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CHEMORECEPTION OF SUGARS AND AMINO ACIDS IN FEMALE FOURTH AND SIXTH INSTARS OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

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ABSTRACT

CHEMORECEPTION OF SUGARS AND AMINO ACIDS IN FEMALE FOURTH AND SIXTH INSTARS OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

Maria Panzuto

Gustatory responses to several sugars and l-amino acids in two age groups of *Choristoneura fumiferana* (Clem.) larvae were studied using electrophysiological methods. Sensitivity to the stimuli was assessed by stimulating the medial and lateral styloconic sensilla of the galea. Of the twelve common sugars tested on the lateral sensillum, fourth instars responded to melibiose, sucrose and raffinose. Sixth instars exhibited an order of sensitivity that was in accordance with earlier behavioural work on feeding responses; sucrose > fructose > m-inositol.

A neuron in the lateral stylconic sensillum was found to be sensitive to all l-amino acids except l-proline. The latter was detected by a neuron on the medial sensillum. Sixth instar larvae were significantly more responsive to the l-amino acids than the younger instars. However, there was no correlation between the neurophysiological input measured in this study and behavioural output from a previous experiment with sixth instars.

L-proline and l-valine proved to be the most stimulating, and dose-response curves for these two amino acids were plotted. Maximal responses for l-proline and l-valine were 50 mM/l and 1 mM/l, respectively. The threshold of the response for both compounds was lower than 0.001 mM/l.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Herbivorous insects, using their chemosensilla, are quite selective at discriminating an acceptable host plant from one that is unpalatable (Schoonhoven, 1968). These sense organs act as filters and relay information from some specific conditions in the environment to the brain (Schoonhoven, 1987). Although several factors, such as temperature (Chalupa and Fraser, 1968), moisture (Wellington, 1949) and leaf surface (Chapman, 1976) play an important role in host plant selection by phytophagous insects, this study will focus on chemosensory information from their mouthpart sensilla.

Caterpillars are equipped with chemosensory structures located primarily in the maxilla, known as lateral and medial styloconic pegs (Schoonhoven and Dethier, 1966). Removal of these structures from the tobacco hornworm (*Manduca sexta*) have shown that they are necessary for unimpaired food selection (DeBoer, 1993). In larvae of *Choristoneura fumiferana* (Clem.), the two lateral and medial styloconic sensilla on the maxillary galea are each innervated by four chemosensory neurons (Albert, 1980). The sensory function of six of the eight chemosensory neurons is known. In the medial sensillum, two of the cells are salt-sensitive and one responds to water (Bond-Toufexis, 1994). The fourth cell has not been characterized. The lateral styloconica contains a sugar-, a water- and a salt-sensitive cell (Bond-Toufexis, 1994). Again, the chemosensory function of the fourth neuron is unknown.

The question arises as to how these cells are characterized. The basic principle behind electrophysiology is that it enables one to record the electrical activity of the

neurons that innervate the chemosensory structures (Hodgson, 1955). The receptor sites for each cell are located at the tip of the dendrites, and when the pegs are contacted with a stimulating solution, the result is a series of chemical and electrical reactions (Wolbarsht, 1965). Hence, the cells can be differentiated by the characteristic shapes of their action potentials in response to stimulation by appropriate chemicals. For instance, the salt neuron recordings from the spruce budworm larvae have action potentials that are basically bi-phasic, i.e., possess a steep negative flank (Schnuch and Hansen, 1990), action potentials of small pulses, whereas those from recordings of water cells from both styloconica are monophasic (no prominent negative flank) and large. The action potentials of proline-, sugar- and amino acid-sensitive cells are distinguished by their different amplitudes. It is interesting to note that, in flies (Schnuch and Hansen, 1990), this basic shape of the neurons' action potentials is conserved.

Nutritional studies of *Choristoneura* feeding on sugars (Harvey, 1974; Clancy, 1992) and amino acids (Neish, 1958; Durzan and Lopushanski, 1968; Mattson, 1980) have shown that its survival and fecundity are affected by the quality of these food sources. Additional studies on the spruce budworm's principal hosts, white spruce (*Picea glauca*) (Bauce *et al.*, 1994) and balsam fir (*Abies balsamea*) (Kimmins, 1971), provide further evidence for the synchronicity of host-tree phenology with the emergence of the second instars in the spring (Morris, 1963). The young larvae mine old needles following emergence from hibernacula. However, once the vegetative buds have opened, the now older larvae mine the new shoots (McGugan, 1964).

Heron (1965) conducted pioneering work on the role of chemotactic stimuli in the

feeding behaviour of spruce budworm larvae. Among the substances extracted from host-plant tissues, a number of sugars and the amino acid l-proline induced feeding responses. Also of relevance are studies that compare responses between third and sixth instars (Guertin and Albert, 1992) and fourth and sixth instars (Albert and Bauce, 1994) where significant age-related differences in feeding preferences were found. It was hypothesized that young instars are nitrogen-limited, and, therefore, prefer balsam fir extracts from terminal shoots that are rich in nitrogen. Older, sixth instar larvae are energy-limited, and, therefore, prefer lateral shoots that are rich in sugars.

Recent work on feeding in *Choristoneura* has been centered on the behavioural aspects of host-plant preference (Albert and Jerrett, 1981; Albert and Parisella, 1988). Studies by Albert *et al.*, (1982) and Albert and Parisella (1988) tested the response of the spruce budworm caterpillars to several sugars and amino acids. They found that sucrose, fructose and m-inositol were the most behaviourally stimulating sugars to sixth instar larvae. Of the l-amino acids, proline, alanine and, serine stimulated feeding, while valine deterred feeding.

Of noteworthy mention, are studies that investigate the correlation of feeding behaviour and neurophysiological action of the chemosensilla in insects such as the Colorado potato beetle, *Leptinotarsa decemlineata*, (Mitchell, 1987) with alkaloids, in the spruce budworm, *Choristoneura fumiferana*, with reference to the sucrose-sensitive cell (Albert and Parisella, 1988), and in the blowfly, *Phormia regina*, with perception of carbohydrates (Hodgson, 1957).

Until recently, little work has examined the neural basis of feeding behaviour in

caterpillars of the spruce budworm. Here I propose to take advantage of this wealth of information of *Choristoneura*'s feeding behaviour, and determine the mechanisms involved in the detection of stimuli at the neuronal level in order to provide insight into how acceptance and avoidance responses are modulated in fourth and sixth instar larvae.

PROJECT GOALS AND OBJECTIVES

This project will investigate two aspects of insect biology: the correlation between feeding behaviour and neural input from the mouthparts of lepidopteran larvae, and the effect of a phagostimulant and a deterrent on the physiology of the nerve cells. I will use an electrophysiological approach to examine chemoreception of sugars and amino acids from maxillary styloconic sensilla by female fourth and sixth instar spruce budworm, *Choristoneura fumiferana.*

Previous behavioural experiments have elucidated *Choristoneura*'s feeding preferences in response to twelve common sugars and fourteen l-amino acids found in spruce, *Picea glauca*, their host plant (Albert *et al.*, 1982; Albert and Parisella, 1988). Of the sugars, sucrose, fructose and, m-inositol were the most stimulatory. The amino acids l-proline, l-serine and l-alanine stimulated feeding; l-valine was a deterrent. The present neurophysiological study of fourth and sixth instar larvae will be divided into three parts: (1) stimulation of lateral styloconic peg with 25 mM/l solutions of sugars; (2) stimulation of lateral and medial styloconic sensilla with various concentrations of l-amino acids; (3) establishing dose-response curves for l-proline, a known phagostimulant, and l-valine, a deterrent.

This work will reveal some of the mechanisms underlying feeding behaviour in larvae of the spruce budworm. It will also shed some light on a much-discussed area of neurobiology, i.e., the perception of phagostimulants and deterrents by caterpillars.

CHAPTER 1

Physiology of Carbohydrate Chemoreception from the Lateral Styloconic Sensillum of the Galea in Fourth and Sixth instar Larvae of Female Spruce Budworm, *Choristoneura fumiferana*

A. Abstract. Female fourth and sixth instar larvae *Choristoneura fumiferana* (Clem.), were tested individually for the response of the sugar cell on the lateral styloconic sensillum to 25 mM/l concentrations of twelve carbohydrates. The spruce budworm showed an age-related change in responsiveness of the sugar cell. The order of stimulating effectiveness for fourth instars was melibiose > sucrose > raffinose. These storage di- and trisaccharides are present in the host plant at the beginning of budbreak. Sixth instars responded to sucrose > fructose > m-inositol. These findings are in accordance with a previous behavioural study by Albert *et al.* (1982) on feeding preferences of sixth instars. The response for both melibiose and raffinose did not change from fourth to sixth instars; however, it did for sucrose, fructose and m-inositol. The results are discussed in relation to the hypothesis that there exists a "plasticity" allowing for the change in sensitivity that occurs between instars (deBoer, 1993).

B. Introduction

Harvey (1974) discussed the importance of carbohydrates to the spruce budworm larvae. When these were reared on diets that lacked sugars, they had lower pupal weights,

longer development times and poorer survival. However, diets containing sucrose, fructose, glucose and raffinose, which are readily utilizable by the insect, were considered good sugar sources. Diets containing galactose and trehalose were slightly inferior. Larvae reared with melibiose, xylose and arabinose did not vary from the sugarless control.

The preferred hosts of *C. fumiferana* are balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) (Heron, 1965; Mattson *et al.*, 1991). The principal sugars of developing conifer foliage are fructose, glucose and sucrose (Little, 1970). Since sucrose and raffinose are the main sugars present during the winter (Neish, 1958), the second instars may encounter high levels of raffinose at least during the first two weeks of budbreak (Chalupa and Fraser, 1968; Little, 1970).

The most "acceptable" sugars to the spruce budworm caterpillars are fructose, maltose, raffinose and sucrose, and although D-glucose is one of the main components of the host plant tissue, it does not appear to induce feeding (Heron, 1965). With artificial diets containing sucrose and fructose, larvae produced significantly more dry weight of frass than with diets containing either glucose, galactose, or arabinose (Albert and Jerrett, 1981), suggesting that sucrose and fructose are the more nutritious sugars. Among the soluble sugars, raffinose and sucrose are the most abundantly distributed in plants (Dey, 1990). Harvey (1974) showed that sucrose was present in spruce in an approximately constant concentration throughout the year; however, raffinose disappears with the onset of warmer temperatures.

Phenological age of host and insect are crucial factors affecting host preference by *C. fumiferana*. The insect overwinters as a second instar larva and in the spring molts to

the third instar to mine either needles or buds. Feeding on the buds continues in fourth through sixth instars (Morris, 1963). The foliage of young trees has a higher N:tannin ratio than that of older trees, whereas the latter possess greater amounts of soluble sugars (Bauce *et al.*, 1994). Sugars were shown to be particularly important during the later period of larval development; high protein diet during early instars has a significant effect on developmental rates (Harvey, 1974). Albert and Bauce (1994) examined the feeding preferences of fourth and sixth instar spruce budworm larvae for foliage extracts from young and old balsam fir hosts. Fourth instars preferred extracts from terminal shoots of young and old trees, and sixth instars preferred extracts from lateral shoots of young trees. This preference was attributed to the fact that younger instars need nitrogen sources during the early developmental stages. The N content is higher in terminal than lateral shoots. Later instars require a high energy source to undergo the final molt into the pupal stage, and this energy is ingested by the caterpillars in the form of sugars as lateral shoots of trees possess higher sugar content than terminal shoots (Albert and Bauce, 1994).

Caterpillars base their food selection on sensory input from mouthpart sensilla, the chemosensory structures located primarily in the antennae, maxillae and epipharynx (Schoonhoven, 1987). In particular, the medial and lateral styloconic sensilla on the galea have been shown to mediate host-plant choice in caterpillars. Ablation experiments with the tobacco hornworm have shown that information from these chemosensory organs is necessary for unimpaired food selection (DeBoer, 1993).

Each styloconic sensillum in the spruce budworm larva is innervated by four chemosensory and one mechanoreceptor neuron (Albert, 1980). Sugars were detected by

a cell in the lateral styloconica. The effect of sucrose on this cell was examined using electrophysiological methods (Albert and Parisella, 1988).

The labellar taste hairs of calyptrate flies possess multiple receptor binding sites on the sugar cell's dendritic membrane; the most common are the pyranose or sucrose-binding site and a furanose or fructose-binding site (Shimada *et al.*, 1974). There is no reported evidence that lepidopteran caterpillars possess such sites. However, larvae of the spruce budworm seem to have no problem distinguishing between their preferred sugar, sucrose, and L-arabinose, a much less-preferred carbohydrate. This sensory capability of differentiating between two sugar molecules can be partly attributed to some kind of structure-recognition mechanism occurring at the level of the receptors. Hansen (1978) described several properties of sugars as stimulating substances. Briefly, the most significant observations were: (1) binding to protein receptor sites is accomplished by hydrogen bonding of the hydroxyl groups of the sugars; the furan and pyran rings are not involved in binding; (2) very small trioses and tetroses and, large heptoses and polysaccharides are ineffective at eliciting a response; (3) disaccharides and glycosides with an a-glycosidic linkage are much more effective than sugars with a b-linkage. Moreover, Birch (1977) suggested that sugar molecules seem to be "polarized" with distinct sweet and bitter "ends".

The object of this study is to test electrophysiologically the response of the female spruce budworm lateral styloconic sensillum to equimolar concentrations of twelve carbohydrates. The results will be compared to a previous behavioural experiment (Albert *et al.*, 1982) to see whether the behaviour and physiology are correlated.

C. Materials and Methods

Insects

Larvae were obtained as unfed second instars from the Forest Pest Management Institute, Sault-Ste-Marie, Ontario and were maintained with an *ad libitum* access to an artificial diet (Grisdale, 1984). The rearing and test conditions were LD 16:8 h at 27 ± 3°C and 40% r.h. Fourth and sixth instar females were selected for study. Age was determined on the basis of head capsule width (McGugan, 1954).

Stimulants

The test solutions consisted of twelve equimolar concentrations of carbohydrates (25 mM/l), each dissolved in distilled water containing 25 mM/l KCl to ensure adequate electrical conductance. The control solution was 25 mM/l KCl.

All chemicals were obtained from Sigma Chemical, St. Louis, MO, USA.

Electrophysiological Procedure

Fourth or sixth instar larvae were selected from the food cups prior to an experiment. The head and the three thoracic segments were sectioned from the body and mounted on a glass pipette filled with saline (Schnuch and Hansen, 1989). To avoid excessive movements from the mouthparts, the reference pipette was inserted into the lobe of the hypopharynx. This caused the head to tilt dorsally, thus putting pressure on the lobes of the maxilla, and as a result, the galea with the sensilla styloconica were exposed.

The preparation was positioned on the reference electrode under a compound

microscope, with the animal ventral side up to facilitate viewing and contact. The lateral styloconic sensillum was contacted with a glass recording electrode containing the stimulating solution. Each stimulus was presented in a different order with each insect, and between each two stimulations, there was a three-minute waiting period to avoid adaptation effects. The stimulation time was 1 s. All experiments were performed in the morning. There were on average 10-20 insects tested for each sugar. For more details on the electrophysiological procedure refer to Frazier and Hansen (1986).

Data Analysis

Data from electrophysiological experiments were recorded on digital audio tape. The spikes were digitized using Sapid Tools (Smith *et al.*, 1990) and then analyzed by counting the total number of action potentials obtained from the response of each cell. Direct current recordings were made to facilitate the task of distinguishing spike shapes, since the impulses were not distorted as in filtered recordings (Schnuch and Hansen, 1990). The four cells were identified according to their characteristic shapes. Results from the analysis were plotted in the same order as those in Albert *et al.*'s (1982) to allow for comparison.

Statistical Analysis

Statistical analysis was done with Number Cruncher Statistical Software (J.L. Hintze, 865 East North, Kaysville, UT 84037, USA). To test the effect of carbohydrate and instar on the response of the lateral styloconic sensillum, a two-way ANOVA ($p <$

0.05) was used. A two-sample unpaired T-test ($p < 0.05$) was used to determine whether there was a significant difference between the response of Cell 1 in fourth and sixth instars for each carbohydrate. A Pearson correlation ($p < 0.05$) was used to compare the response of the sugar cell in sixth instars for each of the sugars to "% Mean Consumption" from an earlier behavioural study by Albert *et al.* (1982).

D. Results

Response of fourth instars to carbohydrates

The results of tests with 12 equimolar concentrations (25 mM/l) of sugars for fourth instars are shown in Figure 1.2. All of the sugars were found to stimulate Cell 1 located in the lateral styloconic sensillum. The maximal firing rate response is seen in melibiose, followed by sucrose and raffinose (Table 1.1.).

Response of sixth instars to carbohydrates

Representative recordings of the response of the sugar-sensitive cell from a sixth instar larva to three sugars are shown in Figure 1.1.

The order of maximal stimulation changes in sixth instars (Figure 1.3.). Sucrose produced the highest response, followed by fructose and m-inositol (Table 1.1.). A similar trend was seen by Albert *et al.* (1982) study when they measured the "% Mean Consumption" for each of the twelve carbohydrates tested with the sixth instars. Preferences were

determined in two-choice tests, i.e., the carbohydrate versus water. There was a significant correlation (r=0.8193, p=0.011) between Cell 1 response and "% Mean Consumption" data in sixth instar larvae.

Comparison of response of fourth and sixth instars

There was a significant interaction for instar*sugar (df=1,11; F=6.1; $p < 0.0001$) (Table 1.1). In 7 of the 12 sugars, there was a significant difference in response of the sugar-sensitive cell. Among these, responses to sucrose, fructose, m-inositol and D-sorbitol were significantly higher in sixth instars, and the opposite was true for the remaining, β–D-glucose, galactose and xylose.

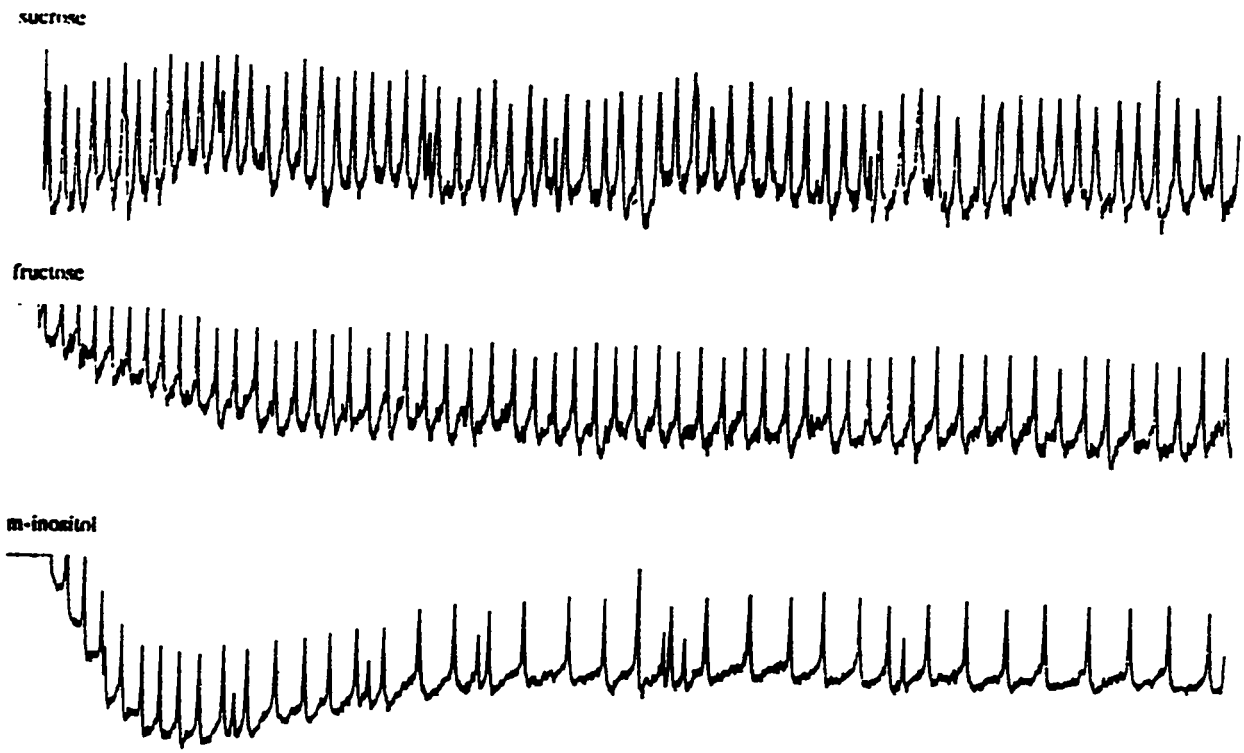

Figure 1.1. Representative traces of Cell 1 responses in lateral styloconic sensillum of female sixth instar spruce budworm larva to 25 mM/l solutions of sucrose, fructose and m-inositol in 25 mM/l KCl. All traces are 500 ms. Cell 1 (sugar cell) responses are large, monophasic pulses; bi-phasic pulses are from the salt-sensitive cell.

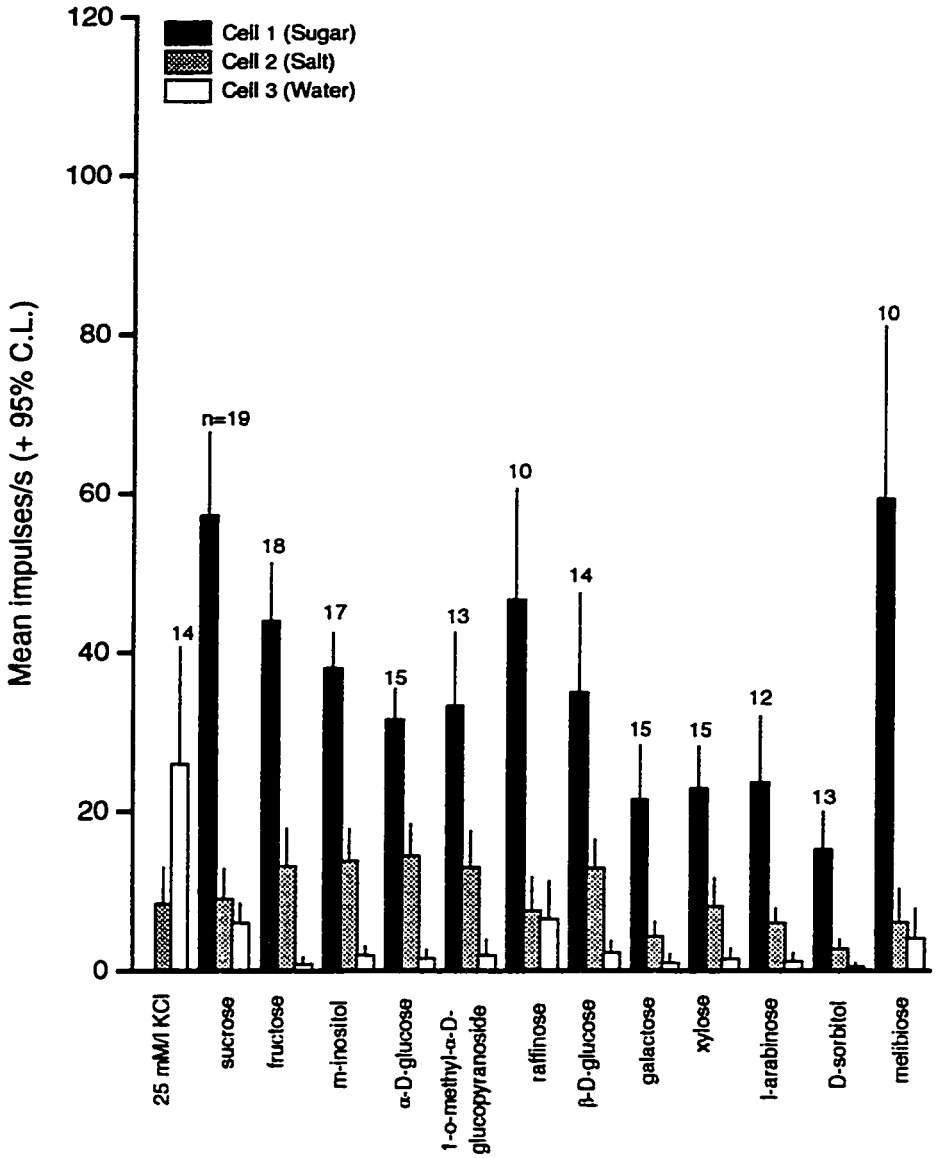

Figure 1.2. Response in mean impulses/s (+95% C.L.) of cells 1, 2 and 3 in the lateral styloconic sensillum of fourth instar larvae to stimulation with 25 mM/l equimolar concentrations of ten carbohydrates dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

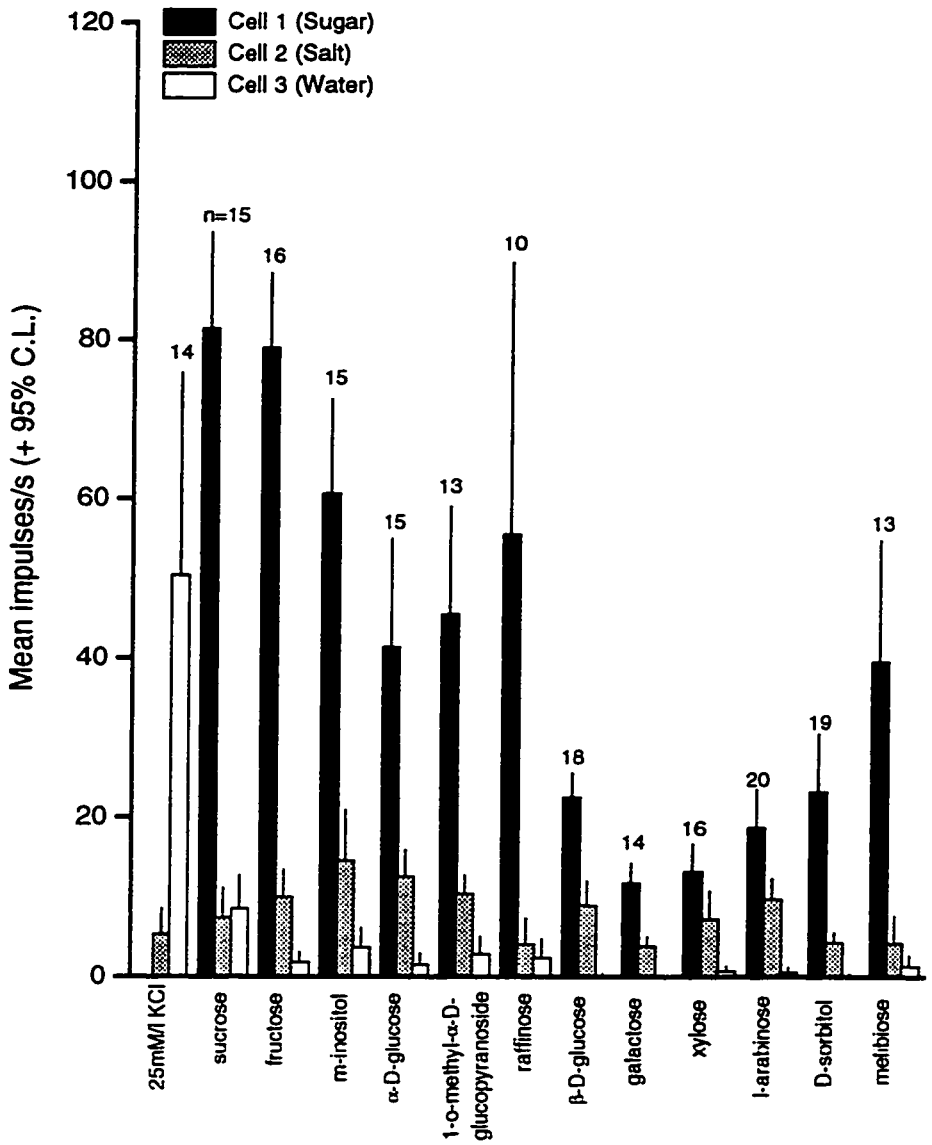

Figure 1.3. Response in mean impulses/s (+95% C.L.) of cells 1, 2 and 3 in the lateral styloconic sensillum of sixth instar larvae to stimulation with 25 mM/l equimolar concentrations of ten carbohydrates dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

Table 1.1. Two-sample t-test analysis comparing mean impulses/s ± S.E. from Cell 1 in fourth and sixth instars in response to stimulation with 25 mM/l solutions of carbohydrates.

Carbohydrate (25mM/l)	(Mean ± S.E.) 4th instars	(Mean ± S.E.) 6th instars	p values from Two-sample t-test Analysis
sucrose	54.58 ± 5.85	81.33 ± 5.73	0.001*
fructose	43.89 ± 3.50	78.94 ± 4.47	0.000*
m-inositol	38 ± 2.13	60.53 ± 5.61	0.000*
α-D-glucose	31.53 ± 1.85	41.27 ± 6.43	0.082
1-o-methyl-α-D-glucopyranosidase	33.31 ± 4.26	45.46 ± 6.25	0.060
raffinose	46.6 ± 6.23	55.5 ± 15.22	0.299
β-D-glucose	35 ± 5.76	22.34 ± 1.54	0.025*
galactose	21.47 ± 3.24	11.71 ± 1.19	0.005*
xylose	22.87 ± 2.49	13.12 ± 1.67	0.001*
L-arabinose	23.58 ± 3.85	18.65 ± 2.32	0.874
D-sorbitol	15.23 ± 2.19	23.16 ± 3.52	0.033*
melibiose	59.3 ± 9.63	39.46 ± 7.08	0.942

E. Discussion

One of the four chemosensitive cells of the lateral styloconic sensillum of spruce budworm larvae responds to sugars (Albert, 1980); its sensitivity to the various sugars tested differs between fourth and sixth instars. The stimulation order for fourth instars is melibiose > sucrose > raffinose. These results seem to contradict the order found in another behavioural study where sucrose is unequivocally the most "acceptable" sugar followed by fructose, maltose and raffinose (Heron, 1965); Heron used penultimate instars in those experiments. Chalupa and Fraser (1968) showed that during the period of budbreak, white spruce seedlings were still exposed to low temperatures, and consequently, a greater amount of oligosaccharides from the raffinose family were present. In particular, raffinose and melibiose were observed in the needles and roots, and the levels of melibiose in plant tissues are usually low. Increases occur during periods when storage carbohydrates are actually consumed, such as during seed germination and fruit ripening (Dey, 1990). These are the conditions that fourth instars encounter during their development.

The stimulating order found in sixth instar *C. fumiferana* larvae in the present results, is closely correlated with the one found in Albert *et al.*'s (1982) behavioural paper on the feeding preferences of sixth instar larvae to these same carbohydrates. This suggests a relationship between the behaviour and the response of the sugar-sensitive cell. In particular, the correlation stresses the notable role of sugars as phagostimulants as well as nutritious for the spruce budworm.

There is evidence for an age-related variability in chemosensitivity between fourth and sixth instars. The responses of sixth instars to sucrose, fructose and m-inositol are significantly higher than in fourth instars. Changes in receptor sensitivity between individual instars have been reported in other insects (Clark, 1980; Schoonhoven *et al.*, 1991). Simmond's *et al.* (1992) found that as larval weight increased, a subsequent increase in neurophysiological response to sucrose occurred in *Spodoptera littoralis*. According to them, the regulatory mechanism involved in receptor sensitivity is either centrally or peripherally controlled. The former is induced by endocrine or neural pathways, whereas the latter by a peripheral factor that causes an increase in receptor sites or a change in the threshold. There have been few reports in lepidopteran larvae of central regulatory processes as likely candidates affecting chemoreceptor activity. On the other hand, peripheral control may explain changes in sensitivity occurring between instars. Since not all of the twelve sugars exhibit an elevated response in sixth instars, as compared to fourths, it is unlikely that an increase in the number of receptor sites is occurring. Rather, the system may possess a certain "plasticity", possibly resulting in a change in "the threshold of the spike generating mechanism" (Simmonds *et al.*, 1992).

In fourth instars, the enhanced stimulatory role of sucrose and fructose has not yet been been developed and the switch in sensitivity has not yet occurred. The caterpillars are still responsive to the sugars present during budbreak, namely the oligosaccharides. Levels of these will eventually decrease with the onset of warmer temperatures, although, at this later date, the sensitivity to both raffinose and melibiose is not altered in the younger instars ($p > 0.05$). Moreover, sucrose sensitivity increases significantly ($p < 0.001$) in sixth

instars. This observation is in accord with the known feeding behaviour (Albert *et al.*, 1982). In Heron's (1965) study with fifth instar larvae, the feeding preference of the spruce budworm was similar to that for sixth instars, seen by Albert *et al.*'s (1982), implying that between the end of the fourth instar and the beginning of the fifth, there is a mechanism mediating this change in preference caused by a decline in the threshold of the firing rate response.

Another aspect of sugar chemoreception that needs to be addressed is the "sweetness" of the sugar molecule, translated in terms of binding affinity to the receptor proteins of the membrane sugar-sensitive cell. Regardless of the instar, certain sugars, namely, galactose, xylose, L-arabinose and D-sorbitol, did not elicit a pronounced response. This low sensitivity may be partly attributed to the insect's higher threshold as a result of their stereochemistry that may not allow them to be "ideal" stimulators. Perhaps these sugars are too small compared to the di- and trisaccharides (Hansen, 1978), and in order for excitation to occur, two molecules are required to combine with one receptor site.

CHAPTER 2

Chemoreception of l-Amino Acids by Female Fourth and Sixth instar Larvae of the Spruce Budworm (Lepidoptera: Torticidae)

A. Abstract. The correlation between behaviour and neural input from the mouthparts of *Choristoneura fumiferana* (Clem.) was studied. An electrophysiological approach was used to record the response of female larvae to stimulation of the maxillary styloconic galea with several l-amino acids in fourth and sixth instars. One cell on the lateral styloconic sensillum was characterized as an amino acid-sensitive neuron. All of the amino acids tested, except for l-proline and l-arginine, were detected by this cell. Fourth instar *C. fumiferana* were sensitive to l-valine > tyrosine > l-leucine. Overall, sixth instars were significantly more responsive to the amino acids than the younger instars. The results obtained from sixth instars were compared to a previous study on feeding preferences to see whether there was a correlation between neural input and behavioural output. These findings reveal the cell's neural mechanisms underlying feeding in larvae of the spruce budworm.

B. Introduction

Amino acids are among the many compounds found in plants that elicit a feeding response in phytophagous insects (Heron, 1965; Ma, 1972). In general, it is the L- as

opposed to the D-forms that produce a positive response (Sugarman and Jakinovich, 1985). There seems to be no correlation between their quality as a amino acid source and the stimulating effectiveness. In the spruce budworm, *Choristoneura fumiferana*, the amino acids l-proline, l-glutamic acid and hydroxyl-l-proline are said to be non-essential for growth; however, they influence feeding (Heron, 1965). L-arginine is non-stimulatory in these larvae even though it is considered essential (Gilmour, 1961). The amino acid requirements for conifer-feeding tortricid caterpillars have not been defined.

Durzan and Lopushanski (1968) described the amino acid composition of larvae fed on their host trees, balsam fir (*Abies balsamea*) and red and white spruce *(Picea glauca*), at different sampling dates (Instars 5 and 6). Overall, the most prevalent free amino acids were glutamine, glutamic acid, alanine and proline. Of the bound acids, aspartic and glutamic were the most abundant in protein. Total free amino acids per larva did not differ with instar; however, larvae fed on balsam fir had significantly greater concentrations of these than spruce-fed larvae.

A study on the seasonal changes of amino acid composition of the needles in the spruce has shown that arginine, glutamine and proline were the most common nitrogen-containing components and that they exhibited complementary patterns to each other (Durzan, 1968). In May, at budbreak, glutamine, glutamic acid, and proline were predominant, whereas arginine levels were minimal. From the beginning to the end of the period of shoot elongation, the amino acid profile was such that alanine, serine and asparagine occurred in higher percentages replacing the amino acids found in May. The fall and winter months were characterized by high levels of arginine. These reported

seasonal changes are in accordance with a later study on conifers which found that levels of arginine, glutamic acid and glutamine fluctuated cyclicly (Nasholm and Ericsson, 1990).

Kimmins (1971) investigated the amino acid composition of current and old foliage of balsam fir and white spruce; he found that new foliage possessed higher levels of amino acids. Behavioural experiments with early- and late-instar *Choristoneura* larvae showed that the two instars did not differ in their preferences for current and one-year foliage; however, third instars consumed significantly greater amounts than sixth instars from disks containing amino acids (Guertin and Albert, 1994). A similar behavioural trend was observed in another study that compared feeding preferences of fourth and sixth instars which correlated higher nitrogen content to the preferred fourth instar foliage (Albert and Bauce, 1994). Clearly, age of insect and quality of food source have significant effects on host preference.

Albert and Parisella (1988) examined "Mean % consumption" and "Feeding Rates" of sixth instar *C. fumiferana* larvae on filter paper containing several l-amino acids at concentrations equal to those found in balsam fir and spruce foliage. They found that l-proline, l-alanine, l-lysine and l-serine stimulated feeding, l-valine was a deterrent, and the remainder had no effect.

Physiological investigations of chemoreception of amino acids by insects are sparse. Mitchell (1985) found that a cell on the galeal sensillum of adult Colorado potato beetles is sensitive to l-alanine and γ-amino butyric acid (GABA). Schoonhoven (1981) reported the presence of amino acid receptor neurons in several larval species of Lepidoptera. Such receptors are found in a pair of sensilla styloconica on each maxilla

(Schoonhoven, 1968). Albert (1980) described the mouthpart sensilla in larvae of the spruce budworm. On the galea, the sensory function of six of the eight chemosensory cells is known. The lateral sensillum contains a sugar-, a water- and a salt-sensitive cell. The stimulatory capability of the fourth cell is unknown. On the medial sensillum, there are one water and two salt-receptor neurons. Again, the fourth cell in that sensillum has not been characterized. It is possible that one, or both, of these unknown cells on each of the pegs of the galea may be involved in the detection of amino acids.

Although there are a myriad of compounds that are classified as amino acids, this group is not homogeneous (Van Loon and Eeuwijk, 1989). In fact, various investigators failed to find a relationship between the chemical nature and the stimulating effectiveness of the compounds tested (Sugarman and Jakinovich, 1985). It is quite common for other neurons to respond in a multi-unit fashion to several amino acids, for example, as is seen in calyptrate flies (Shiraishi and Kuwabara, 1970). In this study, amino acids were classified into four groups depending upon their effect on labellar chemoreceptor cells. For instance, proline and hydroxyproline stimulated the salt cell, and valine and leucine, among others, elicited responses from the sugar cell. Nonetheless, in larvae of the spruce budworm, the responses of the chemosensitive cells of the galea seem to be more specific.

The primary object of this work is to test electrophysiologically the effect of several l-amino acids on the lateral and medial styloconic sensillum of the galea. These results will reveal whether neurophysiological input, the present work, and behavioral output, from a study by Albert and Parisella (1988), are correlated. Also, the possibility of finding an amino acid specific neuron is also worth exploring since there have been

instances of these in other insects (Mitchell, 1985; Van Loon and Van Eeuwijk, 1989).

C. Materials and Methods

Insects

Larvae were obtained as second instars from the Forest Pest Management Institute, Sault-Ste-Marie, Ontario and were maintained on an artificial diet (Grisdale, 1984). The rearing and test conditions were LD 16:8 h at 27 ± 3° C and 40% r.h. The age of fourth and sixth instar *Choristoneura* larvae was determined on the basis of a head capsule width of 0.7mm and 1.67 mm, respectively (McGugan, 1954).

Stimulants

The test solutions were fourteen l-amino acids, each dissolved in distilled water containing 25 mM/l KCl to ensure adequate electrical conductance. The concentrations are presented in Table 2.1 and represent concentrations found in nature; the control solution was 25 mM/l KCl. A pilot experiment was done to determine whether l-amino acids were detected by the sugar cell. This was accomplished by stimulating with 2 mM/l glutamic acid and 2 mM/l glutamic acid + 25 mM/l sucrose. Again, these two solutions were dissolved in 25 mM/l KCl. All chemicals were obtained from Sigma Chemical, St. Louis, MO, USA.

Electrophysiological Procedure

Fourth or sixth instar larvae were selected from the food cups prior to an experiment. The head and the three thoracic segments were sectioned from the body and mounted on a glass capillary microelectrode filled with saline (Schnuch and Hansen, 1989). The reference capillary microelectrode was inserted into the lobe of the hypopharynx tilting the head dorsally, thus exposing the galea with the sensilla.

The preparation was positioned on the reference electrode under a compound microscope, with the animal ventral side up to facilitate viewing and contact. The medial and lateral styloconic sensillum were contacted through the use of micromanipulators with a glass recording electrode filled with stimulant plus an electrolyte. Each stimulant was presented in different orders for 1s with a 3-min interval between stimulants to avoid adaptation effects. A humidifier in the recording area prevented evaporation of stimulus solution from the tip opening. An average of 10-20 insects were used for each test. For more details on the procedure refer to Frazier and Hansen (1986).

Data Analysis

Data from electrophysiological experiments were recorded on digital audio tape. The spikes were digitized using Sapid tools (Smith *et al.*, 1990) and, analyzed by counting the total number of action potentials obtained from the response of each cell. Direct current recording of unfiltered signal was used. This method facilitated the task of distinguishing spike shapes since the impulses were not distorted, as they are in filtered recordings (Schnuch and Hansen, 1990). Results from the analysis were plotted, and the

order in which sixth instars are presented in the histogram is the same as that for fourth instar larvae.

Statistical Analysis

Statistical analysis was done with Number Cruncher Statistical Software (J.L. Hintze, 865 East North, Kaysville, UT 84037, USA). To test the effect of amino acid and instar on the response of the lateral styloconic sensillum, a Two-way ANOVA ($p < 0.05$) was used. A two-sample unpaired t-test ($p < 0.05$) was used to determine whether there was a significant difference between the response of Cell 4 for fourth and sixth instars for each amino acid. A Pearson correlation ($p < 0.05$) was used to compare the response of the amino acid cell in sixth instars for each of the amino acids to "% Mean Consumption" and "Feeding Rates" by an earlier behavioural study from Albert and Parisella (1988).

D. Results

Characterization of the Amino acid Cell

Stimulating the lateral sensillum with a mixture of 2 mM/l glutamic acid and, 2 mM/l glutamic acid with 25 mM/l sucrose shows that the sugar cell is not involved in the perception of l-amino acids (Figure 2.1) since the two traces can be superimposed. Cell 4 of this sensillum is sensitive to all l-amino acids tested except proline (Figure 2.2) and arginine. Proline stimulates a cell in the medial sensillum (Figure 2.3). At the concentration

tested, l-arginine does not elicit a response from either styloconic sensilla.

Electrophysiological responses from lateral styloconic sensilla of the spruce budworm larvae show that each of the four neurons responds with a characteristic spike shape: the salt cell is biphasic and fast; the remaining three are slow and monophasic but they differ in impulse height; the order from highest to lowest impulse height is Cell 1 (Sugar cell) > Cell 4 (Amino Acid cell) > Cell 3 (Water cell) (unpublished).

Response of the lateral styloconica to several l-amino acids in fourth instar larvae

The firing frequency of Cell 4, (the amino acid cell), in response to several l-amino acids, is presented in Figure 2.4. The order of stimulating effectiveness for amino acids is valine > tyrosine > leucine for fourth instars. Aspartic and serine were the weakest stimulants, with fewer than 30 impulses/s.

Response of the lateral styloconica to several l-amino acids in sixth instar larvae

Of the amino acids, l-glutamic acid, l-valine and l-leucine give the highest responses in sixth instars (Figure 2.4), whereas l-lysine and l-alanine, give the lowest responses.

There was no significant correlation ($r=0.4110$, $p=0.184$) between the "% Mean Consumption" (Albert and Parisella, 1988) and the responses from Cell 4 (Table 2.1). Similarly, no correlation ($r=0.1642$, $p=0.610$) was found between feeding rate, and mean impulses/s from the amino acid cell.

Comparison of response of fourth and sixth instar larvae to stimulation with l-amino acids

The ANOVA revealed an instar*amino acid interaction ($df=1,11$; $F=5.90$; $p < 0.0001$). The firing rates for the lateral styloconica of fourth and sixth instars showed no difference in response to l-valine, l-tyrosine and l-threonine. Except for l-lysine and l-alanine, the remaining amino acids were significantly more effective as stimulants in sixth instars.

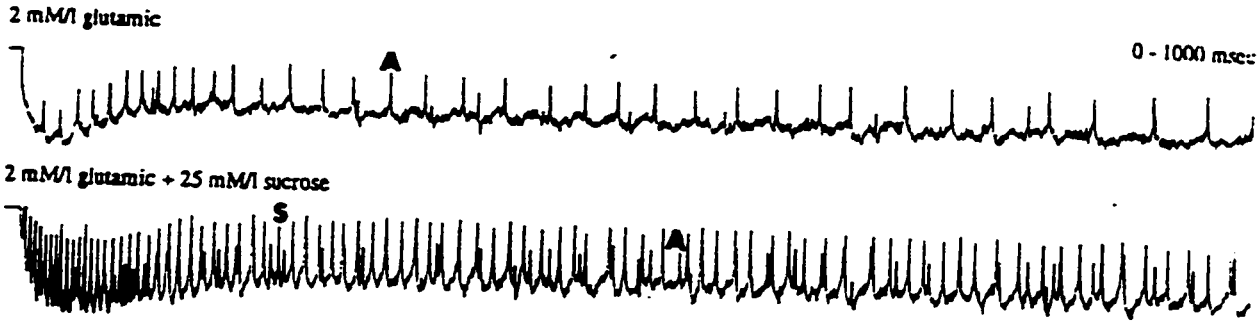

Figure 2.1. Representative traces showing the response of the lateral styloconic sensillum of sixth instar spruce budworm larvae to stimulation with 2 mM/l glutamic acid and 2 mM/l glutamic acid + 25 mM/l sucrose. S=Sugar cell response; A=Amino Acid cell response.

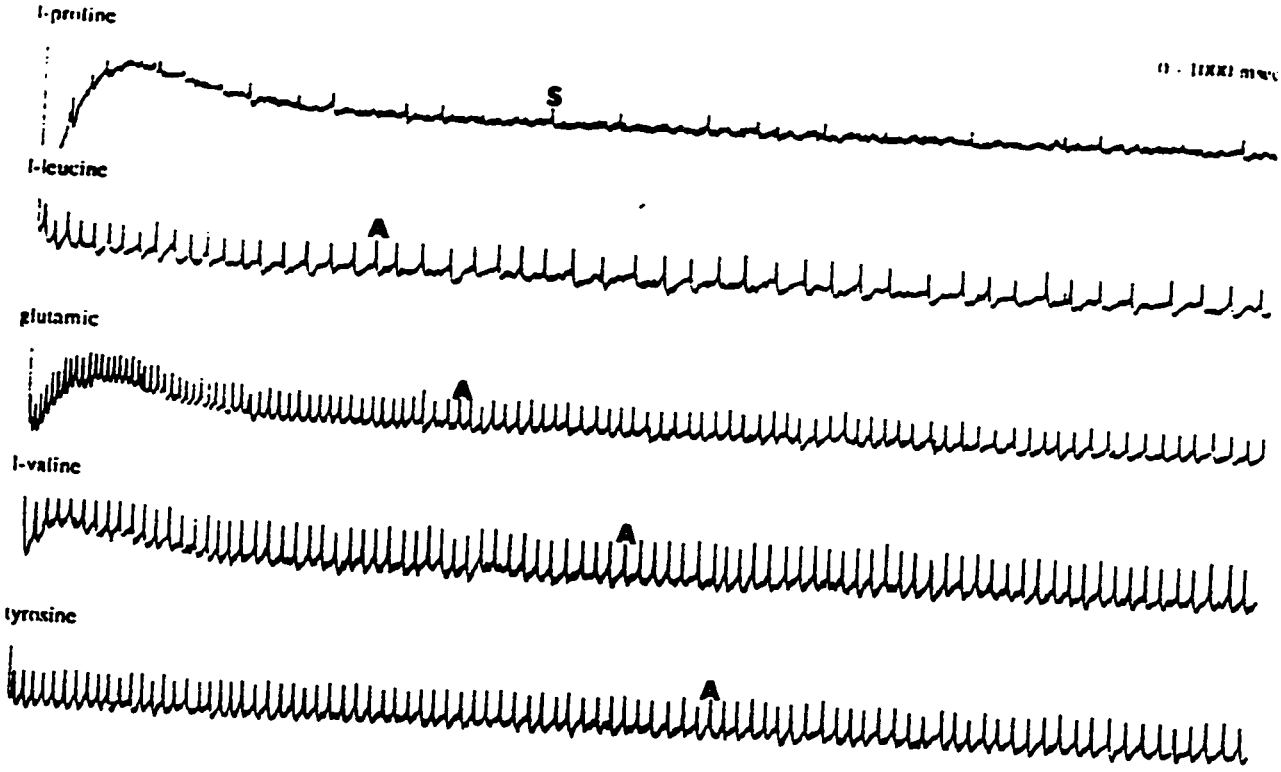

Figure 2.2. Representative traces showing the response of the lateral styloconic sensillum of sixth instar spruce budworm larvae to stimulation with several l-amino acids dissolved in 25 mM/l KCl. A=Amino acid cell response; S=Salt cell response.

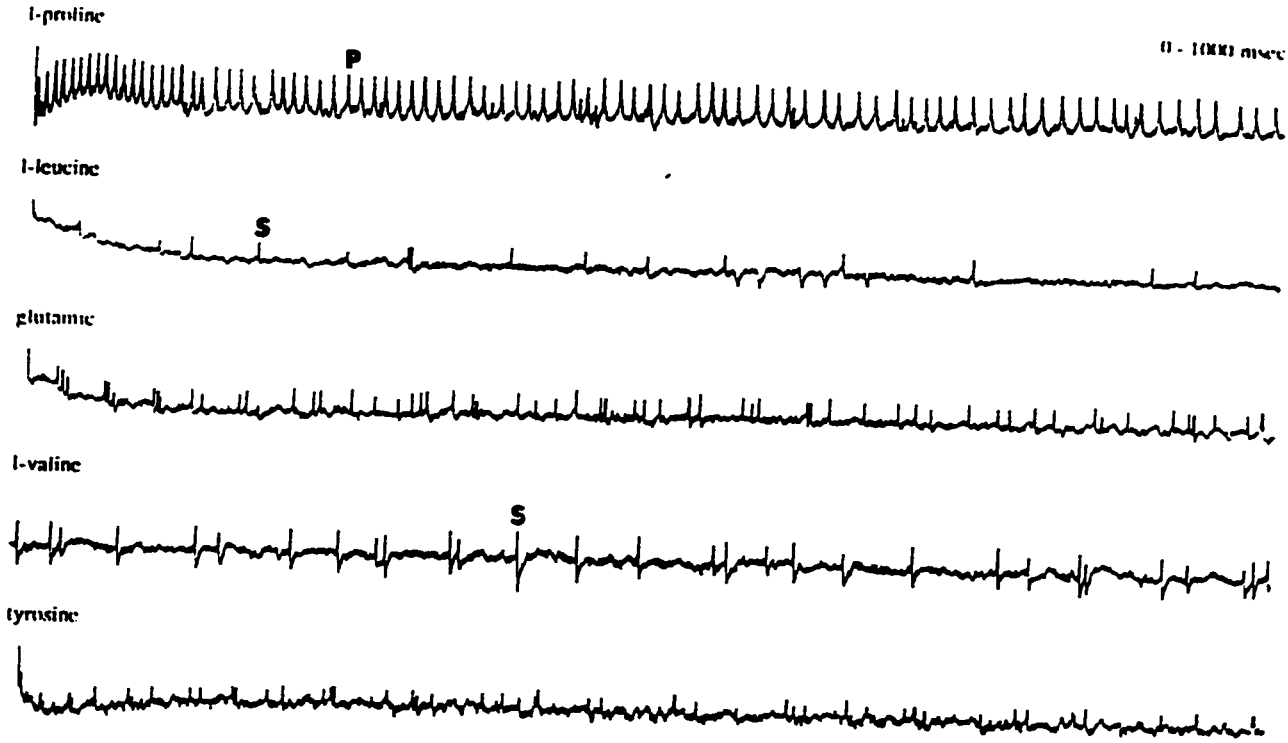

Figure 2.3. Representative traces showing the response of the medial styloconic sensillum of sixth instar spruce budworm larvae to stimulation with several l-amino acids dissolved in 25 mM/l KCl. P=Proline cell response; S=Salt cell response.

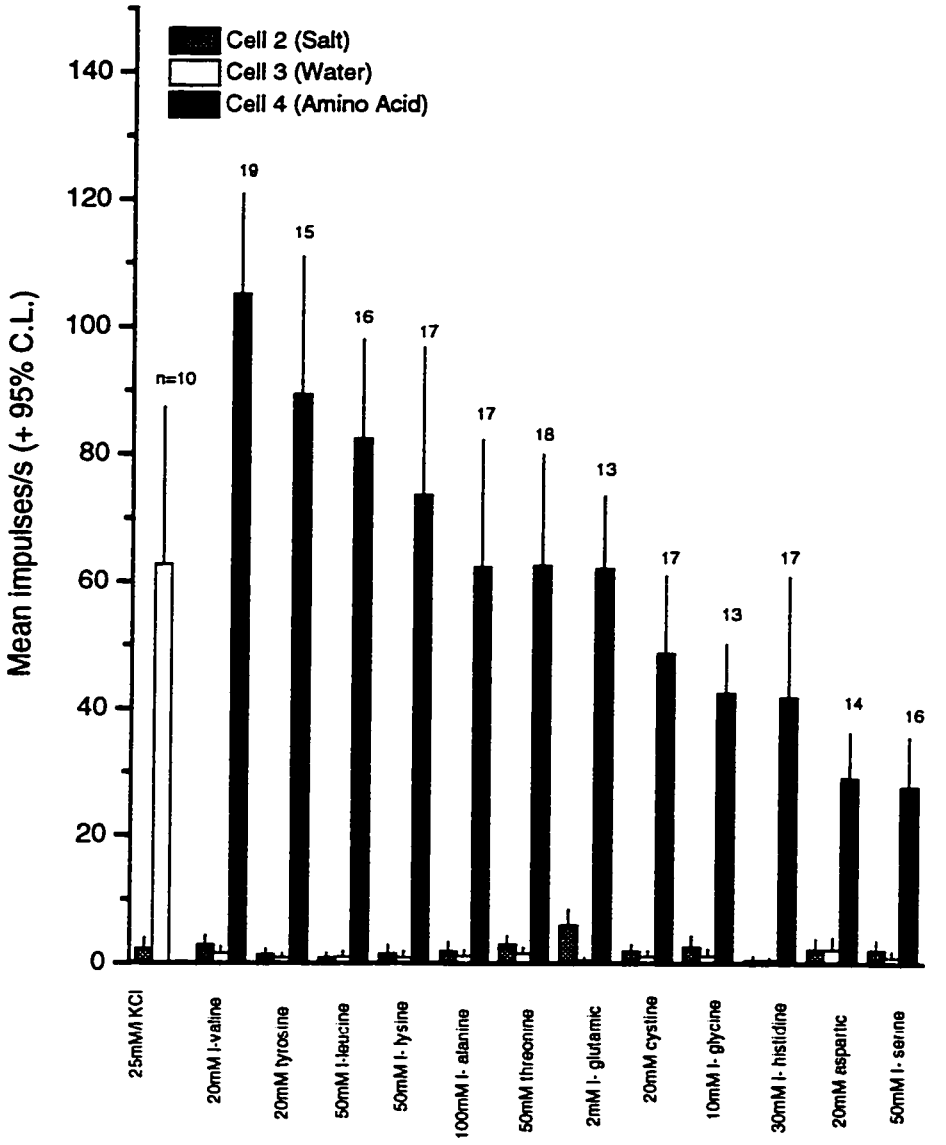

Figure 2.4. Response in mean impulses/s (+95% C.L.) of cells 2, 3 and 4 in the lateral styloconic sensillum of fourth instar larvae to stimulation with various concentrations of l-amino acids dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

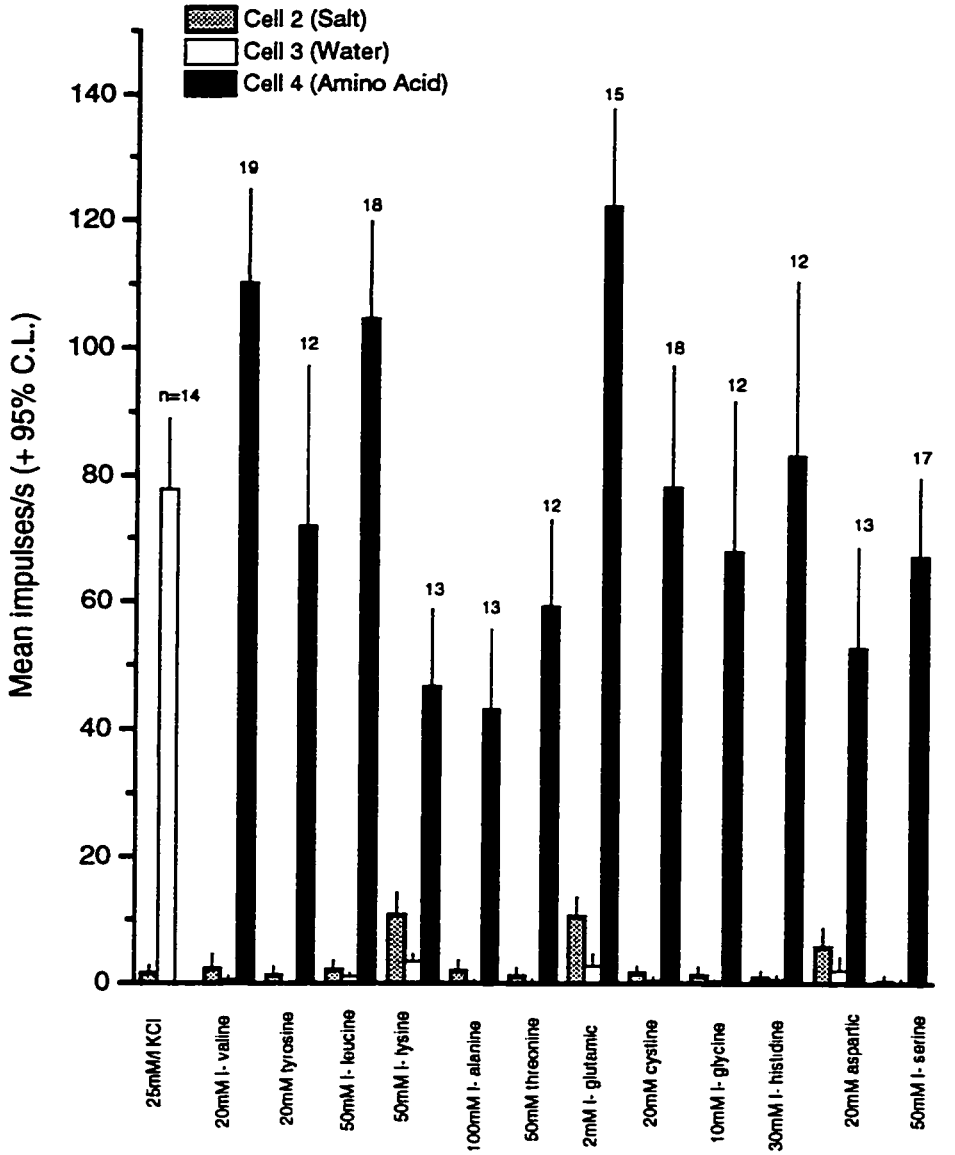

Figure 2.5. Response in mean impulses/s (+95% C.L.) of cells 2, 3 and 4 in the lateral styloconic sensillum of sixth instar larvae to stimulation with various concentrations of l-amino acids dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

Table 2.1. Two-sample t-test analysis comparing mean impulses/s ± S.E. from Cell 4 of the lateral styloconic sensillum in fourth and sixth instars in response to stimulation of various l-amino acids at their natural concentrations in white spruce and balsam fir (Albert and Parisella, 1988).

Amino Acid	Concentration[a] (mM/l)	(Mean ± S.E.) Fourth instars	(Mean ± S.E.) Sixth instars	p values from Two-sample T-tests
l-valine	20	105.10 ± 7.53	110.31 ± 6.96	0.307
tyrosine	20	89.4 ± 10.07	72 ± 11.45	0.868
l-leucine	50	82.44 ± 7.33	104.67 ± 7.22	0.019*
l-lysine	50	73.55 ± 11.00	46.85 ± 5.48	0.019*
l-alanine	100	62.61 ± 8.27	43.23 ± 5.72	0.043*
threonine	50	62.35 ± 9.44	59.33 ± 6.19	0.604
l-glutamic	2	62.06 ± 5.39	122.33 ± 7.22	0.000*
cystine	20	48.69 ± 5.67	78.17 ± 9.05	0.005*
glycine	10	42.47 ± 3.66	67.92 ± 10.79	0.021*
l-histidine	30	41.77 ± 8.75	83.17 ± 12.41	0.005*
aspartic	20	29.14 ± 3.31	52.85 ± 7.19	0.004*
l-serine	50	27.71 ± 3.73	67.18 ± 5.94	0.000*

[a] These were obtained from Table 3 in Albert and Parisella (1988).

Table 2.2. Stimulating effectiveness of amino acids in sixth instar *Choristoneura fumiferana* larvae

Amino Acid	Mean Impulses/s ± S.E. in lateral styloconica	Order of Preference in Albert and Parisella (1988)
l-Glutamic	122.33 ± 7.22	4
l-Valine	110.31 ± 6.96	12
l-Leucine	104.67 ± 7.22	7
l-Histidine	83.17 ± 12.41	8
Cystine	78.17 ± 9.05	9
Tyrosine	72 ± 11.45	10
l-Glycine	67.92 ± 10.79	6
l-Serine	67.18 ± 5.94	1
Threonine	59.33 ± 6.19	11
Aspartic	52.85 ± 7.19	5
l-Lysine	46.85 ± 5.48	3
l-Alanine	43.23 ± 5.72	2

E. Discussion

The gustatory sensilla of *C. fumiferana* contain a cell on the lateral styloconica of the galea which is sensitive to stimulation with most of the l-amino acids tested. Unlike other insects, such as the blowfly (Shiraishi and Kuwabara, 1970) and the Colorado potato beetle (Mitchell, 1985), the *C. fumiferana* neighbouring cells are unresponsive to these compounds and quite specific to the stimulus that is applied; i.e, there is no evidence of across-pattern firing (Schoonhoven, 1987). The finding that one of the cells from the lateral styloconica is responsive to all amino acids except l-proline and that one neuron on the medial sensillum responds to proline, emphasizes the phagostimulatory role of these compounds as factors governing food preference in spruce budworm caterpillars.

Fourth and sixth instar larvae exhibit differences in sensitivity patterns to the l-amino acids tested. Albert and Bauce (1994) established that younger instars of *C. fumiferana* prefer extracts containing higher levels of nitrogen, normally found in current foliage of balsam fir (Kimmins, 1971). However, in the present study, the sixth instar larvae are significantly more responsive to stimulation by l-amino acids than the fourth instar spruce budworms. When compared to the behavioural data mentioned above, our experiment tested the response to pure solutions of l-amino acids, whereas extracts from the (Albert and Bauce, 1994) study, not only consisted of amino acids, but also a whole spectrum of other compounds.

The present results also do not conform with other behavioural work, in particular the Albert and Parisella's (1988) study where sixth instar larvae were stimulated with l-

amino acids whose concentrations taken from Kimmins (1971). A whole issue arises on input/output relationships. Which component of the information from the galea is necessary for the behaviour to occur? And, is this information enough? To answer these questions, one should take into account that there are other chemosensitive sensilla on the mouthparts (Albert, 1980), and the sum of all their inputs, as accomplished in behavioural studies with extracts, should give a more accurate representation on how feeding behaviour is modulated. It is possible that other structures, such as the maxillary palps or epipharyngeal sensilla, possess additional receptors on their membranes that might respond differently than galeal receptors to the amino acids tested. In particular, in the aforementioned study by Albert and Parisella (1988), l-valine is classified as a deterrent for *Choristoneura* larvae, whereas input from the styloconic pegs shows that it is very stimulatory. It is possible that l-valine may interfere with or deter another cell in another receptor field. By the same token, l-alanine and l-serine were classified as stimulating, and yet in the present study they are among the least stimulatory (refer to Figure 2.3).

The present results suggest that the stereochemistry of the compound tested does not necessarily determine its effectiveness. In fact, there is no relationship between the classification of R groups on amino acid molecules as nonpolar, polar, acidic and basic (Lehninger, 1977) and their relative reactivity. For example, of the acidic amino acids tested in sixth instars, glutamic acid contains one more $-CH_2$ group than aspartic and yet is much more stimulatory. Also, glycine, serine, threonine, cysteine and tyrosine, are classified together since they possess uncharged polar R groups and, consequently, we would expect them to exhibit similar stimulatory effects. However, it was found that

tyrosine is second to valine among the most stimulatory amino acids in fourth instars while the others from the uncharged polar R groups are unreactive. Furthermore, of the compounds containing nonpolar R groups, l-proline is the only one that does not elicit a response from Cell 4 of the lateral sensillum.

Another point to consider is the possibility that there are different receptor sites on the amino acid-sensitive cell, comparable to the situation in the fleshfly (Shimada, 1987) and in *Drosophila* (quoted in Hansen, 1978: Isono and KiKuchi, 1974) in which the sugar cell contains pyranose-and furanose-binding sites. In *Phormia*, there is also evidence that valine, leucine, isoleucine and other amino acids bind to the furanose site (Shiraishi and Kuwabara, 1970). In the present work, valine and leucine were among the most stimulatory and might interact with the same site on the amino acid cell. One must also consider that there are other structures on the mouthpart appendages that are known to be chemosensitive. For instance, the maxillary palps in *Manduca sexta* are involved in host-plant selection (De Boer, 1991). It is very likely that these structures play an important role in *Choristoneura* as well. However, further investigations involving chemoreception by the spruce budworm are required to explain the broad specificity pattern exhibited in the perception of l-amino acids.

CHAPTER 3

Proline and Valine Sensitivity in Two Instars of the Spruce Budworm, *Choristoneura fumiferana*

A. Abstract. Larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), possess eight chemosensory neurons on the maxillary galea; one in the medial styloconicum has been characterized as a proline-sensitive cell and another in the lateral sensillum as an amino acid sensitive-neuron. Given the stimulatory properties of l-proline and l-valine, I proceeded to characterize the response of the two cells to these amino acids. Sensitivity in fourth and sixth instar larvae was studied using electrophysiological methods. A dose-response curve for l-proline for both instars showed that it was detected at concentrations as low as 0.001 mM/l and that maximal response was at 50 mM/l. Stimulation of fourth and sixth instar larvae with l-valine showed that the maximum firing frequency was obtained at 1 mM/l. Above and below this concentration, firing frequency decreased. It is known from previous behavioural work that l-proline is a phagostimulant and l-valine inhibits feeding. The dose-response relationships provide some insight into how these two compounds are detected at a neurophysiological level.

B. Introduction

In behavioural observations, l-proline consistently evokes a feeding response in *C.*

fumiferana (Clem.) larvae (Heron, 1965; Albert and Parisella, 1988). It has been suggested that l-proline's primary role is phagostimulatory rather than nutritive (Durzan and Lopushanski, 1968). On the other hand, when l-valine was tested at the concentration found in young balsam fir foliage (Kimmins, 1971), it deterred feeding (Albert and Parisella, 1988). A later study showed that of several amino acids tested, l-proline and l-valine were the most electrophysiologically stimulating (see Chapter 2).

Levels of amino acids vary with a plant's physiological state (Morgan, 1984). During frost, for instance, plants harden, resulting in higher levels of l-proline (Pearce, 1981). L-proline is the most abundant amino acid in water-stressed plants (Cyr *et al.*, 1992), as well as those responding to drought where the accumulation of amino acids may be a protective response (Palfi *et al.*, 1974). It was proposed that black spruce (*Picea mariana*) accumulates osmotically active solutes to tolerate dehydration (Zwaizek and Blake, 1990). Glutamine and l-proline protect enzymes from heat denaturation, suggesting a similar role during drought (Paleg *et al.*, 1981)

Several investigators maintain that stressful conditions in plants usually result in insect outbreaks (Louda *et al.*, 1987; Mattson and Haack, 1987; Larsson, 1989), and the spruce budworm thrives when summers are warm and dry (McGugan, 1965). These same conditions are deleterious to their host trees, especially if prolonged, since it signifies decreases in growth, resistance mechanisms and water content (Mattson and Haack, 1987). However, there are opposing views on the subject (Mattson, 1980; White, 1984). For instance, increases in SO_2 will cause nitrogen in leaf tissue to drop and this is hypothesized to result in less nutritious foliage for herbivores (Lechowicz, 1987).

The two sensilla styloconica of the galea in caterpillars are important in the selection of an acceptable versus an unpalatable plant (deBoer, 1993). In larvae of *C. fumiferana*, each is innervated by four chemosensory neurons that are easily accessible for electrophysiological investigations (Albert, 1980), and the sensory function of each is known. In particular, the medial sensillum contains a proline-sensitive cell and the lateral sensillum a neuron that responds to other amino acids but not to l-proline (see Chapter 2).

Proline-sensitive cells in lepidopteran larvae are not uncommon (Schoonhoven, 1987). However, there have been no reports of l-valine specific neurons. In the blowfly it was shown that l-valine stimulates the furanose-site of the sugar cell (Shraishi and Kuwabara, 1970) and in the fleshfly, the alkyl site of the sugar cell (Shimada, 1987). A separate amino acid-sensitive neuron on the lateral sensillum styloconicum resposive to l-valine was found in *Choristoneura* caterpillars.

Several studies have successfully used dose-response curves to characterize responses to sugars, amino acids and other compounds (Mitchell and Gregory, 1979; Hara, 1983; Albert and Parisella, 1988; Van Loon and Eeuwijk, 1989). Dethier (1982) claimed that for stimulating substances, as the concentration increases, there is a concomitant increase in stimulation. The concentration-response relationship for this response is sigmoidal. Behavioural responses, however, are characterized by a bimodal curve, indicating that at one level of the curve there is rejection and at the other end acceptance.

The aim of the present work is to examine the effect of increasing concentrations of two stimulatory l-amino acids on the response of the proline- and the amino acid-

sensitive cell on the medial and lateral sensilla styloconica, respectively.

C. Materials and Methods

Insects

C. fumiferana larvae were maintained on test conditions as described in Chapters 1 and 2. Experiments were done with mid-molt, fourth- and sixth instar larvae.

Experimental Procedure

The tip recording technique (Hodgson *et al.*, 1955) was used to record action potentials from the two sensilla styloconica of the maxilla (see Chapters 1-2). Six to twenty insects were used to determine the responses to each concentration of l-proline or l-valine.

Stimulants

The test solutions consisted of l-proline 0.001, 0.01, 0.025, 0.05, 0.1, 1, 10, 25 and 50 mM/l; l-valine 0.0001, 001, 0.01, 0.025, 0.05, 0.1, 1, 10, 25, 50 and 100 mM/l for fourth instars; sixth instar larvae were not stimulated with 0.0001 mM/l l-valine. All solutions were dissolved in distilled water containing 25 mM/l KCl to ensure adequate electrical conductance.The control solution was 25 mM/l KCl. All chemicals were obtained from Sigma Chemical, St. Louis, USA.

Data Analysis

Data from electrophysiological experiments were recorded on digital audio tape. The action potentials were digitized using SAPID tools (Smith *et al.*, 1990), a program that separates the data from the background noise. Direct current recording of unfiltered signals was used, which facilitated the task of distinguishing spike shapes since the impulses were not distorted as in filtered recordings (Schnuch and Hansen, 1990). Responses of four sensory cells were identified visually using the characteristic shape of the spikes.

A double-reciprocal plot (Lineweaver-Burk) was made, assuming that the response to increasing concentrations of l-proline corresponded to Michaelis-Menten enzyme kinetics (Stryer, 1988). This plot was constructed to derive K_b and V_{max} values. The Lineweaver Burk equation was:

$$\frac{1}{v} = \frac{K_b}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

The Michaelis constant (K_b) was equal to the concentration at which the reaction is half of its maximal value. It was obtained by taking the inverse of the X-intercept. The maximal velocity (V_{max}) was derived by taking the inverse of the Y-intercept. 1/v is the response plotted against 1/[S], the substrate concentration which in this case is l-proline.

Statistical analysis was done with Number Cruncher Statistical Software (J.L. Hintze, 865 East North, Kaysville, UT 84037, USA). An analysis of variance (Two-way ANOVA) ($p<0.05$) was used to examine whether there was an effect of instar on Cell 4 responses to the two l-amino acids tested.

D. Results

(1) l-Proline

Dose-response curve for fourth instars in response to l-proline stimulation

A cell in the medial sensillum styloconicum of *C. fumiferana* responds to l-proline in a positive dose-dependant manner (Figure 3.1) with a threshold of 0.001 mM/l for fourth instar larvae. However, the low response rate, 2.33 impulses/s, suggests that it is likely to be spontaneous activity from otherwise silent cells or to the occurrence of one or more cells firing simultaneously. It is common practice to omit data that yield responses of less than 10 impulses/s from calculations of the double-reciprocal plot.

The maximal response was produced with 50 mM/l l-proline (90 impulses/s). The action potentials of this cell are characteristically different from those of the other cells. Unlike the salt cells, the proline cell fires at a slower rate, and has a monopolar spike shape like the water cell, but with a much greater amplitude.

The Lineweaver Burk plot of the response with 0.01 mM/l to 50 mM/l l-proline yields K_b and V_{max} values of 0.117 mM/l and 111 impulses/s, respectively (Figure 3.2). These results are in accord with those shown in Figure 3.1 since at 0.1 mM/l l-proline, the mean firing frequency is approximately 48 impulses/s, i.e., close to half of the V_{max} obtained at 111 impulses/s; hence the Michaelis-Menten model is a good representation of this system.

Dose-response curve for sixth instars in response to l-proline stimulation

The dose-response pattern for sixth instar larvae is similar to that of earlier instars.The threshold of the response can be assumed to be lower than 0.001 mM/l (Figure 3.3). The K_b, is of 0.0011 mM/l, and V_{max} is 46.51 impulses/s (Figure 3.4). The ANOVA revealed an instar*l-proline interaction effect (F=2.13; df=1,8; p=0.034).

(2) l-Valine

Dose-response curve for fourth instar in response to l-valine stimulation

The lateral sensillum styloconicum contains a cell that is sensitive to amino acid stimuli and is particularly reactive to l-valine (see Chapter 2). The peak response frequency is obtained at 0.1 mM/l l-valine and, from 10 mM/l to 100 mM/l, there is a decrease in mean impulses/s rather than the increase that occurs in the proline-sensitive cell (Figure 3.5). The threshold is less than 0.0001 mM/l l-valine, and the maximal velocity is 67.56 impulses/s.

Dose-response-curve for sixth instar larvae in response to l-valine stimulation

L-valine elicits a maximum response from the sensory cell at 1 mM/l (Figure 3.6). At 0.001 mM/l, the spike frequency is 45 impulses/s. This indicates that since the maximum stimulation is obtained at a concentration of 1 mM/l, the threshold is probably much lower than 0.001 mM/l. There was an instar*l-valine interaction effect (F=2.59; df=9,1; p=0.0069).

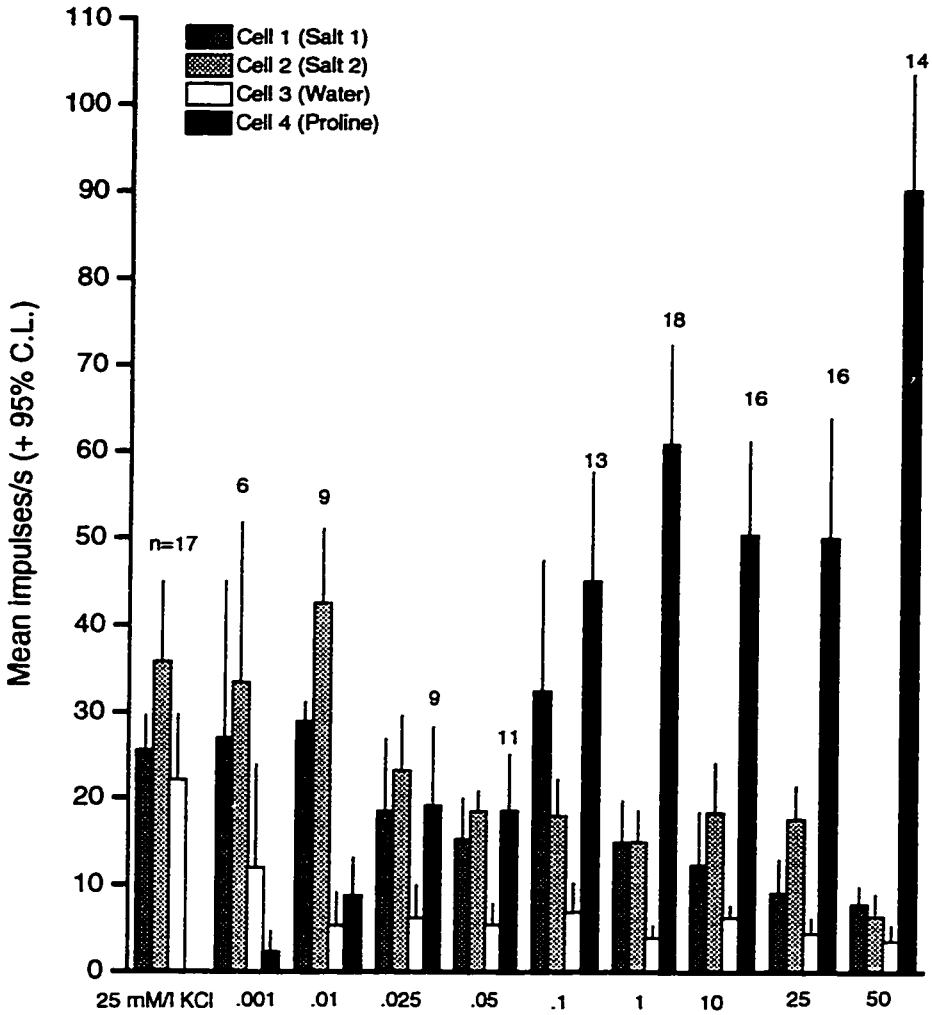

Figure 3.1. Response in mean impulses/s (+ 95% C.L.) of cells 1, 2, 3 and 4 in the medial styloconic sensillum of fourth instar larvae to stimulation with several concentrations of l-proline (mM/l) dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

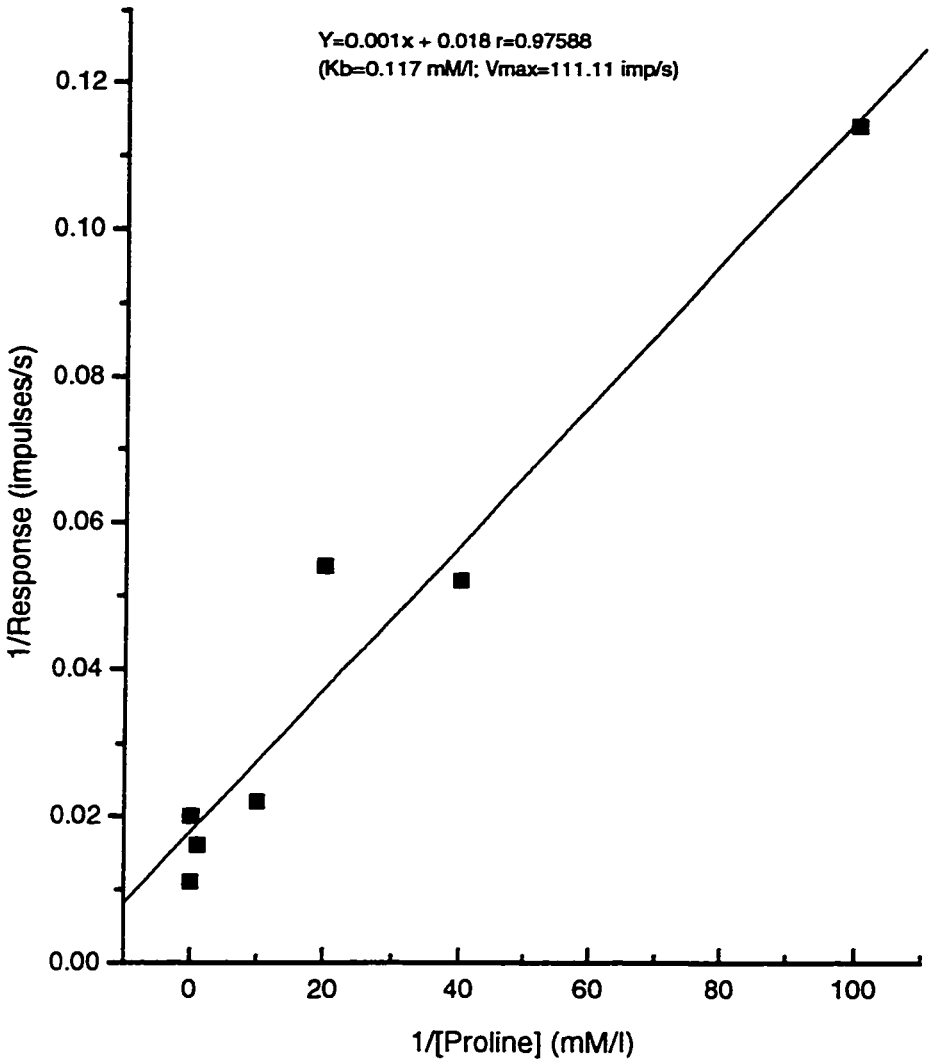

Figure 3.2. Double-reciprocal plot from Figure 3.1.

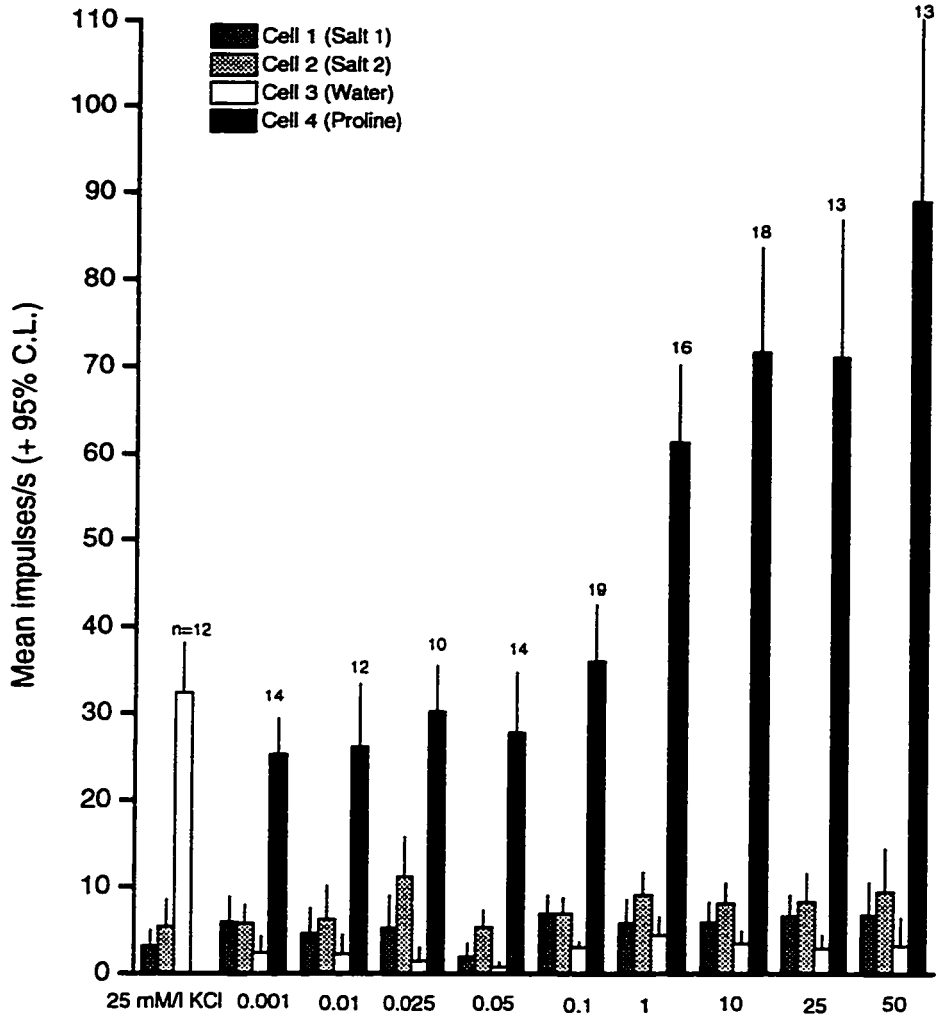

Figure 3.3. Response in mean impulses/s (+ 95% C.L.) of cells 1, 2 , 3 and 4 in the medial styloconic sensillum of sixth instar larvae to stimulation with several concentrations of l-proline (mM/l) dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

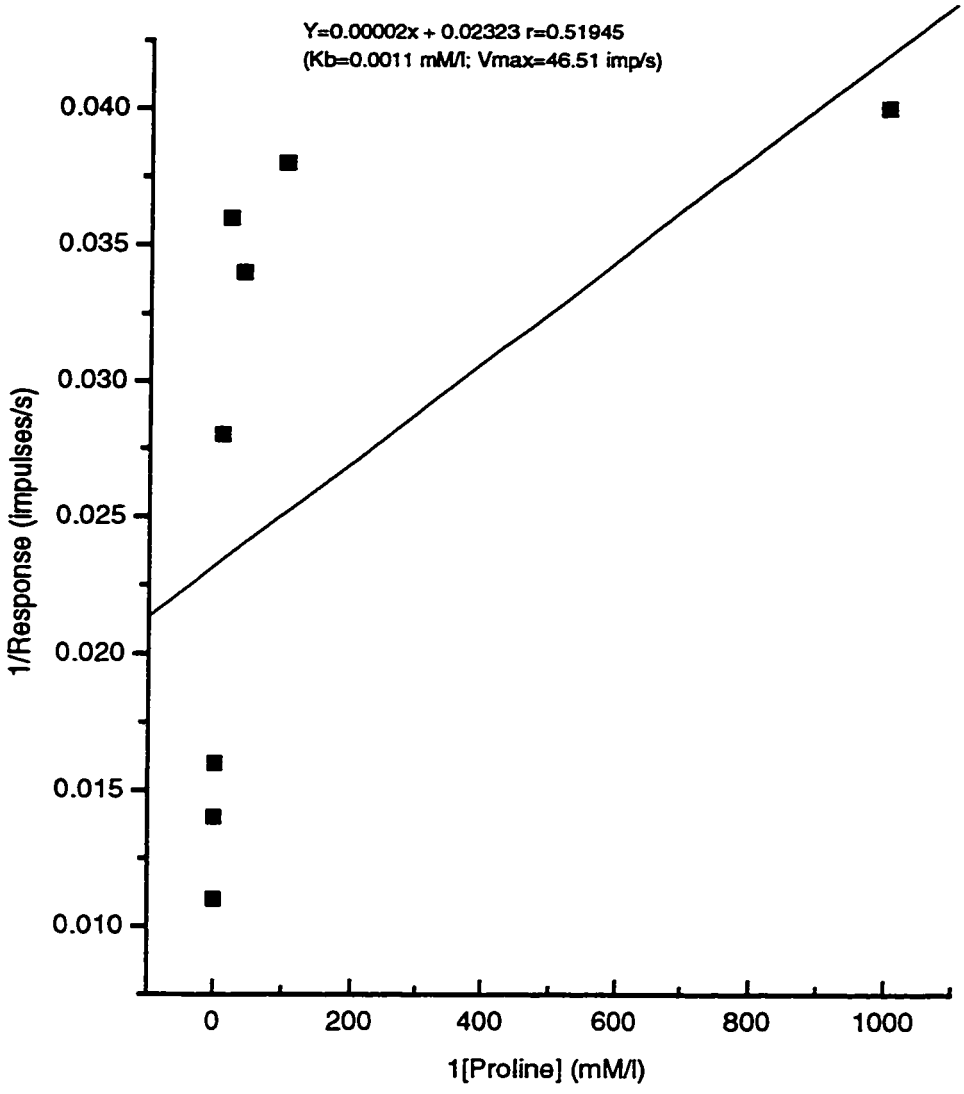

Figure 3.3. Double-reciprocal plot from Figure 3.2.

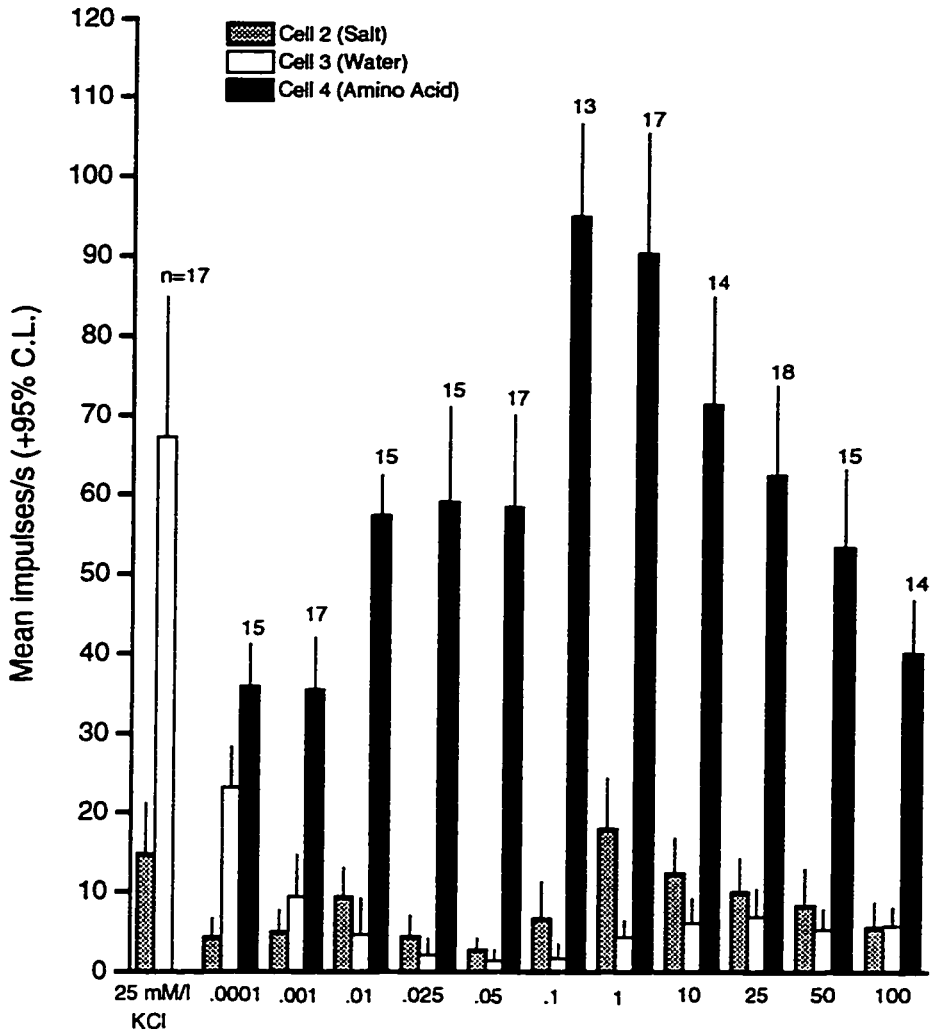

Figure 3.5. Response in mean impulses/s (+ 95% C.L.) of cells 2, 3 and 4 in the lateral styloconic sensillum of fourth instar larvae to stimulation with several concentrations of l-valine (mM/l) dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

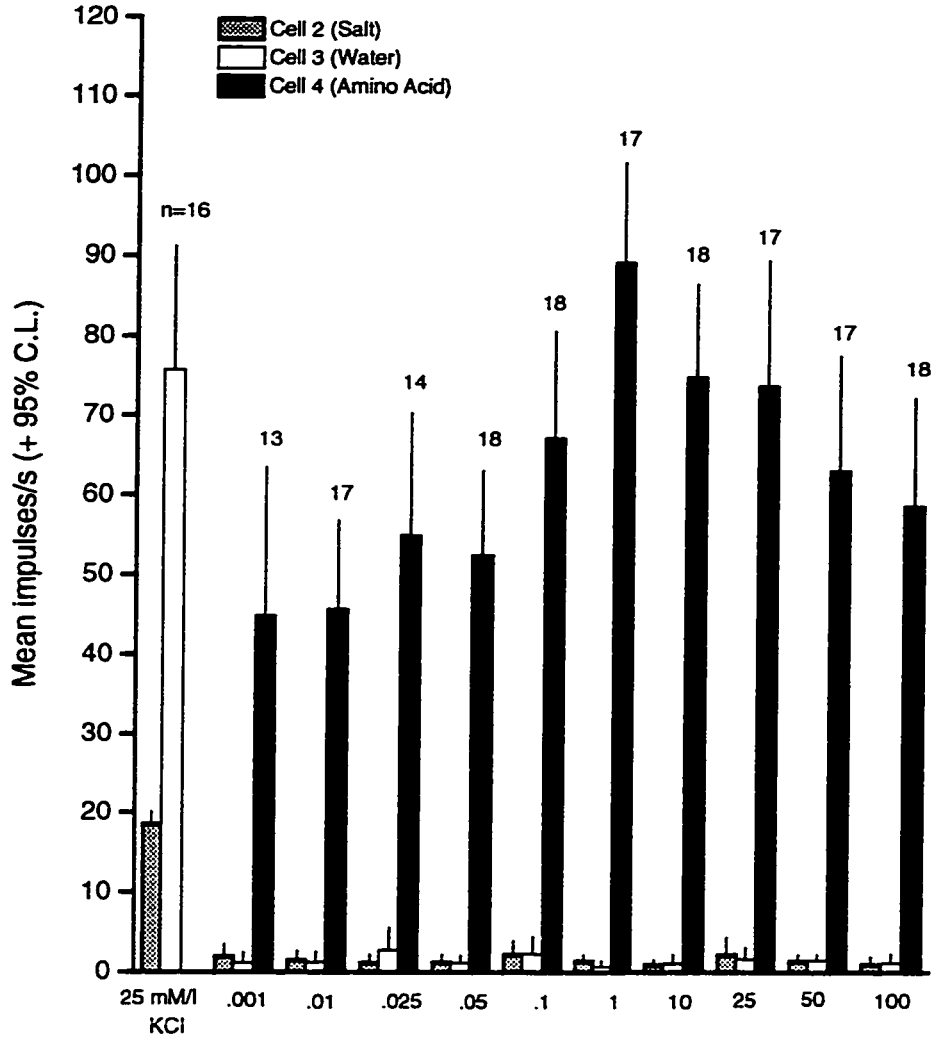

Figure 3.7. Response in mean impulses/s (+ 95% C.L.) of cells 2, 3 and 4 in the lateral styloconic sensillum of sixth instar larvae to stimulation with several concentrations of l-valine (mM/l) dissolved in 25 mM/l KCl. (n=sample size for each stimulus)

E. Discussion

The dose-response curves for l-proline and l-valine yielded different profiles. Maximal firing frequency for l-proline was obtained at 50 mM/l as opposed to 1 mM/l for l-valine, and in the latter, the response decreased after saturation was reached. Response thresholds were lower than 0.001 mM/l for both stimuli. It is interesting to note that the proline-sensitive cell in *Choristoneura* does not respond to valine or to other amino acids. However, in *Pieris brassicae* the proline cell has a broader spectrum and is sensitive to a number of amino acids (Schoonhoven, 1987).

There was a significant difference in the response of fourth and sixth instar larvae to l-proline and l-valine. The earlier instars were generally more responsive to l-valine, whereas, sixth instars were more sensitive to l-proline. Solid inferences about these differences in sensitivity between instars can not be made at the present time.

Water stress has been associated with increased herbivory and attributed to the numerous physiological changes that occur within a given plant (Louda *et al.*, 1987). Saenz *et al.* (1993) found that under severe water stress, an increase in alkaloid content and total amino acid and proline contents in the leaves of *Catharanthus roseus* were observed. Buxton *et al.* (1985) examined physiological responses of conifers to moisture stress and found significant changes in osmotic potentials. In a later study, the same authors (Cyr *et al.*, 1989) found a correlation between decreased turgor and pressure potentials and amino acid accumulation. It has been suggested that in plants, amino acids principally proline, provide a mechanism for the maintenance of osmotic adjustments (Palfi

et al., 1974).

L-proline is not an essential amino acid for *Choristoneura* larvae and yet is behaviourally and electrophysiologically stimulatory (Heron, 1965, Chapter 2). One may speculate that because this amino acid accumulates in stressed plants (Pearce, 1981; Cyr *et al.*, 1989), it may aid caterpillars in detecting those hosts that are vulnerable to attack. In addition, levels of more nutritious compounds such as carbohydrates (Zwiazek and Blake, 1990), organic acids (Cutler and Rains, 1978) and inorganic ions (Cutler et al., 1977) may also increase in those plants undergoing unfavourable conditions. Moreover, if we carry these observations one step further, it is probable that the nutritional properties of stressed-plants are enhanced and, consequently, the foliage is perceived as stimulatory to the phytophagous insects.

Dethier (1980) suggested that there is no generalized deterrent receptor in lepidopteran larvae. Substances such as tannic acid, quinine, caffeine and piperidine have been shown to electrophysiologically inhibit sugar response, and hence they play an important part in mediating host-plant choice (Dethier, 1982). There have been reports of amino acids, such as aspartic and glutamic acids, classified as inhibitors due to their action on chemoreceptor cells (Shiraishi and Kuwabara, 1970). The present data does not provide evidence for a specific deterrent cell as seen in *Bombyx mori* (Ishikawa, 1966). The behavioural deterring effect of l-valine is not very clear. One possibility is that it inhibits a cell in another receptor field. However, it can very well depend upon the concentration at which the response is being tested. In fact, behavioural, dose-response curves should reveal whether this amino acid is perceived by the gustatory receptors as a

feeding inhibitor at all the concentrations tested.

It has been shown that l-valine tastes "sweet" in flies (Shiraishi and Kuwabara, 1970) and "bitter" in humans (Yoshida *et al.*, 1966) and the spruce budworm (Albert and Parisella, 1988). The latter authors also found that when l-valine was combined with sucrose, the mixture was stimulatory. This shows that the responses to individual compounds and to mixtures varies. Taking into account that plants are composed of a spectrum of chemicals, studying insect responses to mixtures would prove most useful.

CONCLUSIONS

More details of the sensory capabilities of the galeal sensilla in *C. fumiferana* are now better known. The four cells of the medial styloconic sensillum of the maxilla are stimulated by salt (2 cells), water and l-proline. The lateral sensillum has cells sensitive to sucrose, water, salt and amino acids. However, these findings do not imply that these gustatory cells are narrowly tuned to one stimulus only. In most electrophysiological studies, there can be considerable overlap in sensitivity (Shiraishi and Kuwabara, 1970; Shimada, 1987). The maxillary palps and epipharyngeal sensilla are also sensitive (Albert, 1980), thus providing a whole new receptor field to explore.

Age-related differences were observed in the responses of larvae. In general, sixth instars were more chemosensitive to the sugars, which could be attributed to a change in "the threshold of the spike generating mechanism" (Simmonds *et al.*, 1992). A relationship was found between the feeding preference in sixth instar larvae of spruce budworm when stimulated with these compounds and the neural input involved in mediating this preference. These results suggest that sugar reception is primarily accomplished in the galeal structures. When fourth and sixth instar *C. fumiferana* were stimulated with various l-amino acids, the older instars were more responsive. No relationship was found between the known behavioural response to these compounds and the neurophysiological data in the present work. Thus, the styloconic sensilla may not be responsible for the overall decision-making process with respect to the perception of the amino acids.

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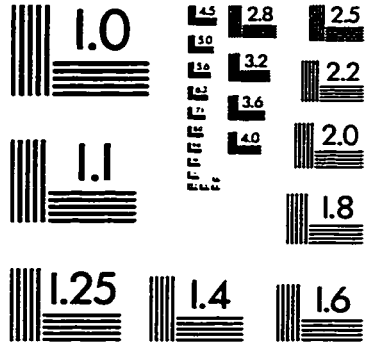

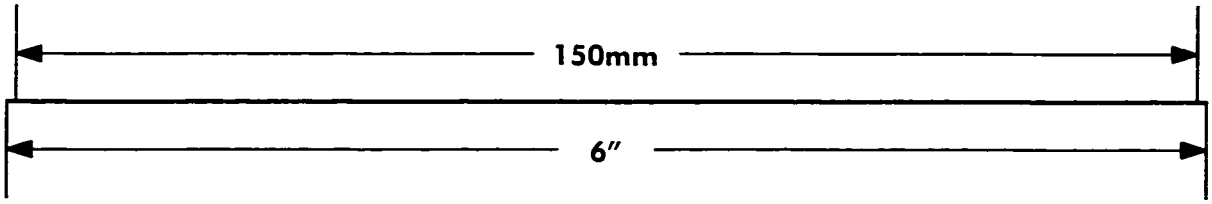

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