliva and regurgitated fluids in the sensory physiology of ingestion is needed (M.J. Rice, pers. comm.).

The results of the behavioural preference experiments on cue-lure concentrations correlated well with the firing rate of neurone "c" in electrophysiological tests. However, there were some discrepancies, in that nanomolar and 10 nM cue-lure were nonstimulatory to neurone "c" but sometimes stimulated a small number of flies to feed on the lure/agar medium. The behavioural threshold, found to be between micromolar and 5 µM, is also higher than the electrophysiological threshold which was between 0.1 µM and micromolar cue-lure. The difference may be explained by the observation that, threshold measured electrophysiologically was exclusively for

longest and long labellar gustatory sensilla, stimulated individually. The behavioural threshold of freely moving flies results from the input of a large number of different types of labellar gustatory sensilla that contact food simultaneously. In addition, the input is compounded with that of the visual, antennal, palpal, and tarsal gustatory sensilla. Shiraishi and Tanabe (1974) obtained a median behavioural acceptance thres hold for sucrose in Ph. regina which was higher by a factor of 1.2 in tarsal D-type sensilla (1.8 mM) than in largest labellar gustatory sensilla (1.5 mM). Correspondingly, an average of 11 and 2.5 spikes per first 0.1 second were suggested necessary to elicit the threshold response in tarsal and labellar gustatory sensilla respectively. This seems to be an arrangement which ensures that concentrations of acceptable substances contacted, slightly exceed the behavioural and/or electrophysiological thresholds of the labellar gustatory sensilla prior to extension of the labellum.

Although no "c" spikes were recorded with 10 mM cue-lure in longest and long labellar gustatory sensilla, a substantial number of male D. tryoni were attracted to and fed on lure/agar medium of this concentration. This shows that, despite a recorded lack of "c" spikes in these sensilla, probably caused by instantaneous adaptation or temporary disruption of the spike generating mechanism in the receptor membrane, there are neurones in other types of labellar gustatory sensilla that are still active at this concentration. This may serve as a means of broadening the range of cue-lure concentrations that attract male D. tryoni and on which they may or may not feed. Another possible explanation is

from the observation that when flies are feeding on solid and/or semisolid substances, they secrete saliva and regurgitate gut fluids. The food materials are suspended or dissolve in the fluid forming a solution of relatively low viscosity which is easily ingested (Hansen-Bay 1978). When cue-lure was impregnated in filter paper discs, feeding male D. tryoni regurgitated copious amounts of fluid when extracting the lure. Such regurgitation of fluid and/or secretion of saliva onto a 10 mM cue-lure/agar medium may have caused localised dilution of the lure to a lower, electrophysiologically effective concentration, leading to the observed feeding. It is beyond the scope of this thesis to consider the many factors regulating ingestive physiology of insects, a good review of this is Bernays and Simpson (1982).

Longest and long labellar gustatory sensilla of male D. tryoni are innervated by four gustatory neurones with their dendrites extending through the lumen and terminating close to the tip of each sensillum. Of these, two were positively sensitive and one negatively sensitive to increase in LiCl concentration. The remaining neurone (out of the four) is presumably predominantly sensitive to sugars although this has not yet been experimentally demonstrated for males of D. tryoni. Since insect gustatory neurones can usually respond to a spectrum of stimuli, there is a high probability of cue-lure acting on either one of the neurones sensitive to LiCl or the fourth neurone suspected to be sensitive to sugars. Results of a cross-stimuli test between 0.5 M LiCl and millimolar cue-lure in 0.5 M LiCl, to determine whether neurone "c" was also sensitive to LiCl, are reported in section 6.4 of this thesis.

6.3. AN INVESTIGATION OF THE MOLECULAR CHARACTERISTICS OF THE CUE-LURE RECEPTOR USING RELATIVE ELECTROPHYSIOLOGICAL ACTIVITIES OF CUE-LURE ANALOGUES

6.3.1. INTRODUCTION

Gustatory and olfactory neurones have specific receptor sites that accept specific stimulant molecules and some of their analogues. These chemicals are usually in the same class, or have some common molecular components, as has been shown on various gustatory neurones: for amino acids (Shiraishi and Kuwabara 1970; Shimada and Isono 1978); for sugars (Morita and Shiraishi 1968; Jakinovich *et al*. 1971; Hanamori *et al*. 1974; Shimada *et al*. 1974, 1985; Wieczorek and Koppl 1982); and for glucosides (Wieczorek 1976; Mitchell and Gregory 1981; Sutcliffe and Mitchell 1982). Different compounds that stimulate have different ranges of stimulatory concentrations, different numbers of neurones sensitive to them, and different frequencies of spike discharge generated in the neurones at each concentration. Any of these differences, or a combination of them, may reflect differences in the stereochemistry of the molecules of the series. Thus, "specificity", as used hereafter is with respect to a group or class of stereochemically related compounds rather than a single chemical *per se*.

Monosaccharides and disaccharides that have been shown to be stimulatory to the labellar gustatory neurones of flies *e.g*. *B. peregrina*, *Ph. regina*, and *S. bullata* have certain molecular configurations and conformations. These features enable their effective binding onto either the pyranose or the furanose site of the sugar-sensitive neurone. Shimada *et al*. (1974) concluded that only three successive equatorial -OH

groups, in the chair form of the pyranose ring, are necessary for binding onto the pyranose site. Positions of -OH were not regarded as important, contrary to previous suggestions (Evans 1963; Jakinovich et al. 1971; Hanamori et al. 1974). For stimulation at the furanose site, the necessary structures tentatively suggested were: -OH groups at the C1 and C2 positions, plus a residue (varies with different sugars) orientated in a direction not likely to cause steric hindrance. There were some exceptions that could not be explained by the presence of these features. Aromatic amino acids that stimulated the furanose site had an alpha-amino group, a carboxyl group, and a carbon chain of adequate length (dimensions unspecified) (Shiraishi and Kuwabara 1970; Shimada and Isono 1978). Those stimulatory at the pyranose site, e.g. valine, had no polar group (-OH) in the aliphatic side chain.

Metcalf et al. (1975, 1979, 1981) and Mitchell et al. (1985) have done comparative studies on the attraction and feeding of male D. dorsalis on methyl eugenol (3,4-dimethoxyallylbenzene) and a large number of its isomeric analogues. They recognised several molecular parameters that contribute to methyl eugenol's higher potency compared to all the analogues. Presence of the primary substituent methoxy groups, particularly at the ortho-position with respect to the aliphatic side chain, were characteristic of the most active derivatives (Metcalf et al. 1975, 1979). Para-substituted derivatives were less active whilst the meta- derivatives were inactive. The odour intensity (to humans) of these chemicals, their attractancy to flies and stimulation of feeding by male D. dorsalis positively

correlated with the electron donating properties (σ) and strongly with the hydrophilic characteristics (Π) of the primary substituent group. The receptor site for the allyl moiety of methyl eugenol has been suggested to be a specialised lipophilic patch on the receptor membrane. The atomic composition and size of the primary substituent groups also affected the potency of methyl eugenol analogues (Metcalf et al. 1975, 1981). The size of the receptor site suggested seems somewhat flexible, because it accomodated alkoxy groups of van der Waals radii 0.2-0.6 nm (methyl-propyl). Other substituents of three atomic diameters (close to that of the methoxy group) e.g. -Cl and -OH gave maximum relative response whereas butyl and propyl had lower stimulatory effectiveness.

Kikuchi (1973) tested 64 odorant chemicals, comprising mono- and diketones, aldehydes and carboxylic acids, for attractancy to the olfactory mutant (HPB-1) of D. melanogaster. Seven chemicals, out of the 12 that were regarded as specifically attractive, had a proton acceptor (=O) and a proton donor (-OH) separated by a mean distance of about 31 nm (range, 28-33 nm). Overall molecular size and shape were not considered to have been important.

Wright (1963) explained the attractancy of cue-lure and its analogues to male melon flies (D. curcubitae) on the basis of the molecular vibrational energy theory (Wright 1954; Wright and Serenius 1954a,b). Doolittle et al. (1968) tested the validity of these explanations by deuterating the cue-lure molecule at six different points. This caused significant shifts in the far infra-red (500 - 50 cm^{-1}) absorption maxima, to lower frequencies. No accompanying significant changes in the

physical constants (volatility, molecular shape and size) of the lure molecule were observed. Also, behavioural tests with deuterated cue-lure did not show any significant changes in the attractancy relative to the nondeuterated chemical. Thus no experimental data was obtained supporting Wright's vibrational theory.

Therefore, in testing for the specificity of the electrophysiological response of the c-neurone, sensitive to cue-lure, in the labellar sensilla of male D. tryoni, the molecular structure-activity model has been adopted as the basis for discussion of the results. The size, atomic components and the molecular constants of the cue-lure molecule and another five, closely-related chemicals, have been considered here. Of the five chemicals: 4-phenyl-2-butanone (abbreviated 4-PBN) is the radical of which 4-(p-acetoxyphenyl)-2-butanone (cue-lure abbr. p-APBN) and 4-(p-hydroxyphenyl)-2-butanone (p-HPBN) are derivatives. Toluene (TLN) and acetophenone (ACP) were considered to be analogues of 4-PBN with shorter aliphatic side chains. An aliphatic ketone, 4-decanone (4-DCN), with same number of carbon atoms as p-HPBN, was also tested with the aim of evaluating whether the planar benzene ring is necessary for the stimulatory effectiveness of the lures. Although a relatively small number of chemicals were tested, all of them are such that differences in the electrical responses of the c-neurone could be attributed to the presence or absence of some substituent groups and the possible resultant modification of molecular constants with respect to 4-phenyl-2-butanone. Clearly the number of chemicals used needs to be increased, when

time for further experiments is available.

This approach is one way to attempt to understand the molecular components that may individually, or together with others, determine the stimulatory effectiveness of a chemical on the receptor. From the information obtained, the possibility of maximizing the potency of a lure can be assessed and, where possible, the chemical synthesized and tested.

Millimolar cue-lure elicited near maximum firing rate in the c-neurone and had the highest behavioural response in preference tests (section 6.2). To test the behavioural significance of 10 mM 4-PBN and millimolar p-HPBN, which also elicited peak firing rate in the c-neurone, two sets of preference tests on feeding and attraction were carried out: between millimolar cue-lure (p-APBN) and 10 mM 4-PBN and between millimolar cue-lure and millimolar p-HPBN.

Using the results of electrophysiological and behavioural tests, plus physicochemical data on the molecules, a model for the receptor site for the cue-lure molecule has been hypothesized. Based on this and the tabulated values of molecular constants for aromatic substituents (Hansch et al. 1973), a possible substitution with an acetonyl group on 4-phenyl-2-butanone is proposed.

6.3.2. MATERIALS AND METHODS

4-Phenyl-2-butanone and 4-decanone (98% pure) were from Aldrich Chemical Co.(USA). Acetophenone from Ajax Chemicals (Sydney, Aust.) and p-HPBN from Dragoco Co. (appendix II). Equimolar concentrations between nanomolar and 50 mM, in decade steps, were made up in 50 mM LiCl. Electrophysiological testing and recording for short term stimulus applications ($\leq$ 2.0 sec.) was as in section 6.2 of this thesis. Longest labellar sensilla (LH2, LH5, and LH8) of 7-15 days old male *D. tryoni* were recorded from. Results are presented as dose/response curves for the c-neurone.

For the feeding preference tests, concencentrations of cue-lure, 4-PBN, and p-HPBN were made up in 2% agar in 50 mM LiCl and tested as in section 6.2.

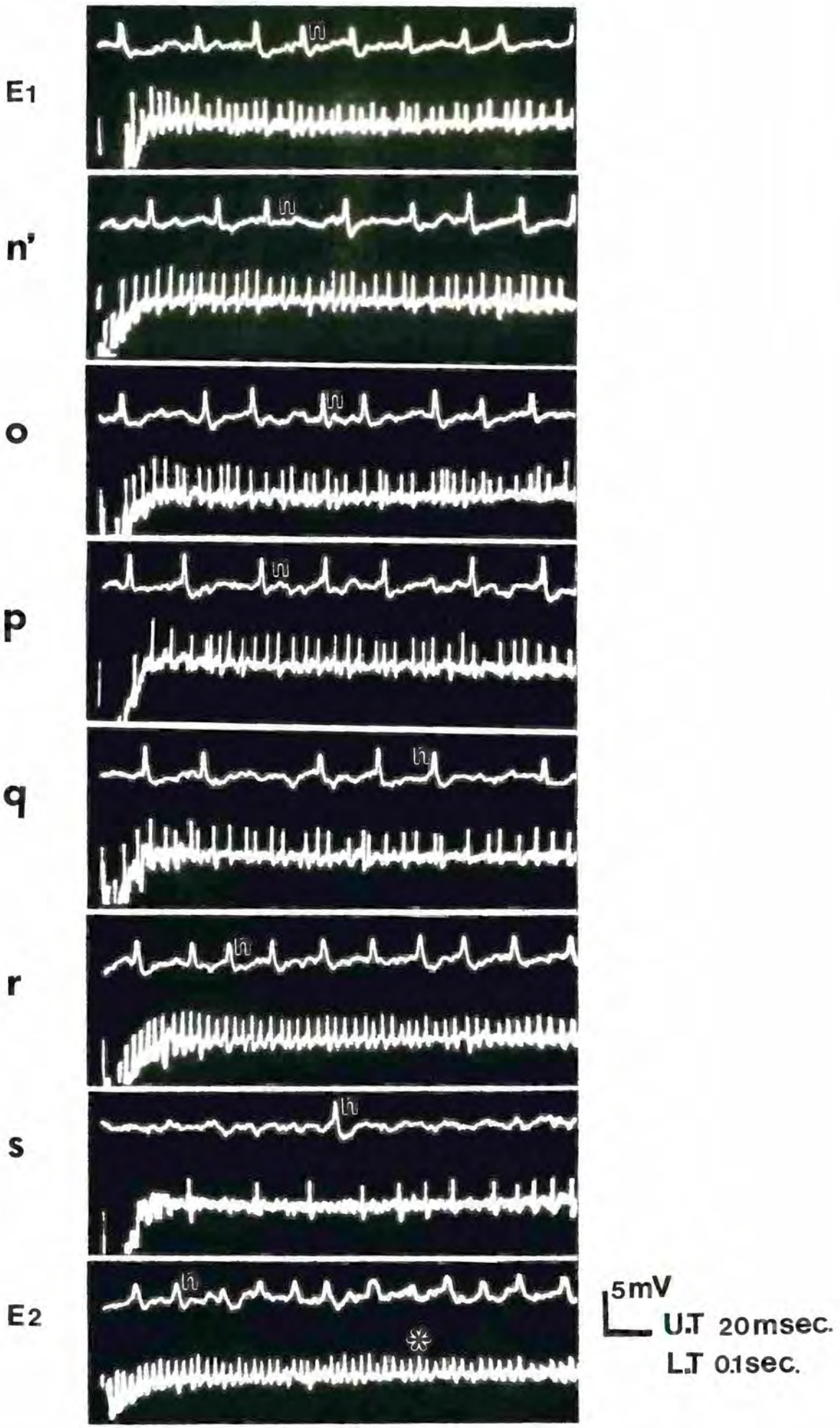
6.3.3. RESULTS

6.3.3.1. Dose-response relationships

There was no detectable firing of spikes from the c-neurone in response to 4-DCN, TLN and ACP for all the concentrations tested (fig. 6.12; 6.15,A-C). With millimolar 4-DCN there was a single spike in one test, which was not considered to be relevant to this study. The mean firing rate of the n-neurone in response to the carrier electrolyte (50 mM LiCl) did not change significantly from that recorded with the control, for all concentrations of the three chemicals. In contrast, 4-PBN and p-HPBN were stimulatory to the c-neurone, in a manner comparable to that obtained with concentrations of cue-lure greater than micromolar (fig. 6.13; 6.14; 6.15,D-F).

FIGURE 6.12

Representative spike traces of electrophysiological recordings from LH2 on the right labellar lobe of a male D. tryoni with a series of concentrations of acetophenone (ACP) in 50 mM LiCl: n'. 0.1 µM; o. 1.0 µM; p. 10 µM; q. 0.1 mM; r. 1.0 mM; and s. 10 mM. E1,E2 are the control recordings with 50 mM LiCl. This is an example of a chemical nonstimulatory to the c-neurone; only the n-neurone was responding to 50 mM LiCl. Decline of amplitude of the n-neurone spikes at the end of this series (* on E2) may be due to deterioration of the preparation. Time base scale: 20 msec. for upper traces (U.T.) and 0.1 second for lower traces (U.T.).


E1
n'
o
p
q
r
s
E2
5mV
U.T 20msec.
L.T 0.1sec.

6.3.3.1.1 4-Phenyl-2-butanone (4-PBN)

Eight concentrations of this chemical in the range 0.1 µM - 20 mM were tested and only three (above 0.1 mM) were stimulatory to the c-neurone (fig. 6.13; 6.15D). At millimolar, "c" had a mean firing rate of about 5 spikes (Nx = 9) in the first second, which was about 10% that elicited by cue-lure and by p-HPBN in longest gustatory sensilla at the same concentration. Unlike cue-lure, for which there were no spikes from the c-neurone at 10 mM, this concentration of 4-PBN caused firing of "c" spikes at a mean rate of 19.9 $\pm$ 8.2 in the first second (Nx = 7). This was the highest rate of all the 4-PBN concentrations but was only about 50% of the rate evoked by 10 mM p-HPBN (fig. 6.14; 6.15E). The firing rate declined and was lower at 20 mM, which was near the saturated emulsification concentration of 4-PBN in 50 mM LiCl. There was no significant change of mean firing rate of the n-neurone in presence of 4-PBN in the electrolyte, except for the slight drop at 10 mM when the firing activity of "c" was maximum (fig. 6.15D).

6.3.3.1.2. 4-(p-hydroxyphenyl)-2-butanone (p-HPBN)

The dose/response curves for the c-neurone, in response to concentrations of p-HPBN in the range 1.0 nM - 50 mM (fig. 6.14; 6.15E) were similar to those for tests with cue-lure, except for the presence of the smaller peak at micromolar of the latter (fig. 6.15F from 6.2). Nanomolar and 10 nM p-HPBN were not stimulatory and it was only in two sensilla (out of a total 18 recorded from) that any activity of "c" was observed at 0.1 µM. Mean firing rate increased gradually with concentration, up to 10 µM, after which there was a large increase at

FIGURE 6.13

Representative traces of electrophysiological recording from sensillum LH5 of a male D. tryoni with concentrations of 4-phenyl-2-butanone (4-PBN) in 50 mM LiCl: g. 0.1 µM; h. 1.0 µM; i. 10 µM; j. 0.1 mM; k. 1.0 mM; l. 10 mM. E1, E2 are control recordings with 50 mM LiCl. Spikes of the c-neurone initially occured at millimolar 4-PBN (k, arrow). A spike with very low amplitude (arrow heads) was also conspicuous. *: a break in the recording due to mechanical vibration during application of micromolar 4-PBN. Time base scale: 20 msec. for upper traces (U.T.) and 0.1 second for lower traces (L.T.).

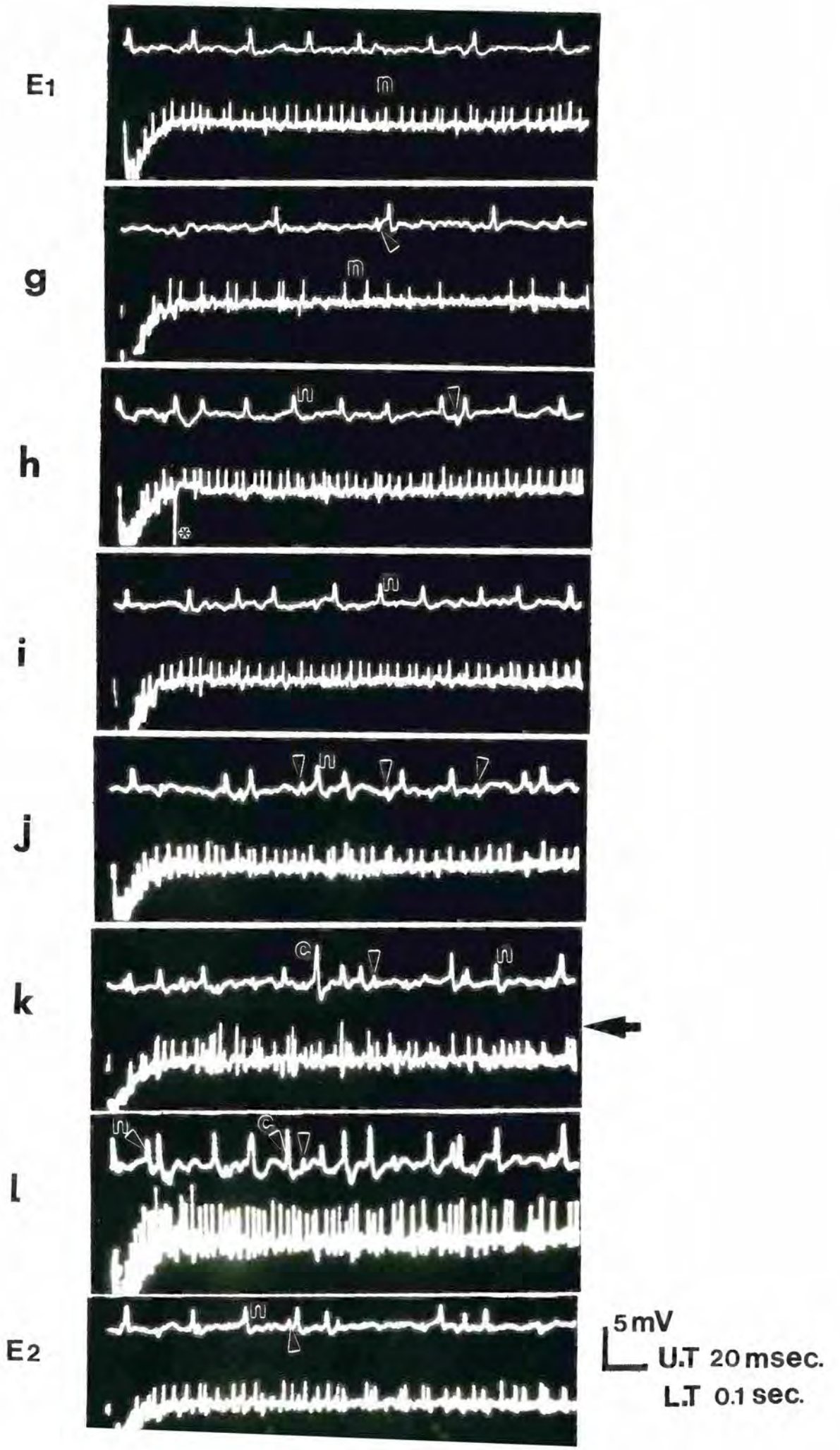

E1
g
h
i
j
k
l
E2
n
c
5mV
U.T 20msec.
L.T 0.1 sec.

FIGURE 6.14

Representative traces of electrophysiological recordings from sensilum LH2 on the right labellar lobe of male D. tryoni with a series of concentrations of 4-(p-hydroxy-phenyl)-2-butanone (p-HPBN): a. 0.1 μM; b. 1.0 μM; c. 10 μM; d. 0.1 mM; e. 1.0 mM; f. 10 mM. The c-neurone started responding at 10 μM (c, arrow) and had very high firing rate at 0.1-10 mM p-HPBN. Summations (S+) occured between spikes from the n- and c-neurones. Time base scale: 20 msec. for the upper traces (U.T.) and 0.1 second for lower traces (L.T.).

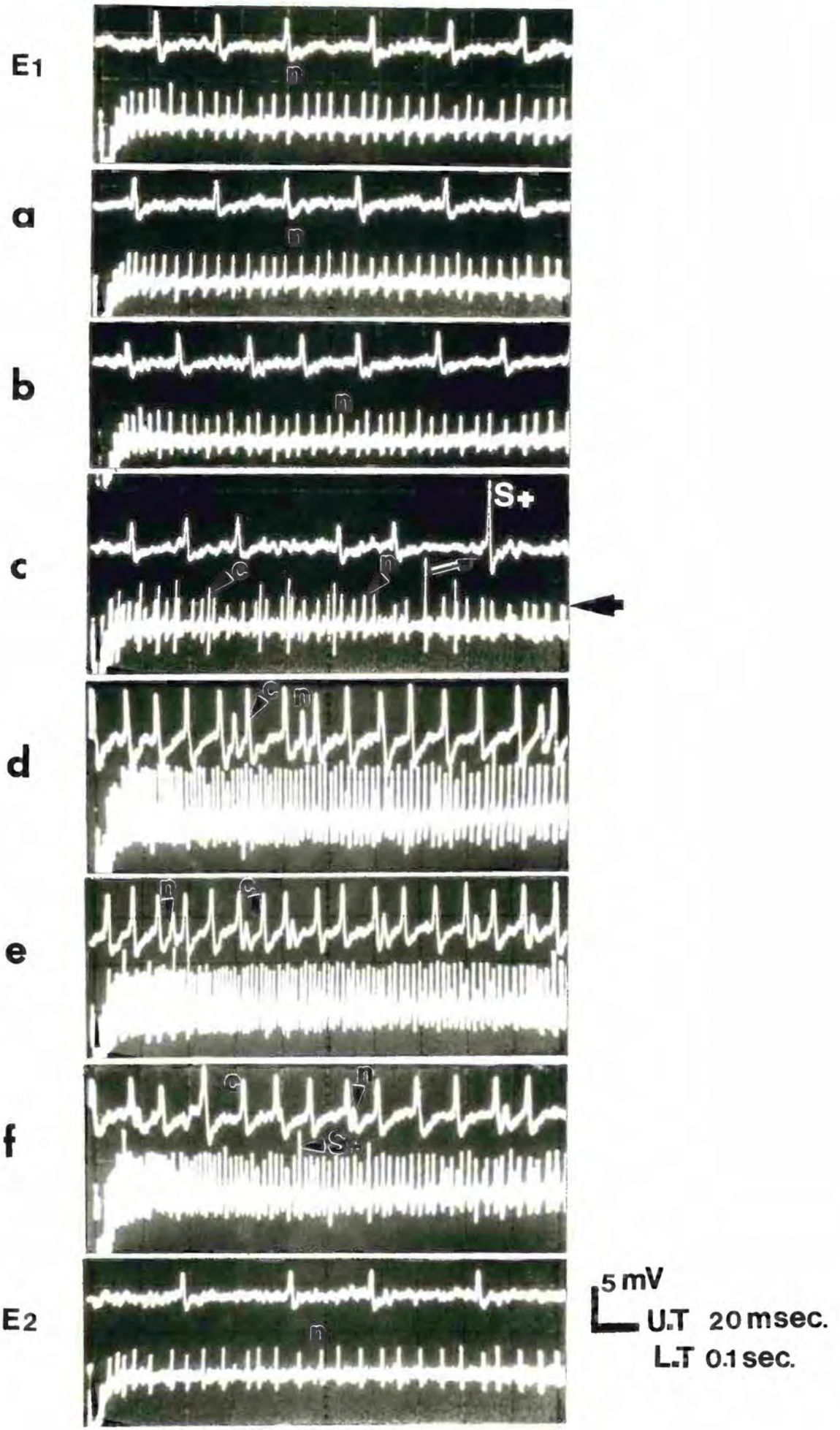

E1
a
b
c
d
e
f
E2
S+
5 mV
U.T 20 msec.
L.T 0.1 sec.

FIGURE 6.15,A-C

Dose/response relationship for neurones "c" (—•—) and "n" (--o--) in longest labellar sensilla of male D. tryoni to concentrations of:

A. 4-decanone;

B. Toluene; and

C. Acetophenone.

Six concentrations (0.1 μM - 10 mM in x10 steps) were tested together with the control (⊙ , 50 mM LiCl).

Number of sensilla tested are shown above the points.

Error bars are ±1 s.e.

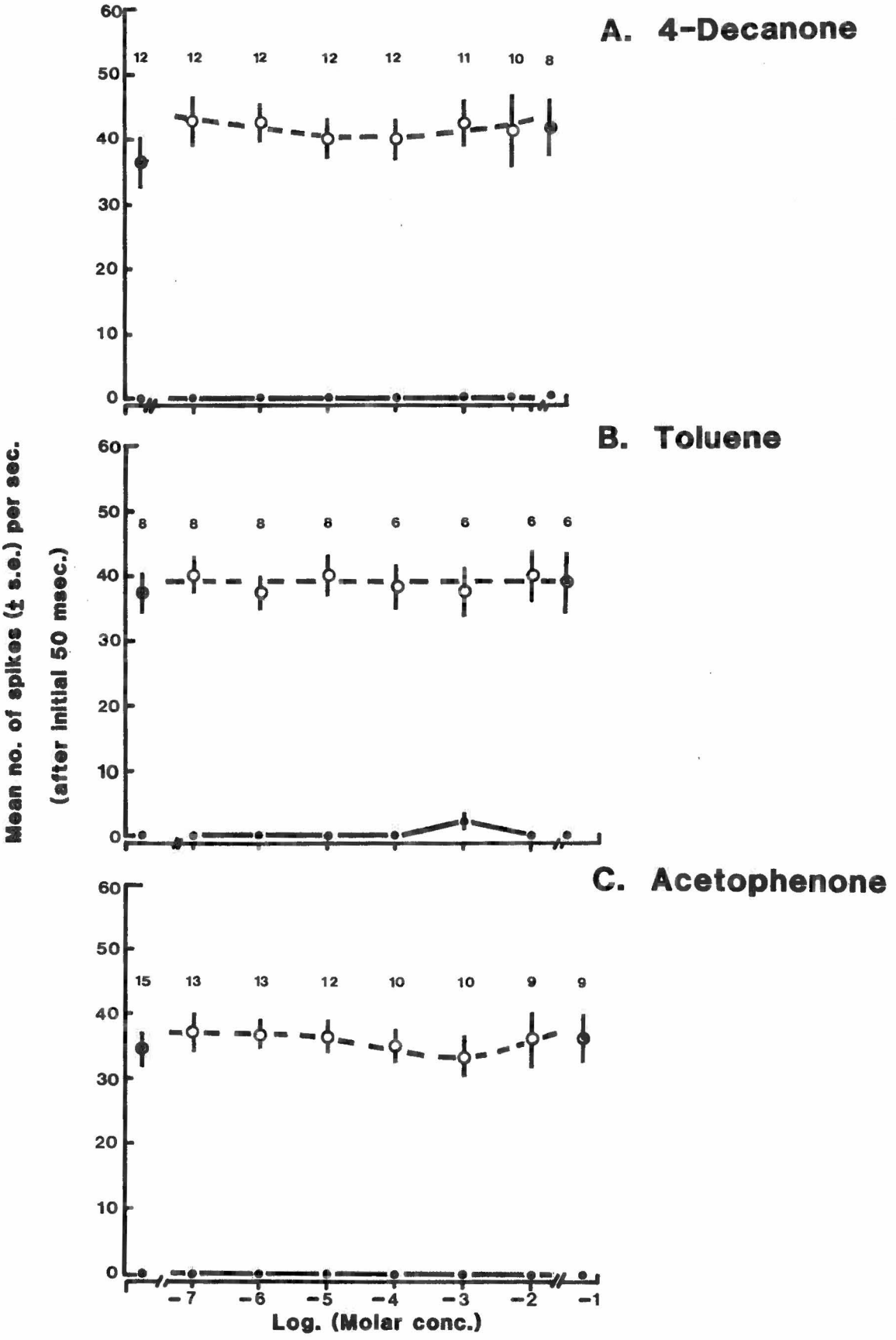


FIGURE 6.15 A-C

FIGURE 6.15,D-F

Dose/response relationship for neurones "c" (—•—) and "n" (--o--) in longest labellar sensilla of male D. tryoni for concentrations of:

D. 4-phenyl-2-butanone;

E. 4-(p-hydroxyphrenyl)-2-butanone (p-HPBN);

F. Cue-lure (cf. section 6.2).

There were extra concentrations tested: nanomolar and 10 nM for cue-lure and p-HPBN, and 20 and 50 mM for 4-phenyl-2-butanone and p-HPBN. Point X in graph E indicates mean firing rate at 50 mM p-HPBN which is thought to have been that high because of reduction in concentration by crystallization of p-HPBN. Number of sensilla tested are shown on ordinates above the points. Error bars are ±1 s.e.

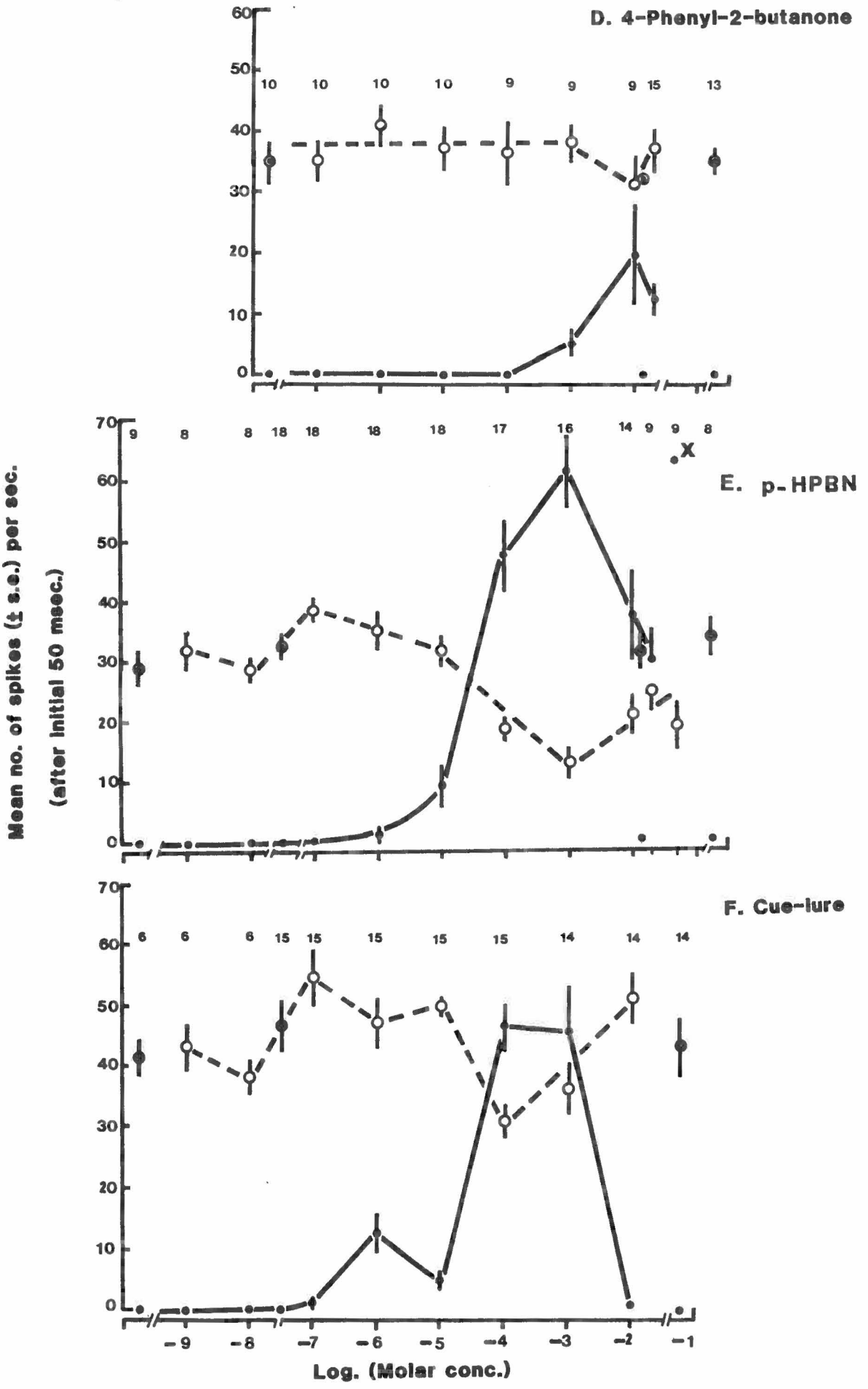


FIGURE 6.15 D–F

0.1 mM reaching a maximum of slightly over 60 spikes in the first second at millimolar. Compared to the mean firing rate with millimolar p-APBN, peak firing rate of "c" with p-HPBN was not significantly higher ($t = 1.53$, $p = 0.05$; $df = 28$). There was a decline in firing rate at higher concentrations, dropping to about 50% of the maximum at 20 mM, similar to that with 4-PBN. At 50 mM p-HPBN, the mean firing rate was higher than at 20 mM and of a similar magnitude as the peak value at 10 mM (point X on fig. 6.15E). This was considered not to be the actual concentration because the solution was saturated and flakes of p-HPBN had crystallised out in the glass micropipette in the course of stimulation. The remaining solution was therefore of much lower p-HPBN concentration probably in the range of those (0.1 mM - millimolar) evoking peak firing rate.

Neurone "n" had fluctuations of mean firing rate with increase in concentration of p-HPBN similar to those observed with cue-lure (section 6.2; fig. 6.15F). Although there were some summations and cancellations between spikes from "n" and "c" neurones during recording, the reciprocity between the firing rate of the two neurones may reflect an inhibitory effect of spikes in the c-neurone on those of the n-neurone or, a direct effect of the chemical on "n". Further work is needed (cf. sections 6.2 and 6.4).

6.3.3.2 Behavioural preference tests: millimolar p-APBN versus millimolar p-HPBN and millimolar p-APBN versus 10 mM 4-PBN

Calculation of the relative percent response for concentrations of two chemicals was carried out as for different concentrations of cue-lure using the procedure outlined in 6.2.

Mean percent of behavioural responses (attraction and feeding) of male D. tryoni to concentrations of the electrophysiologically stimulatory lures are presented in Table 6.7. Millimolar p-APBN was slighlty more potent than p-HPBN of the same concentration. Almost all flies attracted by these two chemicals (those within the 10 cm diameter circle around the lure/agar disc), were seen feeding on the medium (fig. 6.16).

For the preference test between millimolar p-APBN and 10 mM 4-PBN, mean numbers of flies attracted during the 5 minute intervals were not significantly different (Table 6.7). However, numbers of flies feeding on 10 mM 4-PBN/agar was only about 10% of those attracted, which was very significantly lower than that of flies feeding on millimolar cue-lure (Table 6.7, fig. 6.16B). This, together with the observation that mean number of flies responding to millimolar p-APBN in experiment 2 was up to about 50% of that in experiment 1 (highly significant $t = 7.9, 7.2$ for attraction and feeding respectively, $p = 0.05$; $df = 8$), demonstrates a repellent effect on flies by 10 mM 4-PBN. The repellent effect may be mediated through olfactory stimulation at a distance from the source and/or gustatory stimulation when contacted. For flies that landed on the 4-PBN/agar medium, it is more likely to have been through tarsal gustatory sensilla and to a lesser extent the labellar sensilla.

In the latter case, flies explored the substrate with pulsating movements of the labellum and moved to the outside of the plastic dish within a short period after arrival (fig. 6.16B).

Table 6.7 Mean percent response of male D. tryoni attracted to (A) and feeding on (F) millimolar p-HPBN and 10 mM 4-PBN relative to millimolar p-APBN. 40 flies were used for each replicate. Nx: number of replicates.

Exp.	Nx	response	Mean % rel. Response (± s.e.)		Student's t test: t	p
1	5		mM p-APBN vs. mM p-HPBN			
		A	60.4 ± 8.35	46.5 ± 5.33 *	3.33	0.05
		F	58.4 ± 9.12 [a]	44.9 ± 5.91 *	2.77	0.05
2	5		mM p-APBN vs. 10 mM 4-PBN			
		A	20.8 ± 7.43	17.2 ± 7.06 n.s.	0.78	0.05
		F	20.2 ± 7.53 [b]	3.59 ± 1.44 **	4.84	0.05

Differences between means; p-APBN and p-HPBN:
* slightly significant for both attraction and feeding.
p-APBN and 4-PBN: ** highly significant for feeding; n.s. not significant for attraction.
a,b A and F values in column 4 for p-APBN statistically different.

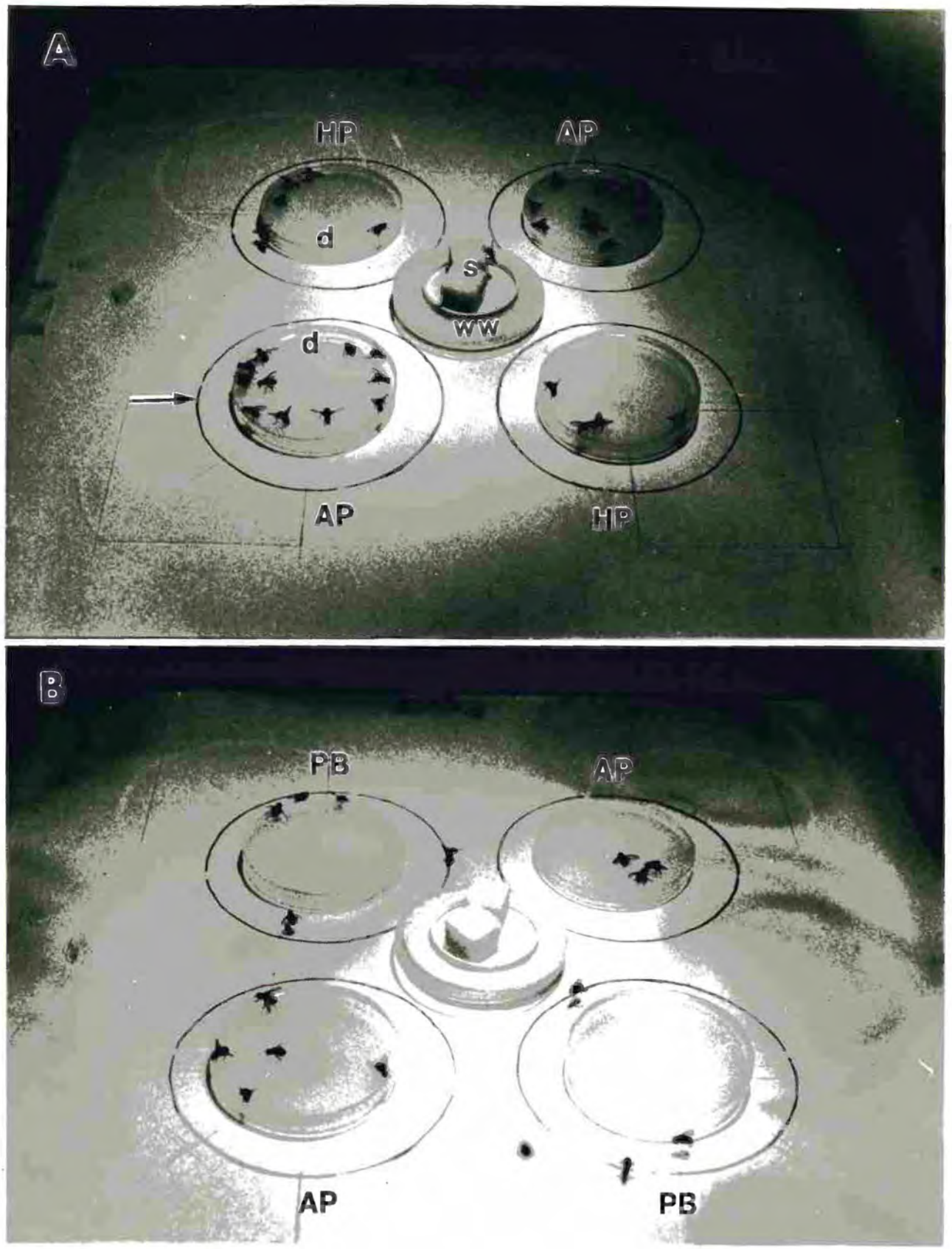
FIGURE 6.16

Front top-view photographs of the floor of the perspex cage used for feeding preference tests with male D. tryoni showing arrangement of lure/agar discs in plastic dishes (d). All flies within the 10 cm diameter circle around each dish (arrow) were scored as those attracted. Those on the medium were invariably feeding.

A. Millimolar cue-lure (AP) versus millimolar p-HPBN (HP). s: sugar cubes; ww: absorbent material (Wettex®) containing water.

B. Millimolar cue-lure (AP) vs. 10 mM 4-PBN (PB).

The photographs were taken 20-25 minutes after starting each experiment.


A
HP
AP
d
s
ww
d
AP
HP
B
PB
AP
AP
PB

6.3.4. DISCUSSION

The chemicals and lures tested ranged from highly stimulatory to nonstimulatory to the c-neurone. Stimulatory effectiveness of the chemicals seems to be a property of the molecular structure, size of molecule is also important. Only 4-phenyl-2-butanone and its two derivatives (p-APBN and p-HPBN) stimulated neurone "c" in accord with a dose/response relation. Some properties of 4-PBN which seem important when compared to the nonstimulatory ACP and TLN are worthy of consideration:

The phenyl ring may be envisaged to fit onto a specific, complementary area on the receptor membrane (cf. Metcalf et al. 1975) such that, active functional groups on the side chains are appropriately orientated to complementary regions on the macromolecules of the receptor site. The $-CH_2$ groups in the aliphatic side chain of 4-PBN, may have two main effects that might contribute to the stimulatory effectiveness of the molecule, discussed below.

A. Length of the molecule and liposolubility

Increasing a hydrocarbon chain length by addition of $-CH_2$ groups increases the liposolubility of a compound through increase in the short range van der Waals forces (Dethier 1951; Ottoson 1958; Diamond and Wright 1969; Metcalf et al. 1981). This may enhance binding of 4-PBN molecules onto the receptor membrane. Dethier and Chadwick (1950) and Dethier (1951) observed that, increase in carbon chain length of alcohols which correspondingly lowered their water solubility (increased liposolubility), decreased the behavioural tarsal rejection threshold in Ph. regina. Likewise, Ottoson (1958)

recorded a gradual increase in amplitude of the slow potential change in the nasal mucosa of frogs for primary aliphatic alcohols, aldehydes and ketones with increasing carbon chain from C_3 to C_7.

B. Electronic charge effect

The presence of electrophilic atoms, proton acceptor and donor groups and points of unsaturation in molecules have been suggested to contribute to the stimulatory effectiveness of: lures (Kikuchi 1973; Metcalf et al. 1975, 1979, 1981); pheromones (Roelofs and Comeau 1971; Liljefors et al. 1987); and sugars (Evans 1963; Jakinovich et al. 1971; Hanamori et al. 1974; Shimada et al. 1974).

Monoketones are almost 100% in the keto form (Geissman 1968) and have an induced dipole on the oxygen of the carbonyl group. In aqueous solutions, a monoketone might be transformed to a hydroxy compound by the electron-deficient carbonyl-carbon accepting electrons of one -OH groups (Diamond and Wright 1969):

An aqueous acidic or alkaline solution of a monoketone at equilibrium has a significant proportion of -OH substituted molecules with an induced dipole on each -OH.

Addition of $-CH_2$ groups between the phenyl ring and the carbonyl (or hydroxy) group(s) displaces the latter further from the phenyl ring. Positioning of an induced dipole adjacent to an oppositely charged one in the receptor membrane may facilitate an interaction between the two, which might be a major contributing factor to changes of the receptor potential

(den Otter 1972c).

The resultant increase in the size of the molecule may determine how well it fits into a receptor site and the nature of any membrane conformational changes that may be induced. Therefore, some or all of the above factors, among others no doubt, act in a way that enhances effective binding of 4-PBN molecules onto the receptor membrane of labellar gustatory sensilla of male D. tryoni. Such factors will be significant in determining the stimulatory effectiveness of the molecule.

6.3.4.1 Nonstimulatory chemicals: Acetophenone, Toluene and 4-Decanone

Although acetophenone (ACP) might be expected to have similar induced dipole(s) in the carrier electrolyte, as suggested for 4-PBN above, their close proximity to the phenyl ring may modify their strength. They would also be several atomic lengths away from possible fixed dipoles on the receptor site suggested to interact with induced dipoles on the stimulus cue-lure molecules (fig. 6.17). Thus, appropriate short range van der Waals force interaction may be very weak.

Toluene is an aromatic compound that lacks both the $-CH_2$ and carbonyl groups. Therefore, even if the phenyl ring was to fit into the hypothesized complementary area of the receptor site membrane, no further steric and/or electronic charge interaction seems possible, such as those expected to cause a receptor potential.

The aliphatic ketone 4-decanone (4-DCN) is nonstimulatory to the labellar gustatory c-neurone of male D.

tryoni; it has seven $-CH_2$ groups and an induced dipole on the carbonyl oxygen (or substituted -OH groups, in aqueous solution). Assuming the molecular length of 4-DCN to be within the limits of that which could effectively be accomodated on a possible cue-lure receptor site, absence of the phenyl ring may cause improper fitting. In addition, random orientation of a molecule on a receptor site minimizes the probability of occurence of charge interaction between induced dipoles and localised ones on the receptor membrane. Regarding the end methyl group, closest to the carbonyl group in 4-PBN and its derivatives, as a reference for size of the molecule; presence of two $-CH_2$ groups between these two in 4-DCN causes a displacement of the induced dipole(s) to the left (fig. 6.17). Even if the molecule was to fit snuggly into the receptor site, the 4-DCN dipole would most likely not align with the suggested fixed dipoles. The remaining weak van der Waals forces and other molecular parameters such as size, might not be sufficient to cause adequate conformational changes in the receptor membrane.

Longer and shorter analogue molecules are less potent behaviourally and often nonstimulatory (Roelofs and Comeau 1971; present work). Metcalf et al. (1975) postulated a receptor site length of about 1.4 nm, equivalent to that estimated for the 3,4-dimethoxyallylbenzene molecule. Shorter molecules without the alkyl or alkoxy had a 10^4 increase in their response threshold (attraction and feeding). Klopping and Meade (1971) observed that, octylformate with a chain length 0.25 nm shorter than that of the very attractive (to Conioscinella melancholica) decylformate, was not attractive. Undecenyl-formate with chain length 0.12 nm longer had lower

attractancy. While shorter molecules are nonstimulatory, partly because they occupy a small part of the receptor site, longer molecules than normal might not fit into a receptor site.

Therefore, it is suggested that acetophenone, toluene, and 4-decanone molecules do not bind effectively onto the hypothesized receptor site of the c-neurone that is activated by 4-phenyl-2-butanone and its derivatives. These chemicals also did not stimulate any other neurone(s) in the longest labellar gustatory sensilla of male D. tryoni. Conformational changes in the receptor membrane, that increase conductance to the inorganic ions which generate receptor potentials, either do not occur or are of subthreshold level.

6.3.4.2 Stimulatory chemicals: Lures; 4-PBN, p-HPBN, p-APBN

4-(p-acetoxyphenyl)-2-butanone (cue-lure) and 4-(p-hydroxyphenyl)-2-butanone were more potent both behaviourally and electrophysiologically than the parent radical 4-phenyl-2-butanone. The 4-phenyl-2-butanone component of the molecules of these two lures is therefore only partly responsible for their stimulatory effectiveness. Para-substitution with $CH_3(O)CO-$ and -OH (for p-APBN and p-HPBN respectively), with respect to the aliphatic side chain of the 4-PBN moiety, seems to affect several molecular parameters such that, threshold concentration and concentration for maximum firing rate are lower by a factor of several magnitudes. The para-substituted side chains, although different in atomic composition, may have some common properties in aqueous solutions. The p-APBN molecule has an additional induced dipole (or two -OH dipoles in aqueous

solution) which may be of modified strength because of the electrophilic oxygen atom between the carbonyl group and phenyl ring. Presence of this oxygen causes a displacement of the dipoles further from the ring and may therefore determine the proximity of the latter to fixed dipoles on the receptor membrane. In addition, this oxygen forms a pivot for the rest of the side chain allowing for flexibility of rotation which might facilitate proper fitting and orientation for optimum interaction with receptor site components. The p-HPBN has a single dipole on the para-substituent -OH group. Though attached to the phenyl ring, it is likely to cause changes at the receptor site comparable to those of the carbonyl group; resulting in an increased firing rate of the c-neurone and in the feeding responses. These responses, together with the attraction of flies to p-HPBN, were of a magnitude close to that obtained with cue-lure. Considering molecule length (fig. 6.17), the acetoxy group makes the cue-lure molecule longer than that of 4-HPBN by several atomic lengths. However, separation distance between induced dipoles of the two side chains of each, which could be quite important for their stimulatory effectiveness, does not seem to differ much. Kikuchi (1973) suggested the bifunctional unit, comprising -OH and -O groups, to have been characteristic of molecules of chemicals most attractive to a mutant (HPB-1) of D. melanogaster. The separation distance between the two groups he considered to be more important than the actual size of the molecules. Roelofs and Comeau (1971) concluded that, very potent synergists of the female sex pheromone (cis-n- tetradecylacetate) of the red-banded leaf roller Argyrotaenia velutinana, had molecular structures of very close resemblance to the pheromone

in size and composition (12-14 carbon atoms, carbonyl and/or C=C groups and electrophilic groups with oxygen or sulphur atoms). Similar factors apply to analogues of 3,4-dimethoxyallylbenzene, which elicit feeding in males D. dorsalis (Metcalf et al. 1975, 1979, 1981).

A slightly longer molecule of cue-lure might possibly facilitate closer fitting onto the receptor site, causing enhanced conformational changes in the receptor membrane contributing to a lowering of threshold. This and other factors such as the electronic charge parameters of the substituents (e.g. $CH_3(O)CO-$ is a proton donor whereas -OH is an acceptor) (Table 6.8), might modify the properties of the whole molecule. Such differences may explain the slightly higher potency of cue-lure in attraction and feeding, though differences observed between the electrophysiological data from the c-neurone in longest labellar gustatory sensilla, with the two lures, were not very significant. Two receptor sites or two subunits of a receptor site on the c-neurone were suggested to explain the occurence of two peaks in the dose/response curve with cue-lure (section 6.2). Molecules of both 4-PBN and p-HPBN seem to bind the site/subunit to give the higher threshold response only.

Different firing rate patterns from those reported here for longest and long sensilla, might occur in neurones in other types of labellar gustatory sensilla of male D. tryoni that were not tested (cf. Maes and den Otter 1976; Maes and Vedder 1978). There was probably also a contribution to behaviour from the olfactory receptors. These may be the subject matters of other, later studies.

As a summary of the above observations, and in

explaining the possible molecular structural basis of the relatively higher potency of cue-lure in feeding (and attraction) of male D. tryoni, a model of a receptor site for the cue-lure molecule on the dendritic membrane of labellar gustatory receptors has been proposed. If some features of this model, derived from gustatory electrophysiology, prove correct, they may also apply to the olfactory neurones that are sensitive to these lures.

6.3.4.3. A hypothetical model of a receptor site for molecules of lures in labellar gustatory neurones of male D. tryoni

Three assumptions were made on parameters of the molecular structure of cue-lure and possible interaction with the receptor membrane at specialised sites. Under suitable conditions (both environmental and physiological), these may generate the sensory input to the CNS that contributes to the elicitation of the observed behaviour.

1. At low concentrations (less than millimolar), interaction between lure molecules and the receptor site is considered to be largely a surface phenomenon. It probably involves close range van der Waals forces (summary, den Otter 1972c). At higher concentrations a relatively large number of molecules might be absorbed into the general matrix of the receptor membrane, probably causing a temporary disruption of the receptor potential mechanism.
2. Receptor potential magnitude may depend on several parameters of the lure molecule: size and shape; induced dipoles on electrophilic groups; points of unsaturation; and liposolubility (cf. Metcalf et al. 1975, 1979, 1981). Fixed polar groups in the receptor membrane facilitate electronic charge interaction with stimulus molecules of apolar compounds (den Otter 1972c).
3. A single cue-lure molecule is likely to fit properly onto a receptor site with its phenyl ring occupying a specific complementary area (cf. Metcalf et al. 1975).

These three factors will facilitate optimum

interaction between receptor site and stimulus molecule, leading to the generation of receptor potentials in a c-neurone, according to the dose/response relationship discussed in this section of the thesis.

Figure 6.17 is a two-dimensional representation of the fitting of cue-lure and related molecules onto a hypothetical receptor site on the c-neurone membrane surface. It depicts a possible orientation of a cue-lure molecule perfectly fitting the site. Points of matching are marked as either electronic or physical. The other chemicals are shown referenced to the position of the phenyl ring complementary patch on the receptor site (fig. 6.17). As the molecular length decreases, displacement of the active groups from complementary patches on the receptor site weakens the van der Waals forces and other physical interactions. While this should be viewed in a kinetic context, it will no doubt cause a reduction in the effectiveness of the molecule in evoking a receptor potential. For the nonstimulatory chemicals, the effect is so strong that insufficient receptor potential for the triggering of action potentials is generated.

The lower stimulatory effectiveness of 4-PBN compared to cue-lure (higher threshold and higher concentration for peak firing) stems from the dynamics of interaction of its molecules with the receptor sites. Two of the most likely mechanisms are:

1. Each 4-PBN molecule, with one or two induced dipoles, could fit onto about half of the lure receptor site, with respect to the position of the phenyl ring. A large number (high

FIGURE 6.17

Diagram (not to scale) of a hypothetical receptor site or receptor site subunit for the cue-lure (p-APBN) molecule on the receptor membrane of a lure-sensitive labellar gustatory neurone in D. tryoni. The site is within a few atomic diameters of the membrane surface and orientation of the side chains of the molecule are referenced to the positioning of its phenyl ring on a matching area. Vertical dotted lines indicate projection for the fitting of molecules of the other chemicals onto the receptor site: 4-DCN (4-decanone); TLN (toluene); ACP (acetophenone); 4-PBN (4-phenyl-2-butanone); p-HPBN {4-(p-hydroxyphenyl)-2-butanone}.

Dark patches indicate supposed areas of physical matching between membrane and molecule and cross hatched areas indicate supposed electronic charge matching.

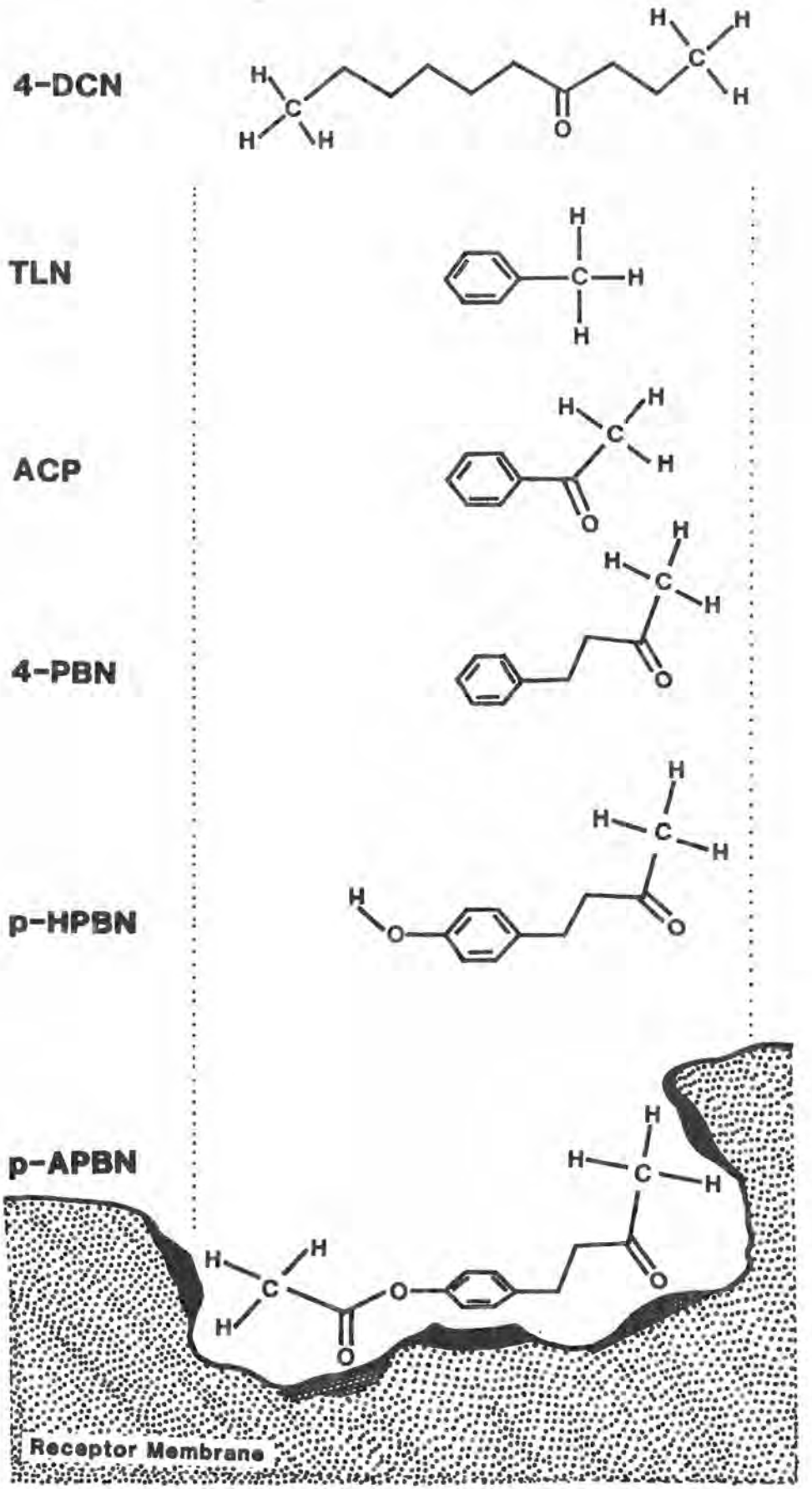


FIGURE 6.17

concentration) of these molecules may have to occupy the receptor sites to cause a supra-threshold receptor potential necessary for firing of action potentials.

However, the maximum firing rate of spikes at 10 mM 4-PBN was still less than 50% of that with millimolar cue-lure indicating a smaller receptor potential. This was also demonstrated in the behavioural choice experiment in which though 10 mM 4-PBN attracted flies, the concentration was so high that flies were repelled from feeding on it as well as from millimolar cue-lure in the adjacent dishes (fig. 6.16B).

2. Cooperativity between two molecules:

Amoore (1952) suggested that dipole association or hydrogen bonding may facilitate "co-operation" of two molecules of the same compound to fit into a receptor site. At the interface of the receptor membrane and the dendritic fluid, distribution and orientation of apolar stimulus molecules are envisaged to be random. Two molecules of 4-PBN might fit onto a single receptor site similar to a single molecule of cue-lure (fig. 6.18). It requires that, in addition to one molecule (no. 1 in fig. 6.18) fitting as postulated above, a second molecule (no. 2) superimposes over the first one at a planar rotation of about 180°. With minor horizontal adjustments and some conformational changes of the membrane, the side chain of the second molecule could fit onto the unoccupied part of a receptor site. It may occur <u>vice versa</u> so that, in either case, the induced dipoles on the two lure molecules match with charged groups on the receptor site. However, contrary to cue-lure, the magnitude of interaction might be lower depending on a number of

FIGURE 6.18

Diagram of a hypothetical lure receptor site on the c-neurone in labellar sensilla of male D. tryoni occupied by two molecules of 4-phenyl-2-butanone similar to a cue-lure molecule. A second (2) 4-PBN molecule at 180° horizontal rotation (curved arrow) relative to (1) has the side chain fitting into the unoccupied part of the site after horizontal adjustment (double-ended arrow). d: minimum separation distance between phenyl rings and Δd that between induced dipole on carbonyl oxygen and charged groups in the membrane (cross hatched). Dark areas denote physical interaction.

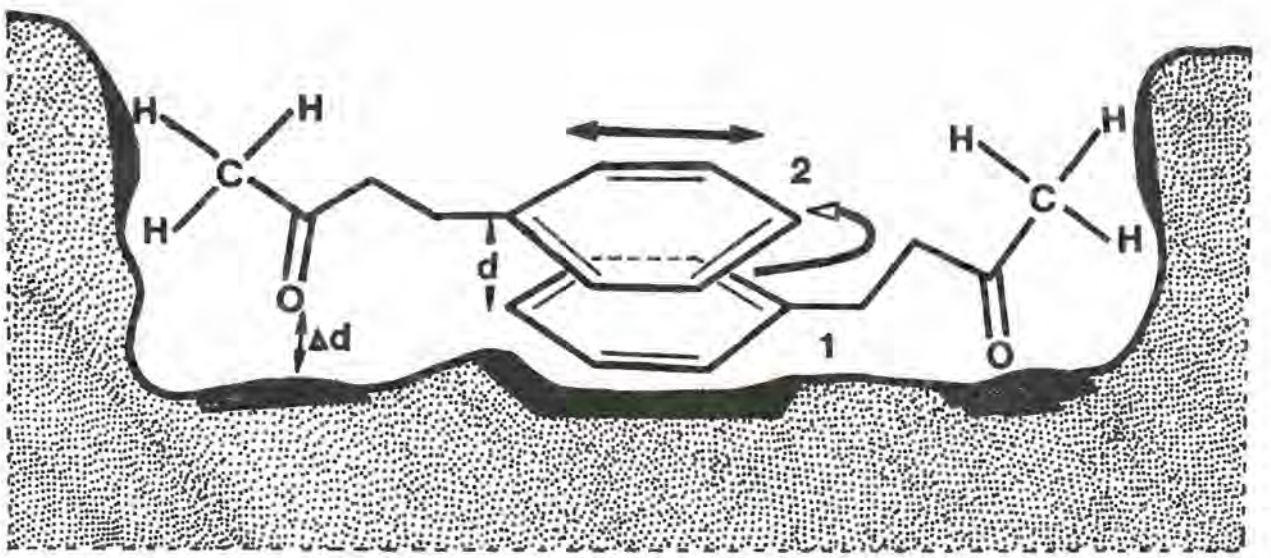


FIGURE 6.18

factors. There is a minimum separation distance (d) between the phenyl rings within which intermolecular repulsive forces come into play. This would in turn determine the closeness (Δd) of the induced dipole(s) of the second molecule, to those on the membrane; thus supposedly affecting the strength of the interaction (fig. 6.18). The probability that two molecules will superimpose effectively out of their random distribution as suggested may require a relatively larger number (higher concentration) of 4-PBN, to elicit spikes from "c". Even then, the proportion of receptor sites occupied may still be small. Kinetic factors are likely to be important in lure molecule/receptor site interaction, but these are beyond the scope of this thesis.

Though only six chemicals were tested electrophysiologically, some specificity has been demonstrated for firing of gustatory neurone "c" with respect to the molecular structure of 4-phenyl-2-butanone and its two derivatives. Stimulatory effectiveness seems to be characteristic of the molecular structure of a class of compounds. This is from the observation that, additional substituent functional groups or absence of others (that affect the size of a molecule) compounds the effects of shape and size and those of hydrophobicity and electronic charge interaction. Therefore, variation in electrophysiological and hence behavioural responses cannot be wholly attributed to the effect of any one of the molecular parameters. Para-substitution (with respect to the aliphatic side chain) on the phenyl ring of 4-phenyl-2-butanone with -OH and $CH_3(O)CO$- groups has the effect of lowering the electrophysiological threshold and the concen-

tration evoking peak firing rate of "c". Increased attractancy and feeding are subject to the presence of these groups on the 4-phenyl-2-butanone molecule, probably through modification of some of its molecular parameters. In addition to increase in length of the molecule, these electrophilic groups (with induced dipoles) might modify the electronic charge constants of the whole molecule and its hydrophilic characteristics (lipophilic constant π).

With reference to tabulated values of these constants and the above observations, further putative change in the activity of 4-phenyl-2-butanone may be achieved by para-substitution with an acetonyl group (CH_3CO-) as suggested below.

6.3.4.4 PUTATIVE ACETONYL DERIVATIVE OF 4-PHENYL-2-BUTANONE

Hansch et al. (1973) compiled a table of constants of some molecular parameters of substituents of aromatic compounds that are useful in drug manufacture. These constants are usually useful in correlating the molecular structure with reactivity of drugs. The lipophilic constant (Π), electronic charge constant for substitution at meta- (σm) and para-position (σp) and molar refractivity (MR) for four substituent groups are quoted in Table 6.8. Molar refractivity is defined as tolerance of the substrate to bulkiness of a drug binding onto it. In the case of gustatory receptors, it may analogously refer to the effects of the size of the stimulus molecule on the receptor site that lead to conformational changes in the receptor membrane. These constants have been selected in connection with three attractants of male D. tryoni of which, chemicals with substituents 1 and 3 (table 6.8) were tested behaviourally and electrophysiologically.

Presence of other substituents on the same phenyl ring may modify the overall electronic and hydrophobic parameters of the lure molecule. However, for the substituents under consideration there is a pattern in relation to the attractancy and lure feeding by flies. Functional groups nos. 2 and 3, which are substituents for 4-(p-methoxyphenyl)-2-butanone (anisylacetone) and 4-(p-hydroxyphenyl)-2-butanone, are proton acceptor/electron donor groups ($-\sigma p$). Substituent no.1, as in cue-lure, is a proton donor/electron acceptor. The molar refractivity of 1 is higher than that of 2 and 3 despite close similarity of the lipophilic constants of 1 and 3 (Table 6.8). Since cue-lure is more potent, differences in these constants,

especially the electronic charge parameters might play a role in rendering the molecule more stimulatory at the receptor site.

Table 6.8 Some aromatic substituent constants (after Hansch et al. 1973)

	Fn. group	Π	σm	σp *	MR	Representative lure
1.	$OCOCH_3$	-0.64	0.39	0.31	12.5	cue-lure
2.	OCH_3	-0.02	0.12	-0.27	7.9	anisylacetone
3.	OH	-0.67	0.12	-0.37	2.9	p-HPBN
4.	$COCH_3$	-0.55	0.38	0.50	11.2	**

* σm, σp positive for proton donors/electron acceptor and negative for proton acceptor/ electron donor groups.

** suggested acetonyl derivative of 4-phenyl-2-butanone.

Functional group 4 (Table 6.8) has all constants similar to 1 except that 4 is a stronger proton donor/electron acceptor at the para- position. To test the contribution of the electronic constants to the lures of male D. tryoni, I suggest substitution of the acetonyl group at the para- position (with respect to the butanone side chain) on the phenyl ring of 4-phenyl-2-butanone. If this is possible, comparative behavioural and electrophysiological tests between the acetonyl para-substituted 4-phenyl-2-butanone and cue-lure may provide some data useful in correlating the magnitude of electronic charge constant, stimulative effectiveness of the c-neurone and the resultant behaviour.

6.4 ELECTROPHYSIOLOGICAL RESPONSES TO CHANGING STIMULI WITH A FLOW SYSTEM (A joint project with Dr M.J. Rice)

6.4.1 INTRODUCTION

Longest and long labellar gustatory sensilla of male D. tryoni have four gustatory neurones and a mechanoreceptor (Chapter 5 of this thesis). Two neurones (P1 and P2) have been shown to be positively sensitive to concentrations of LiCl, whereas one (n) was negatively sensitive. The remaining chemosensitive neurone is suspected to be positively sensitive to sugars (S), although this has not yet been tested.

Cue-lure stimulated a single neurone denoted as "c", whose spike amplitude was similar to that of the P2-neurone, positively sensitive to LiCl concentrations greater than 100 mM. It is likely that either P1, or P2, or (S) is the c-neurone that responds to cue-lure. This implies either that cue-lure stimulates the neurone at the same receptor site as another effective stimulus, or that the neurone has a separate receptor site for the lure molecule. Although stimulation mechanisms on gustatory receptors by electrolytes may differ from those by apolar compounds (den Otter 1972b,c), salt-sensitive neurones can be responsive to other ionising compounds or have receptor sites that bind apolar compounds (Shiraishi and Kuwabara 1970).

There are several methods that have been used to determine responses when two chemicals or two odours are used simultaneously to stimulate a neurone that is sensitive to both: use of mixtures of chemicals (Morita and Shiraishi 1968; Shiraishi and Kuwabara 1970; Bowdan 1984; Sutcliffe and Mitchell 1985); and cross-adaptation (Payne and Dickens 1976). Sulfhydryl compounds that selectively react with receptor proteins blocking

some of the receptor sites have been used for investigations on the number of different receptor sites on the sugar-sensitive neurone of B. peregrina (Shimada et al. 1974, 1985).

Cross-adaptation has been widely used in olfactory studies in man and other animals in attempts to classify odorant compounds into groups with common molecular components that presumably stimulate specific receptors (reviews by Engen 1971; Beets 1971, 1975). In this method, two different chemical stimuli are used. The adapting (conditioning) stimulus is applied continously until the responding neurones are fully adapted. The test stimulus is then applied immediately, within the adaptation period of the receptors. Lack of response to the test stimulus previously known to be stimulatory has been taken to mean that the receptor is sensitive to both stimuli. However, Beidler (1975), Payne and Dickens (1976), and Shimada (1987) observed that adapted neurones still responded to the test stimuli. They suggested presence of different receptor sites, with different adaptation properties, for the two chemicals on the same neurone.

Payne and Dickens (1976) developed a technique for applying odours in succession, to determine specificity of the antennal olfactory neurones of Dendroctonus frontalis to pheromones and host plant volatiles. They used a three-way air valve, which was switched from delivering the conditioning odour after appropriate exposure to a second outlet for the test odour. EAG's elicited from groups of differentially adapted olfactory receptors were measured.

A flow technique, to enable changing of test

solutions, may be very useful in electrophysiological studies of gustatory neurone responsiveness. Responses to a range of stimulus concentrations can be done efficiently, without changing any of the parts as happens with the standard glass micropipette technique (Hodgson et al. 1955). However, unlike in olfaction, where test odours are transported in an air stream into an open space; the low compressibility of solutions is a major problem. Small changes in volume of solution during the change of stimulus in an almost closed flow system causes overflow, which often floods the insect gustatory sensillum preparation. Sturkow (1965) used a flow system in electrophysiological studies on the labellar sensilla of Ph. regina. The system comprised of a probe made of glass micropipettes with 2-7 channels emptying into a wider common tip chamber. Each channel had a grounded platinum electrode and the required solutions were switched by taps. Test solutions flowing over the tip of a sensillum and out of the pipette were absorbed by a strip of filter paper.

This section of the thesis is a report on the design, construction and testing of a simple flow system for use in applying different test solutions to the tip of gustatory sensilla of insects. Some results on switching from 0.5 M LiCl to a millimolar cue-lure solution are given.

An open flow system was designed in order to minimize the overflow problem. A recording electrode is embedded in the centre of the flow system, so that different test solutions make contact with it. The system allows for a change from one stimulus solution to another, without breaking contact between the sensillum and the flowing solution. This eliminates

disadaptation problems, which are often encountered with rapidly disadapting neurones. When the adapting solution is also the carrier electrolyte of the test chemical, continued adaptation of the responding neurones is maintained during the change of solutions. Response of any other neurones, recruited by the test chemical, can readily be recognised. Different concentrations of the same chemical can also be tested, by changing to another solution after an appropriate disadaptation period.

6.4.2 MATERIALS AND METHODS

D. tryoni males used in the tests were 7-16 days post-eclosion. The test solutions were: aqueous 50 mM and 0.5 M LiCl and millimolar cue-lure in 0.5 M LiCl. The two LiCl solutions were applied in succession to test the working of the flow system. 0.5 M LiCl was switched to millimolar cue-lure in an attempt to determine which neurone (P1, P2, or S) in the gustatory sensillum responded to the lure.

6.4.2.1. Components of the flow system

For a smooth flow of solutions, the flow system was designed to use gravity flow from a raised reservoir (R), aided by the sink effect of a U-tube arrangement, controlled by a tap (T) (fig. 6.19). Further control of the volume of solution flowing was effected by a glass pipette (gp) of 2 mm internal diameter, pulled into a 6 mm long shank with an orifice of about 300 µm. The solution was delivered, through the pipette, into the in-flow well (IW) on the flow stage. Polystyrene tubes of 2 mm internal diameter, electrically shielded with earthed copper braid (b), connected the reservoir to the glass pipette. The

FIGURE 6.19A

Diagrams of the components of the prototype flow system for stimulating insect gustatory sensilla, illustrating:

A. Arrangement of the perspex flow stage (PFS), braided (cross hatch) and earthed flow tubes for delivery of solutions from reservoir (R), and the syringe (Sy) attachment for injection of solutions.
b: earthed copper braid; C: receptacle with absorbent material; e: recording electrode; EH: exit hole; Fg: flow groove; G1: lead from "e" to G1 input of probe and preamplifier; gp: glass pipette; h: head of solution above level of pipette tip; IW: inflow well; J: collecting jar; N: hypodermic needle; s: shallow inflow groove; T: tap. The microscope (M) was positioned to give a clear view of Fg.

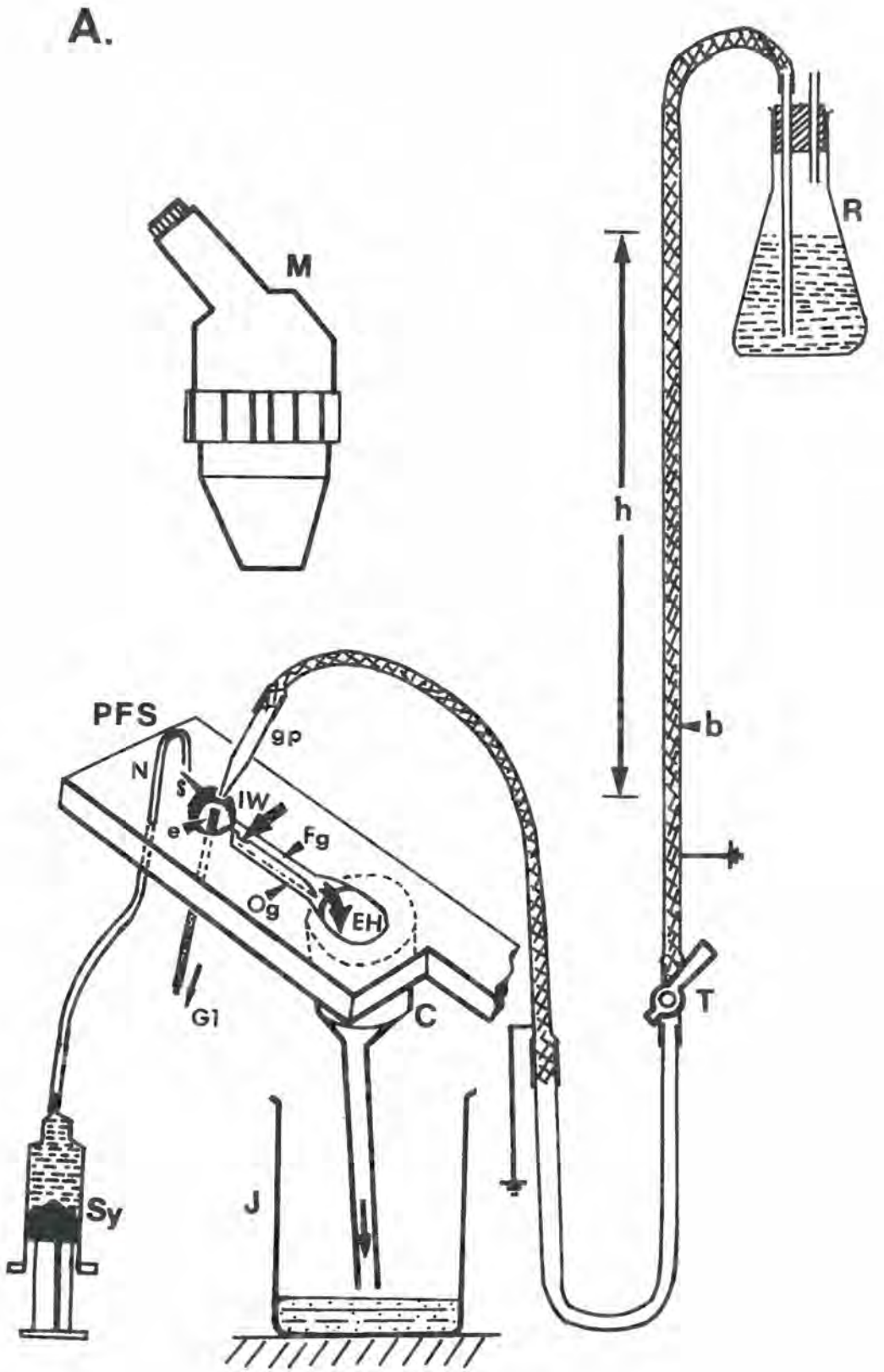


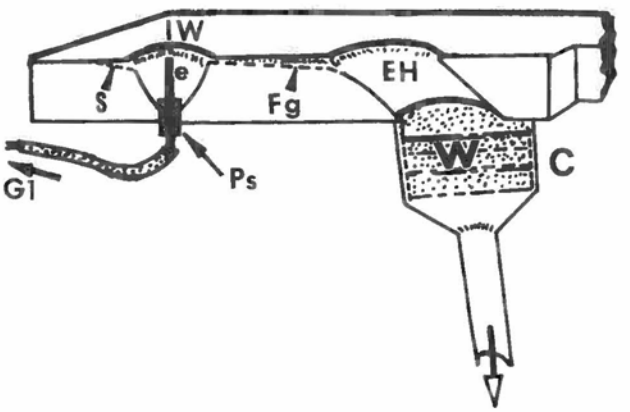
FIGURE 6.19 A

FIGURE 6.19 B,C

B. Section of the flow stage along the flow groove showing placement of the recording electrode in IW and the flow grooves. Ps: polystyrene septum holding the electrode; W: absorbent material (Wettex®).

C. Preparation holder (Ph) with a male D. tryoni preparation (p) attached to the underside of the perspex block (ss) at one end. G2: lead from electrode in the preparation to the G2 input of the probe and preamplifier.

B.



C.

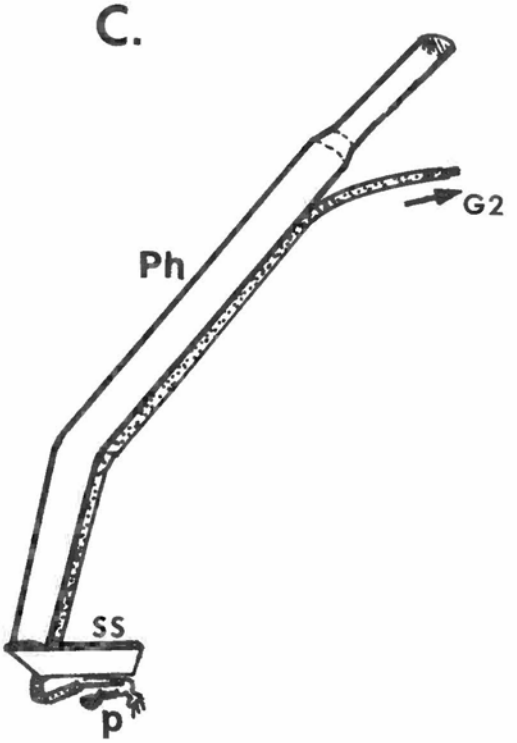


FIGURE 6.19B,C

in-flow well was 4 mm in diameter at the surface and tapered to a depth of 2 mm at the point of insertion of the recording electrode (e) (figs. 6.19A, B). The solution filled IW and then flowed down a groove (Fg) of 1 mm width and increasing depth from 0.5 mm at IW to 1 mm at the exit hole (EH). Solutions drained through EH into a receptacle (C), filled with layers of absorbent material (W, Wettex®) and into a collecting jar (J). To stablise the level of solution in Fg, a groove (Og), which was 2 mm wide and 1 mm deep, parallels Fg and connects it via a notch (arrow) in the 360 µm wide strip separating the grooves. The shape of the flow area of EH and the absorbent material also helped to ensure a flow of uniform level (fig. 6.19B). Breaks in the surface tension of the solution caused the formation of drops at EH, which generated electrical noise at the recording electrode.

The second solution was in a 5 ml syringe (Sy), connected via a polystyrene tube of 0.5 mm bore, to a 24 gauge hypodermic needle (N), passing through a hole drilled close to IW (fig. 6.19A). The needle was bent so as to deliver the test solution into a shallow 1 mm wide groove (S) emptying into IW but not immediately connecting with the solution. This lack of continuity prevented the transfer of electrical interference from the syringe system and premature diffusion of chemicals in the test solution into that on the flow stage.

6.4.2.2. Orientation of the preparation

The preparation holder was a perspex rod, 6 mm diameter, with a small perspex block (ss) glued at right angles at one end (fig. 6.19C). The other end was clamped on a

micromanipulator, orientated so that the L-shaped end of the holder (broad side of ss) was parallel to the flow stage (PFS). Side movements of the micromanipulator arm moved ss and the fly (p) (attached on its dorsum to the underside with sticky tape), parallel to Fg (figs. 6.19A,C). An Ag-AgCl electrode, connected to the G2 input of the preamplifier probe via a lead, was positioned through the thorax of the fly and halfway into the extended proboscis. Viewing through the microscope (M) and using the micromanipulator, the tip of a longest sensillum was placed into the flowing solution in Fg, over the edge of the notch.

The electrodes were connected, via a probe, to a Grass P16 preamplifier. Recording and visual display were as in the other electrophysiological tests reported. The flow system and the preamplifier were in a Faraday cage and all the components were grounded, to minimize electrical interference.

6.4.2.3. Application of test solutions

To change from one test solution to another, four main steps were carried out:

1. Opening of tap T to a set point, to allow a gentle flow of the adapting (control) solution through gm into IW and down Fg. Mean flow rate estimated from the volume of solution flowing into EH without the absorbent material was 18.9 $\pm$ 1.60 µl/second (range, 8 - 31.7, Nx = 15) at a mean head (h) of 19.5 $\pm$ 0.11 cm (range, 18.8 - 20, Nx = 15).
2. Lowering the preparation such that the tip of only one "longest" sensillum contacted the flowing solution.
3. Turning the tap to near off position after a period of stimulation. IW and Fg remained filled with solution and

contact of the sensillum with the solution resulted in a continous discharge of spikes.

4. A gentle release of the test solution from the syringe, with the plunger. The test solution displaced the control solution from IW and Fg, flushing it out into C. A tape recorder recorded the spike trains generated by the changeover of the two solutions.

During change of solutions, the preamplifier sometimes needed re-balancing, depending on the difference between concentrations. The control solution was used to flush out the test solution prior to the start of another test run.

6.4.3. RESULTS AND DISCUSSION

Observations on neuronal activity during change of solution were as follows:

6.4.3.1. Change from 50 mM LiCl to 0.5M LiCl

Fig. 6.20A show parts of the traces of spikes recorded from flies on the flow system with 50 mM LiCl when it was changed to 0.5 M. There was a discontinuity in firing at the time of change of LiCl concentration at the tip of the sensillum. This indicates that, although there may be some dilution due to mixing of the two solutions at the front, subsequently the solution of higher concentration reached the sensillum tip as a front, rather than a gradual change. At the onset of firing after the discontinuity, at least one other neurone of medium amplitude (P1) was firing in addition to the n-neurone (fig. 6.20A).

The temporary discontinuity of firing during change of

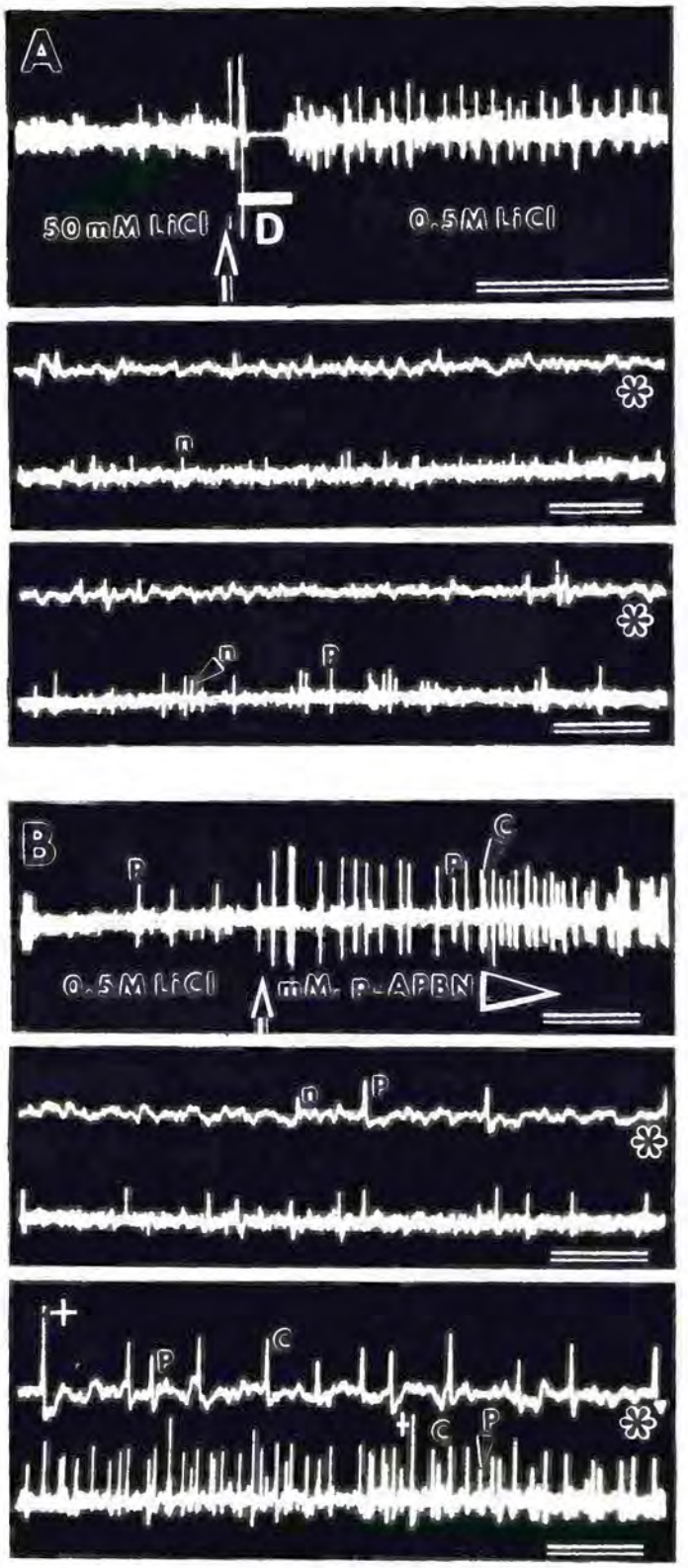
FIGURE 6.20

Traces of spikes recorded from "longest" sensilla of male D. tryoni on the flow system:

A. Shows the point of change (arrow) from 50 mM to 0.5 M LiCl, at which a discontinuity in firing (D) occured. The n-neurone and both n- and P-neurones were firing in response to the two concentrations shown on the expanded traces (asterisks) for 50 mM and 0.5 M LiCl.

B. A change (arrow) from 0.5 M LiCl to millimolar cue-lure (p-APBN) in 0.5 M LiCl. Spike types are shown in the upper traces c: cue-lure sensitive neurone; P: positively LiCl-sensitive neurone; and (+) spikes resulting from the summations of c and P. Note increase in firing rate (horizontal arrowhead) about 600 msec. after the change.

Each bar represents 0.2 second for traces illustrating change, on lower traces for 50 mM and 0.5 M LiCl, and for millimolar cue-lure. For upper traces (asterisks), the bar represents 50 msec.


A
50 mM LiCl
D
0.5M LiCl
50 mM LiCl
0.50M LiCl
B
C
P
0.5M LiCl
mM. p-APBN
0.50M LiCl
+
mM. p-APBN

LiCl concentrations may have resulted from a number of factors. An abrupt, almost 10-fold, change in concentration in the tip chamber of a sensillum, may cause an osmotic shock to the dendrites. This may lead to temporary streaming of water from the dendritic cytoplasm, which may cause some shrinkage. Broyles et al. (1976) suggested the presence of a partial diffusion barrier between the extracellular ion reservoirs and those terminal portions of dendrites accessible to stimulating fluids. Movements of ions across such a barrier may create a short-term osmotic potential prior to establishment of an equilibrium. The duration of the discontinuity may therefore represent the time period during which the concentration of solution in the tip chamber of a sensillum builds up rapidly from 50 mM (ca. 166 mOs/kg) to 0.5 M (ca. 981 mOs/kg) due to the exchange of ions between the concentrated solution and the receptor fluids (fig. 6.21A). This duration may vary from one sensillum to another depending on whether the pore is partially blocked by a plug of exudates from the tip chamber. Sturkow (1965, 1967) reported variable latency (0.39 - 1.1 sec.) for the NaCl-sensitive neurone, for the change of stimuli from water to molar NaCl, in a flow system. The latency was suggested to be affected by the inertia of the flowing solution, diffusion of solution through the tip pore, which itself was affected by the presence of a viscous substance and the latency of the neurones.

The discontinuity may conceivably have arisen from the electrical characteristics of the preamplifier and the recording equipment and/or the handling of the flow system. The preamplifier may not have adjusted appropriately to the abrupt

FIGURE 6.21

Diagrams of the tip of a sensillum in contact with a solution illustrating the concentration of molecules (density of dots) at three stages in a flow experiment:

A. A change from 50 mM (S1) to 0.5 M LiCl (S2). Blank and filled arrowheads in stage 2 indicate direction of flow of water and ions, respectively.
C: cuticle of the sensillum shaft; D: gustatory denrites (only two of the four are shown); L: receptor lymph; P: pore; TC: tip chamber of sensillum.

B. Change from 0.5 M LiCl (S1) to millimolar cue-lure (p-APBN) in 0.5 M LiCl (S2). Cue-lure molecules (large dots) move into the tip chamber together with a relatively small number of lithium and chloride ions that may be necessary to restore the steady state which may be upset through dilution by the dendritic fluid.

A. 50 mM to 0.5M LICl

B. 0.5M LICl to mM. p-APBN

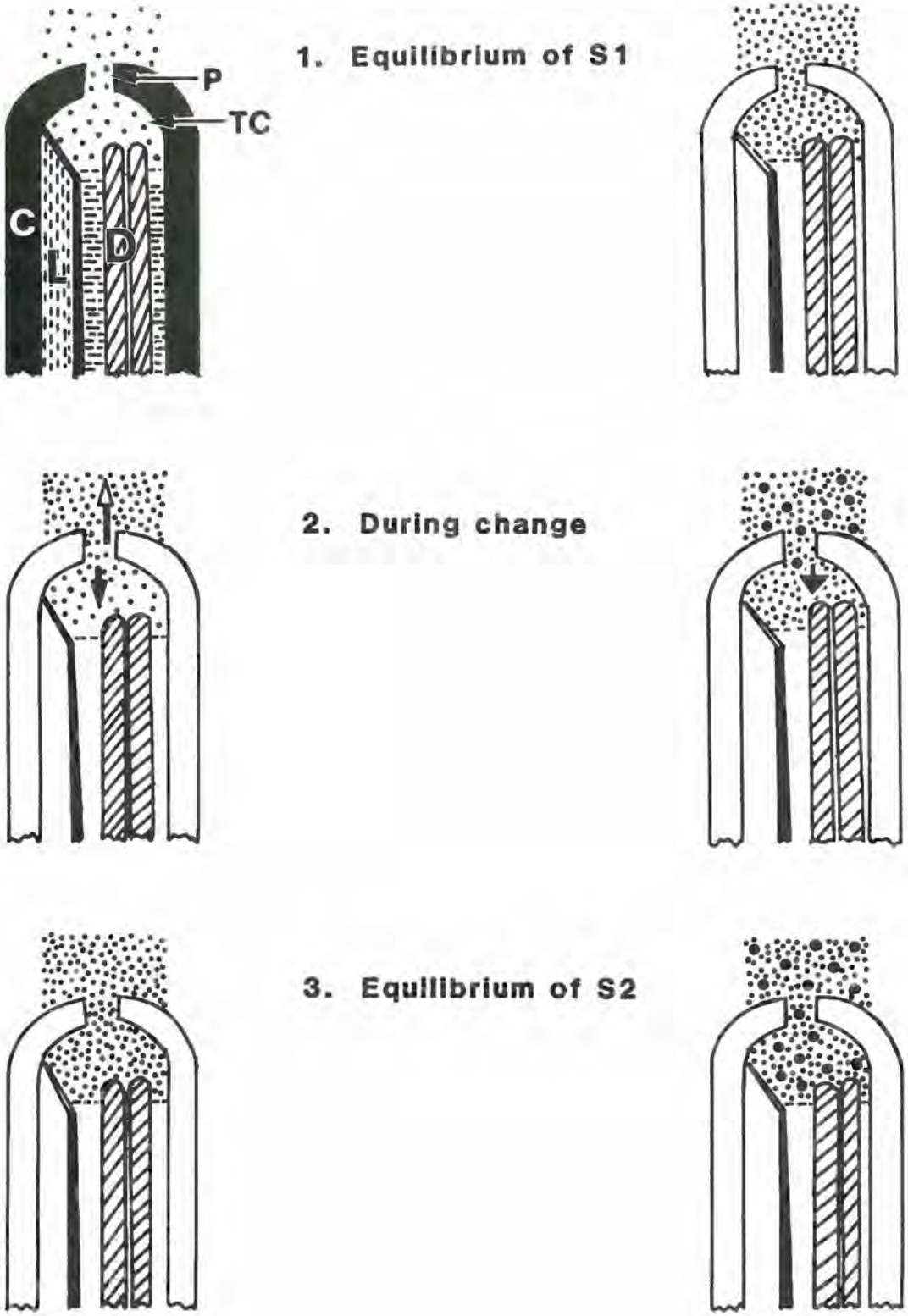


FIGURE 6.21

change of the level of the signal generated by the large change in concentration of the stimulus. However, recording trials with dead (for 2-4 hours) flies, which were presumably of similar haemolymph composition, capacitance and resistance to live flies, showed only a shift of the balance point and decrease in noise level of the baseline. The discontinuity seems, therefore, physiological and may occur in flies in their natural habitat if such steep concentration gradients of salts are contacted.

After osmotic equilibration, the receptors fired at a rate corresponding to the increase in concentration.

6.4.3.2. Change from 0.5 M LiCl to millimolar cue-lure in 0.5 M LiCl

At least two neurones were firing spikes in response to 0.5 M LiCl prior to application of millimolar cue-lure in 0.5 M LiCl (fig. 6.20B). There was no discontinuity during the change and the c-neurone started firing in response to millimolar cue-lure. The firing rate of spikes increased after about 600 milliseconds of firing of the c-neurone. This may be an indication of the occurence of a slight dilution zone of the cue-lure as the changeover front flows along (fig. 6.20 B2). The amplitude of the "c" spikes decreased with increase of the firing rate.

Millimolar cue-lure depressed the osmotic pressure of the aqueous 0.5 M LiCl from ca. 981 mOs/kg to about 854 mOs/kg (with cue-lure). However, this difference did not require re-balancing of the preamplifier, probably because there is little further exchange of ions between the external solution and that inside the tip chamber (fig. 6.21B).

These results show that at least one of the neurones positively sensitive to LiCl is not responsive to cue-lure. It remains to be shown which of the other two neurones (P2 and the putative sugar-sensitive neurone), is the receptor for cue-lure.

At these early stages of development of the flow system, the manually operated tap and syringe injection for changing flow of solutions, and the positioning of the preparation, made it difficult to obtain clear recordings. It is anticipated that, automation of the mechanics of changing solutions by use of balanced pressure in an almost closed system, may solve most of the noise problems encountered. Such improvements will increase the efficiency of the flow system to clarify several other aspects of gustatory function. For a range of concentrations of a chemical or a number of chemicals, more inputs into the inflow well can be added, so that the same recording electrode is used for all of them. The carrier electrolyte (control) can then be used to flush out each solution after testing.

the dimensions of the labellum and the total number of sensilla. In addition, despite the retention of the distinct morphological types of sensilla, larger labella (of flies with larger body size) had significantly longer "longest" sensilla than small flies. These variations have been considered to occur within limits that are characteristic of the range of body size that can be attained by individuals of a species. The body size attained is itself under the control of a number of factors: the juvenile and moulting hormones, availability of nutrients, and the internal metabolic clock(s) that regulate the feeding behaviour. Such correlations have not yet been obtained for the other dipteran species, even where some investigations have been done (Maes and Vedder 1978).

A standard electrophysiological technique (Hodgson et al. 1955) was used for recording from longest and long sensilla. However, only longest sensilla were used throughout the study. The size of these sensilla enabled placement of the stimulus-containing micropipette onto the tip without mechanical disturbance of the other sensilla. Of the four gustatory neurones in the trichoid labellar sensilla, two (P1, P2) were positively sensitive to increase in LiCl concentration whereas a third neurone, n, had a negatively correlated spike frequency (classical water cell) to LiCl concentrations (section 6.1). Decline of firing of the n-neurone, onset of firing of the P1-neurone followed by that of P2-neurone at concentrations of LiCl greater than 50 mM, and firing of P2-neurone exceeding that of P1 ensure continous flow of sensory information into the CNS over the broad range of salt concentrations tested (cf. Evans

and Mellon 1962; Rees 1970a). Thus, the firing of the n-neurone apparently forms a contrasting background against which small changes in the activity of P1 and P2-neurones due to change of stimulus concentration become significant and therefore detectable. Such "push-pull" sensory mechanisms have been observed to occur in other sensory fields in insects and other arthropods: mechanoreceptors (review by Rice 1975); warm-cold thermoreceptors (Davis and Sokolove 1975; Tichy 1979; Loftus and Corbier-Tichane 1981; Hess and Loftus 1984); moist-dry hygroreceptors (Yokohari and Tateda 1976).

Probit analysis was used on the dose/response relationships in the responses of n, P1, and P2 neurones in longest and long sensilla to LiCl. The method has widely been used in toxicological studies of pesticides and potency of drugs (Finney 1971). It has proven to be a powerful and useful tool in the analyses of electrophysiological data especially in correlating the firing rate of neurones to the concentration (dose) of stimulus. It also enables estimation of threshold concentration for each responding neurone, and the median concentration (M50) at which the neurone would be expected to fire at half the maximum rate attained over a certain range of stimulus concentrations. Apart from use of probit plots for estimation of median acceptance and rejection behavioural tarsal thresholds for Ph. regina (Dethier 1953), actual probit analysis does not seem to have been applied before for analysis of insect electrophysiological data.

The rapidly adapting c-neurone, sensitive to cue-lure, in longest and long labellar sensilla had a threshold between 0.1 and micromolar cue-lure and peak mean firing rate at

0.1 - 1.0 mM. There was no response from the neurone at 10 mM cue-lure. The dose-response curve resembles that for chemoreceptors of a freshwater fish to low concentrations of electrolytes (Konishi 1967). However, it was speculated that the decline and cessation of firing in the c-neurone at high lure concentration was due to a large number of lure molecules, temporarily embedding into the receptor membrane, disrupting the ionic fluxes. In behavioural "choice" tests, about 30% of the flies were attracted to and fed on 10 mM cue-lure in 2% agar. This may appear to be at variance with the observed lack of firing of the c-neurone in longest and long sensilla. However, the chemoreceptive field of a large number of neurones, when many sensilla contact the lure, is very different from that of neurones in a single sensillum. A homologous c-neurone in any of the other four types of sensilla, possibly with an extended range of responsiveness above 10 mM may provide the necessary sensory input into the CNS. Similar electrophysiological studies with the other four types of labellar sensilla may give an idea of the spectrum of neuronal responses that are perhaps necessary for the gustatory detection of lures and elicitation of subsequent feeding. There is also possible dilution of the lure to more acceptable, lower concentration, by saliva and/or regurgitated fluids from flies that start and continue to feed.

The characteristic response of the c-neurone was used as a standard in the elucidation of whether its dose/response relationship is specific to cue-lure and cue-lure-like compounds. Cue-lure and 4-(p-hydroxyphenyl)-2-butanone are more potent in feeding and attraction of flies than 4-phenyl-2-

VII. GENERAL DISCUSSION OF THESIS

starting about three days post-eclosion. Development of sexual maturity in these species and other behaviours in other species, e.g. responsiveness of young male P. americana to conspecific female sex pheromone (Schaller-Selzer 1984), and of female Ae. aegypti to lactic acid (Davis 1984), have a similar pattern. In some dipteran species, development of sexual maturity has been shown to be under the control of 20-hydroxyecdysone (Adams et al. 1984; Blomquist et al. 1984). In some orthopteroid species, JH is the releaser hormone (review by Truman and Riddiford 1974). It is therefore most likely that, the same humoral factor releases both mating behaviour and the other behaviours. In male D. tryoni, synchronization of the development of their responsiveness to the lure and mating behaviour does not mean that they use the lure as a precursor for the sex pheromone. A separate detailed study is necessary to elucidate the underlying physiological processes involved in the development of responsiveness of D. tryoni males and other tephritids to lures and its biological significance.

In insects, tarsal and mouthpart gustatory sensilla play a role in the detection of chemicals in food substances prior to and during ingestion. In labellate dipterans, the labellum is highly specialised for food uptake and as a sensory organ. Light and scanning electron microscopy were used to study the morphology, numbers, and distribution of the labellar sensilla of male D. tryoni. Histological methylene blue staining and TEM were used to study their innervation.

The fringe labellar sensilla were classified into six different morphological types on the basis of their

Males of the Queensland fruit fly D. tryoni are readily attracted by 4-(p-acetoxyphenyl)-2-butanone (cue-lure) and feed avidly on it. Other tephritid species that respond to this or other lures show a similar feeding behaviour (Chapters 1 and 2). Despite some speculations (summary by Brieze-Stegeman et al. 1978), the biological significance of lure feeding by males remains unexplained. In the present work, this behaviour was used as a reference for the study of the neurophysiological basis of the feeding of male D. tryoni on cue-lure. The work reported is primarily on the sensory biology of the labellar gustatory sensilla. Various aspects investigated are: development of the feeding behaviour on cue-lure in relation to the chronological age of flies; mapping the distribution, enumeration, and ultrastructure of the sensilla; and the electrophysiological responses of neurones in these sensilla to cue-lure and cue-lure-like compounds.

This discussion is intended to integrate the separate, detailed discussions for the results of each chapter. Some conclusions and possible implications of the results with regard to the relationship between the feeding behaviour of male D. tryoni on cue-lure and the molecular structure of the lure are presented. Also, some important questions that arose and could not be investigated in the course of this study are outlined as suggestions of possible future projects worth pursuing.

The sigmoid pattern of development of the responsiveness of male D. tryoni to cue-lure is not unique to the behaviour itself or this species (Chapter 2). Other tephritids: D. doralis (Umeya et al. 1973, abstract only) and D. opiliae (Fitt 1981b) also respond similarly to methyl eugenol,

lengths: longest, long, medium long, short straight, bristle, and short bristle sensilla (Ch. 3). A large proportion of the sensilla in the shorter types are close to the marginal strip of the oral disc whereas longer sensilla are in the upper rows. This gradation in sensillar length, in relation to their distribution, may ensure that some sensilla of each type are stimulated, when the labellum is extended to contact food substances. Neuronal sensitivities differ from one type of sensillum to another (Maes and den Otter 1976; Maes and Vedder 1978) and most chemoreceptive fields of stimulated sensilla are likely to contain representatives of the range of the various neurones, able to monitor a wide range of chemicals (quality) in the food substance. Such across-fibre (or across-sensillum) coding of sensory information and the possible CNS attributes of frequency band amplification and/or signal averaging, may form the basis of recognition of foods with different chemical composition. When "referenced to the internal model of reality" in the CNS of the insect, this may facilitate acceptance or rejection depending on the ambient physiological and environmental conditions (cf. Rice 1988).

The papillae on the oral surface of the labellum were also mapped, enumerated, and classified into two morphological types; peripheral (PP) and interpseudotracheal papillae (IP). The short, stout PP with slitted tips occur along the marginal border of the oral disc and are more exposed to mechanical displacement. The actual interpsuedotracheal pappilae (IP), occuring between the pseudotracheae and the interpseudotracheal plates, have a limited field of displacement

due to the restricting narrow, deep socket.

Males of D. tryoni have a total of about 250 fringe labellar sensilla and about 132 papillae on the oral surface (Ch. 3 and 4). Longest, long, medium long, short straight, and bristle sensilla are innervated by 4 gustatory neurones and a mechanoreceptor. Short bristle sensilla have only two gustatory neurones whereas the papillae have 3 gustatory neurones and a mechanoreceptor (Ch. 4 and 5). Both the total numbers of sensilla and neurones innervating each type (except the very short bristle sensilla) are similar to those reported for some calliphorids and muscids (Table 3.4). This is interesting, because of the different food range and feeding behaviour of the different families. However, these families may all have evolved from a common polyphagous ancestor, with a large number of sensilla; and because most of them are themselves polyphagous, a large proportion of these sensilla and the ultrastructural organization of their innervation may have been retained. Alternatively, the ancestor may have had a restricted food range but a large number of sensilla. In the course of speciation, the species may have broadened their food ranges, without any accompanying drastic changes in the complement of neurones and the sensilla. Rather, modification of the sensitivity of some neurones to certain chemicals may have been the actual path of natural selection. Occurence of the very short bristle sensilla along the marginal strip and their innervation by two gustatory neurones may be characteristic of D. tryoni. Whether homologous sensilla are present in other tephritids is not known because no records of similar studies are available yet.

There was a strong positive correlation between

butanone. They also have 10- and 100-fold lower electrophysiological thresholds respectively and concentrations eliciting maximum firing in the c-neurone. Para-substitution on the 4-phenyl-2-butanone with respect to the aliphatic side chain with $CH_3(O)CO$- and -OH for cue-lure and 4-(p-hydroxyphenyl)-2-butanone respectively, changes the magnitude of the behavioural and electrophysiological effects of the parent molecule. The change in size of the molecule, the partition coefficients, and the electronic charge due to the additional functional groups may be some of the contributing factors.

The model of the receptor site for the cue-lure molecule, partly derives from the stereochemical theory (Amoore 1952) and partly on the "profile functional group" (PFG) concept (Beets 1964). Both of these emphasize that the size and shape of the molecule are the important features determining its activity at a receptor site. In addition, the PFG concept is more centred on functional groups and their orientation (profile) as determinants of structure-activity effectiveness of molecules. Because cue-lure is the most potent of the lures, its molecule was taken to be of optimum dimensions fitting perfectly onto the receptor site. Other characteristics of the molecule considered important are the two dipoles on the almost symmetrical carbonyl groups of the side chains, relative to the phenyl ring. The phenyl ring was also considered to interact with a particular patch of the receptor site resulting in optimum orientation of the functional groups on the side chains, to complementary active sites on the receptor membrane (cf. Metcalf et al. 1975).

This model is not only applicable to the activity of lures on both gustatory and olfactory receptors of male D.

tryoni but also, to other chemicals that can be detected simultaneously by olfactory and gustatory receptors. Although the transportation media for stimulus molecules external to gustatory and olfactory receptors differ (liquids and air respectively), stimulus molecules in both situations subsequently diffuse through the receptor lymph to reach the dendritic membranes. For a volatile chemical whose molecules exist in liquid and vapour phases, e.g. cue-lure, the transduction mechanism is expected to be essentially similar at the receptor sites of both sensory systems (Ottoson 1958; Tucker 1961 cited in Beets 1964). However, it is the ganglionic synapsing of axons from peripheral neurones, in different centres of the CNS, that determine what processing of sensory information takes place. This, together with the variable internal state of the higher centres in the brain, the general physiological state, and the environment of the organism, may or may not lead to the generation of a motor command that gives rise to the ensuing behaviour.

Toluene and acetophenone with shorter molecules than 4-phenyl-2-butanone are not stimulatory to the c-neurone. Lack of the $-CH_2$ and/or carbonyl groups affect both the physical dimensions, the liposolubility of the molecule, and in the case of the carbonyl group, the electronic charge. These are all important for the stimulus-receptor site interaction (Dethier 1951; Diamond and Wright 1969). The aliphatic 4-decanone, having a similar number of carbon atoms as 4-(p-hydroxyphenyl)-2-butanone, may fit onto the cue-lure receptor site. However, its molecules may suffer nonspecific orientation, due to lack of the phenyl ring, rendering the chemical less potent or nonstimu-

latory.

The number of chemicals tested may appear too small to enable generalisations to be made with regard to all possible characteristics of molecular structures that may be necessary for a chemical to adequately stimulate the cue-lure receptor. However, the consistency of the dose/response relationships for 4-phenyl-2-butanone and its derivatives showed that the characteristics typical of 4-phenyl-2-butanone play an important role in the stimulatory effectiveness of the lures.

Modelling of the lure receptor site leads to a possibility of modifying the potency of lures, by choosing new substituent groups on the basis of atomic composition and the molecular constants of some of the functional groups. Because an acetonyl group (CH_3OC-) has a higher tendency of accepting electrons at the para position (σp) than the acetoxy group (CH_3OCO-) (Hansch et al. 1973), I have proposed the para-substitution of an acetonyl group onto the 4-phenyl-2-butanone, as a major candidate test for the contribution of electronic charge to stimulatory effectiveness of lures. It is hoped that the acetonyl-substituted 4-phenyl-2-butanone will be synthesized with help from chemists in the near future, and tested both behaviourally and electrophysiologically.

Identification of the three LiCl-sensitive neurones leaves one gustatory neurone unaccounted for functionally, out of the four observed ultrastructurally. This neurone is suspected to be a sugar-sensitive (S) neurone. The c-neurone, and therefore the receptor site for the cue-lure molecule may either be one of the P neurones or the putative S-neurone. Some results obtained with an open, prototype, flow system showed

that one of the P neurones (P1) responding at 0.5 M LiCl is different from the c-neurone (section 6.4). Although this may not imply that both the P-neurones do not respond to the lure, the possibility of the cue-lure receptor site being on the putative S-neurone seems likely. If this can be shown, it may explain the neuronal basis of feeding of male D. tryoni on lures. The elucidation of this entails future work to determine whether there are separate receptor sites for sugars and lures.

In the course of this work, other important questions regarding the behaviour of male D. tryoni have arisen, most of which have not been answered. Pursuit of these may provide further understanding of the neurophysiology, behaviour, and ecology of this and other tephritid species.

The possibility of involvement of a hormone in the development of responsiveness of male D. tryoni to cue-lure is readily testable through monitoring levels of the putative hormones (JH, or 20-hydroxyecdysone, or other hormones) (cf. Adams et al. 1984). The effects of boosting these levels with exogenous, synthetic hormones on the responsiveness of flies to lures a few hours after eclosion could also be tested.

Confirmation of the occurence of the relationship between age and responsiveness of male flies to lures, in the field, as observed in the laboratory requires an accurate method of age estimation for flies caught in lure-baited traps. An attempt to use the "pteridine assay" (Mail et al. 1983) was unsuccessful because of the nonlinearity between the total fluorescence of the pigment in the head capsule of male D. tryoni and increase in chronological age. The fluorescence

peaked three days post-eclosion, declined in the following four days, and then started increasing after the seventh day. The homogenate used may have had several pteridine components some of which might have increased with age whereas others may decrease at some stage due to conversion or transfer to other body parts. Initial identification of all the separate components and a follow-up of changes in the abundance of each with age may help isolate those that show a steady increase with age (cf. Mail and Lehane 1988). If present, spectrofluorometry measurements on these components, together with correction factors may provide more accurate estimation of the age range of wild flies attracted to lure-baited traps.

Some factors have been tested electrophysiologically for their effects on the firing rate of neurones: temperature (Gillary 1966b); pH (Shiraishi and Morita 1969); age (Rees 1970; Stoffolano et al. 1978); and humidity (Stadler et al. 1987). Although osmolarity of solutions is proportional to the molar concentration at low ranges, the osmotic effects at high concentrations may have profound effects on the integrity of the dendritic cytoplasm. The observed effects may thus be due to the osmotic pressure rather than the actual concentration of solute. A hypertonic solution may cause loss of water from the cytoplasm whereas very dilute (hypotonic) solutions leach ions (cf. Rees 1970a; Broyles et al. 1976). In both cases, the firing rate of the neurone(s) is reduced or blocked. Evans and Mellon (1962) showed that inhibition of the activity of the water-sensitive neurone in the labellar sensilla of Ph. regina by sucrose, was linearly correlated with log of osmotic pressure. The osmotic effects of chemicals on the activity of gustatory

neurones requires a thorough investigation.

Overall, the present work provides some knowledge on the sensory biology of the labellar gustatory neurones of male D. tryoni with regard to lures. Because it is the first study on the neurophysiology of lures in tephritids that I am aware of, there remains a lot to be done on this and other aspects e.g. behaviour, ecology, and the evolutionary significance of the lures in males of this and other tephritid species.

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APPPENDICES

II. Chemicals tested and some of their properties; FW = formula wt., Δ = density (g/ml.):

Chemical	Properties: State	FW	Δ	Supplier
Cue-lure*	liquid	206	1.05p	Intern. Pheromones Ltd., UK.
Willison's lure **	solid (flakes)	164.2	----	Dragoco Pty. (Aust.) Ltd.
Benzyl-acetone***	Liquid	148.2	0.99s	Aldrich Chem. Co. Inc., USA.
4-Decanone	liquid	156.3	0.82s	Aldrich Chem. Co. Inc., USA.
Acetophenone	liquid	120.2	1.03sc	Selby's Scient., BNE, AUST.
Toluene	liquid	96.14	0.87s	BDH Chem. (Aust.), Pty.,Ltd.

* 4-(p-acetoxyphenyl)-2-butanone.

** 4-(p-hydroxyphenyl)-2-butanone.

*** 4-phenyl-2-butanone.

Letters following density values indicate source:

p Personal calculation.

s Supplier's label.

sc Sigma Chemicals catalogue (1986).

SOLUBILITY:

Acetophenone, 4-decanone, toluene: all very slightly soluble or insoluble in water (CRC Handbook of Physics and Chemistry; Merck Index, 8th ed. 1969). Soluble in organic solvents.

4-(p-hydroxyphenyl)-2-butanone: insoluble in water but soluble in ethanol (Monroe and Richardson 1969).

III. Summary of mean firing rates of the n-neurone and c-neurone to chemicals tested electrophysiologically on "longest" labellar sensilla. All concentrations made up in 50 mM LiCl:

1. 4-(p-hydroxyphenyl)-2-butanone (p-HPBN). Nx = number of sensilla tested.

		Mean no. of spikes (± s.e.) in first second after 50 msec. contact recovery	
Conc.(mM)	Nx	n-neurone	c-neurone
50 mM LiCl	9	28.6 ± 2.48	0
10^{-6} p-HPBN	8	31.5 ± 3.25	0
10^{-5}	8	28.3 ± 1.78	0
50 mM LiCl	18	32.1 ± 1.92	0
0.0001 p-HPBN	18	38.3 ± 1.82	0.11 ± 0.11
0.001	18	34.7 ± 2.95	1.39 ± 0.89
0.01	18	31.3 ± 2.49	9.06 ± 3.49
0.10	17	18.5 ± 1.96	47.4 ± 5.53
1.0	16	12.9 ± 2.18	60.9 ± 5.90
10	14	20.5 ± 3.25	36.7 ± 7.82
50 mM LiCl	13	30.9 ± 2.88	0
20 p-HPBN	9	24.4 ± 3.05	29.0 ± 5.56
50	9	18.7 ± 3.82	62.3 ± 10.8 *
50 mM LiCl	8	33.3 ± 3.14	0

* 50 mM p-HPBN in 50 mM LiCl was an almost saturated solution and flakes of p-HPBN crystallised from solution. Hence, the actual concentration was in the range (0.1-1.0 mM) of those eliciting peak firing rate.

2. 4-phenyl-2-butanone (4-PBN):

		Mean no. of spikes (± s.e.) in first second after 50 msec. contact recovery	
Conc. (mM)	Nx	n-neurone	c-neurone
50 mM LiCl	10	34.9 ± 3.26	0
0.0001 4-PBN	10	35.0 ± 3.35	0
0.001	10	40.9 ± 3.50	0
0.01	10	37.1 ± 3.57	0
0.10	9	36.4 ± 5.23	0
1.0	9	38.1 ± 3.11	5.22 ± 2.18
10	7	31.1 ± 4.98	19.7 ± 8.16
50 mM LiCl	6	32.0 ± 1.45	0
20 4-PBN	13	37.0 ± 3.23	12.5 ± 2.68
50 mM LiCl	13	35.2 ± 1.92	0

3. Acetophenone (ACP):

50 mM LiCl	13	34.6 ± 2.63	0
0.0001 ACP	13	37.1 ± 3.08	0
0.001	13	36.6 ± 2.08	0
0.01	12	36.4 ± 2.82	0
0.10	10	34.9 ± 2.40	0
1.0	10	33.4 ± 3.02	0
10	9	36.1 ± 4.30	0
50 mM LiCl	9	36.2 ± 3.70	0

4. Toluene (TLN):

		Mean no. of spikes (± s.e.) in first second after 50 msec. contact recovery	
Conc. (mM)	Nx	n-neurone	c-neurone
50 mM LiCl	8	37.4 ± 3.09	0
0.0001 TLN	8	40.0 ± 2.51	0
0.001	8	37.3 ± 2.58	0
0.01	8	40.0 ± 3.16	0
0.10	6	38.3 ± 3.37	0
1.0	6	37.5 ± 3.82	2.17 ± 1.05
10	6	40.0 ± 3.96	0
50 mM LiCl	6	39.0 ± 4.71	0

5. 4-Decanone (4-DCN):

50 mM LiCl	12	36.5 ± 3.98	0
0.0001 4-DCN	12	42.9 ± 3.72	0
0.001	12	42.8 ± 2.88	0
0.01	12	40.1 ± 3.06	0
0.1	12	40.1 ± 3.25	0
1.0	11	42.4 ± 3.49	0.09 ± 0.09 *
5.0	10	41.2 ± 5.64	0
50 mM LiCl	8	41.6 ± 4.40	0

* Only one spike was observed in one recording.

IV. Long-term adaptation of c-neurone to millimolar cue-lure for 4 "longest" sensilla:

Time Interval (sec.)	Mean no. of spikes (± s.e.) for each one sec. interval after 50 msec. post-recovery	
	c-neurone	n-neurone
1	49.0 ± 5.50	9.75 ± 4.45
2	28.3 ± 2.40	13.0 ± 4.34
3	19.8 ± 2.53	11.0 ± 3.90
4	15.3 ± 2.38	11.8 ± 3.71
5	12.0 ± 1.92	12.3 ± 3.04
6	9.25 ± 2.84	11.3 ± 2.22
7	7.75 ± 2.25	10.0 ± 3.77
8	7.0 ± 2.45	9.75 ± 3.99
9	6.0 ± 2.49	9.75 ± 2.66
10	7.0 ± 3.03	9.0 ± 3.19
11	4.75 ± 1.60	9.75 ± 2.56
12	4.75 ± 1.89	8.75 ± 2.18
13	5.0 ± 1.83	10.3 ± 1.93
14	4.0 ± 1.69	10.8 ± 2.40
15	2.33 ± 0.67 *	7.33 ± 3.38 *

* Nx = 3.

Best fitting curve using STATIGRAPHICS Programme:

Model	Parameter	Estimate	s.e.	T	P-level
y = exp. (a + bx)	Intercept	3.45	0.13	25.8	1.52
	Slope	-0.17	0.02	-11.2	4.81
					R^2 = 0.92

V. Estimation of flow rate of solutions on the prototype flow system without absorbent material in the receptacle.

T = 21-22° C.

Head (h) cm.	Vol. (ml) flow per 2 minutes	Flow rate (µl/sec.)
19.8	1.70	14.2
19.7	1.50	12.5
19.6	3.80	31.7
19.4	2.40	20.0
19.3	2.60	21.7
19.2	3.20	26.7
19.0	2.80	23.3
18.9	1.0	8.0
18.8	2.70	22.5
20.4 *	2.30	19.2
20.1	1.70	14.2
19.6	2.40	20.0
19.5	1.40	11.7
19.4	2.0	16.8
19.3	2.60	21.7
Mean (± s.e.) 19.5 ± 0.11	2.27 ± 0.19	18.9 ± 1.60

Nx = 15.

* Refilled reservoir to almost initial level.

VI. Number of flies recorded from in electrophysiological tests with different chemicals. Abbreviations of names as in the text.

	Numbers of flies used in recording from:			
Chemical	Longest sensilla			Long sensilla
**	Low conc.	High conc.	Med.conc.	Medium range conc.
LiCl	-	-	15	8
4-APBN	6	-	15	11
4-HPBN	-	9	18	-
4-PBN	-	12	20	-
ACP	-	-	13	-
TLN	-	-	8	-
4-DCN	-	-	12	-

** For cue-lure and related compounds, medium range concentrations were in the range 0.1 μM - 10mM relative to which lower and higher concentrations are denoted.

NB. The useful lifetime of recording from a fly usually lasted to the point of completion of testing a single series of up to eight concentrations of a chemical on one sensillum.