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COMPARATIVE RESPONSES FROM THE GALEA OF TWO INSTARS OF *CHORISTONEURA FUMIFERANA* STIMULATED WITH EXTRACTS OF BALSAM FIR, SUCROSE, AND AMINO ACIDS

Donna Bond-Toufexis

A Thesis
in
The Department
of
Biology

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ABSTRACT

Comparative responses from the galea of two instars of *Choristoneura fumiferana* stimulated with extracts of balsam fir, sucrose, and amino acids

Donna Bond-Toufexis

Electrophysiological studies on the maxillary sensilla styloconica on the galea of fourth- and sixth-instar spruce budworm larvae revealed age-related responses to aqueous extracts of balsam fir foliage, to sucrose, and to proline. An extract-sensitive neuron was found in the lateral styloconic sensilla of both larval age groups. Fourth-instar larvae were stimulated to a greater extent by terminal foliage extracts from young and old trees and sixth-instar larvae were more chemosensitive to lateral foliage extracts from old trees. Fourth-instar larvae were more sensitive to lower sucrose concentrations and less sensitive to higher sucrose concentrations than were the previously tested sixth-instar larvae. A proline-sensitive cell believed to be the water cell was found in the medial styloconica of both instars. The fourth-instar larvae were significantly more chemosensitive to proline than sixth-instar larvae. Receptors sensitive to the feeding deterrent valine were found on a neuron in the lateral styloconica of sixth-instar larvae.

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And to the One who made all of these wonders we study - words do not exist which could express my gratitude.

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INTRODUCTION

Feeding behaviour in insects has been studied in some detail. Investigations on Lepidoptera have shown that larvae are capable of making sophisticated feeding decisions. For example *Trichoplusia ni* (Noctuidae) larvae will preferentially consume leaf disks from their host plant over disks treated with sucrose, even though sucrose is known to be a strong phagostimulant (Sharma & Norris, 1991). Larvae of *Mamestra configurata* (Noctuidae) choose that part of their host-plant which results in their optimum growth and survivorship (Bracken, 1984). When larvae of *Heliothis zea* (Noctuidae) were offered a choice among defined diets, they self-selected the protein:carbohydrate ratio known to be nutritionally the best (Waldbauer *et al.*, 1984). Caterpillars of the moth *Spodoptera frugiperda* (Noctuidae) reared on diets that have been moderately diluted by cellulose and water adjust their consumption and utilization of nutrients in order to compensate for decreased nutrient availability (Wheeler & Slansky, 1991). These studies warrant the conclusion that lepidopteran larvae are seeking out and ingesting food of the quality and in the quantity that delivers them the best nutritional payload.

The spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) is a major insect pest infesting the forests of Canada and the northeastern United States. Its larvae feed on the foliage of red, white, and black spruce, and on balsam fir, resulting in millions of dollars of lost revenue for the lumber industry annually. Recent westward expansion of the species attests to its tenacity in the face of decades of effort to control its population.

In a preliminary study (Albert & Jerrett, 1981), the importance of host plant chemical extracts as feeding stimulants was tested. Petroleum ether, water and ethanol were used as solvents to obtain different chemical fractions from the host plants. Behavioural tests on the ultimate (sixth) instar larvae undertaken with these extracts found that the water fraction produced in the strongest feeding response. Consequently, substances that are extracted along with water; the sugars, organic acids, and amino acids, were the next focus for investigation. These substances were tested in single choice tests against water as a control medium. Subsequently, it was found that the sugars elicited the best response. A group of amino acids including proline, arginine, citrulline, and glutamic acid, those amino acids that exist in the highest concentration in budding host conifers, also produced a strong response. When three-choice tests were done pitting each of the fractions in the water extract against one another, it was found that the sugars and the chemically related glycosides were the strongest feeding stimulants, and that the amino acids were also stimulatory. The organic acids fractions were neutral or deterred feeding.

Albert and Parisella (1985) examined the stimulatory effects of water extracts from the four host species of the spruce budworm sixth-instar larvae. Using extracts from each host, the chemical fractions were tested separately and in various combinations. In tests of separate chemicals, the results were the same as in the previous study, with preference being strongest for sugars/glycosides, then for amino acids, and finally a deterrent effect for organic acids. When the sugars/glycosides were combined with the amino acids, feeding preference was enhanced in all hosts except black spruce. This indicates the possibility of a synergistic effect leading to an increased feeding response when these two stimulants are combined. The presence of organic acids in combination with sugars/glycosides resulted in a reduced feeding preference. Organic acids combined with amino acids had either a deterrent or a neutral effect. This suggests that the presence of a deterrent acts to override the stimulatory effect that may be present. This is particularly intriguing in the case of sugars/glycosides as these are known to be strong feeding stimulants.

In another study, Albert and Parisella (1988b) tested pure amino acids for their effect as feeding stimulants. Serine, proline, lysine, and alanine stimulated feeding, while valine was deterrent. All other amino acids had no effect.

Studies were undertaken to see if there were differences in feeding behaviour between *Choristoneura fumiferana* larvae of different ages (Guertin & Albert, 1992). When tested with sucrose and aqueous extracts from white spruce, no differences were found in preference between the third and sixth instars, although third instars showed a higher feeding rate. Taken together, behavioural studies into feeding preferences indicate that spruce budworms are making clear choices regarding their food. This implies that even a highly specialized insect like the budworm is capable of fine chemosensory discrimination.

While feeding behaviour of the spruce budworm has been studied thoroughly, little is known about the sensory physiology or neurophysiology underlying that behaviour. Studies of the anatomy and morphology of the mouthparts of lepidopterous larvae have revealed an array of cephalic parts and appendages that are involved in the ingestion of food. These consist of the antennae, the labrum, which includes the epipharynx, the mandibles, the maxillae, the labrum, and the hypopharnyx (Zacharuk, 1985). Of those that are chemosensitive, the antennae, the maxillae, including the palpi and galeae, are located externally to the mouth, and therefore provide the initial contact with potential food. The antennae are chemosensory organs involved principally in olfaction. It is the function of the antenna to supply the preliminary signal that a food source is available. Albert (1980) described the morphology and the innervation of the mouthpart sensilla in the spruce budworm larva. The maxillae consist of a pair of head appendages that are positioned ventrolaterally to the mouth. They each contain two articulating basal segments; the proximal one called the cardo, and the distal one called the stipes. Attached to the stipes are the palp and the outer galea.

Emanating out from the side of the stipes is the palp. It contains two large segments that are directed outwards. There are eight multi-innervated sensilla basiconica present on its distal surface. Five of the eight sensilla are thought to be contact chemosensitive, with the remaining three believed to be olfactory in nature. The outer galea, attached to the uppermost portion of the stipes, contains seven sensilla on its distal surface. There is one medium unsocketed peg, two small unsocketed pegs, two large socketed pegs, and two large uniporous sensilla styloconica. The pegs are all involved in mechanoreception, while the sensilla styloconica are contact chemoreceptors. The sensilla styloconica have been designated as the lateral and the medial styloconica based on their relative orientation to the body. Both the lateral and the medial styloconica contain five bipolar neurons, four of which are chemosensitive, while the remaining one is mechanosensitive. The lateral and the medial styloconica have been shown to differ from one another in their responses to various chemicals. The variability in response changes somewhat from species to species, however a cell that responds to sugars occurs universally (so far) in the lateral styloconica of lepidoptera.

The role of the individual external chemosensory mouth organs in feeding discrimination was characterized by de Boer (1991) in a series of studies on the larvae of the lepidopteran *Manduca sexta* (Sphingidae).

The initial study was undertaken to assess if the input from both the right and left sets of chemosensory organs is necessary for proper food discrimination (de Boer, 1991a). He used fifth-instar larvae whose chemosensory organs were either unoperated, unilaterally ablated, or bilaterally ablated in a two choice preference test between a host plant and an unacceptable non-host plant. He found that the ability to distinguish between host and non-host was reduced in larvae that were unilaterally ablated. Results indicated that both sets of chemosensory organs were required in order to make the best feeding choices. In further experiments, the three groups were given two-choice tests where they were allowed a choice between a host plant and water in one instance, or a choice between an unacceptable plant and water in the other. Weaker than usual rejection responses were found in the unilaterally ablated larvae towards an unacceptable plant, and weaker than usual acceptance responses towards a host plant were also observed in the unilaterally ablated group. Also, unilaterally ablated larvae in one experiment consumed just as much of the water disc control as they did from the unacceptable test plant disc indicating a total loss of normal feeding preference. It was concluded that bilateral input was not redundant for the processing of gustatory information by these insects, and that feeding decisions are based on the quantity, as well as the quality of the stimulus.

A follow-up study (de Boer, 1991b) used *Manduca sexta* larvae with particular chemosensory mouth organs ablated. This time the larvae were reared on either one of their natural host plants or on an acceptable non-host plant. When the larvae reached their fifth instar, they were operated and subjected to two-choice tests. The tests involved a choice between a disc with a plant that the particular larvae had not been reared on and a disc containing water. The host-reared larvae with lateral or medial styloconic or epipharyngeal sensilla left intact did not prefer the non-host plant over water. If the maxillary palps were left intact, then the host-reared larvae preferred the non-host over water. The host-reared larvae with only the antennae left intact showed no preference. The intact larvae reared on the non-host plant displayed no preference when given a choice between non-host and water. Non-host reared larvae with only antennae, or maxillary palps remaining preferred the non-host over the water discs. However, larvae with the lateral and medial sensilla left intact preferred water over the non-host plant which they had been reared upon. These results suggest that the galea with its two sensilla styloconica is the component of the gustatory system most critically involved with feeding discrimination.

In a related study, Flowers and Yamamoto (1992) raised tobacco hornworm larvae until their sixth stadium on a natural host plant or on an artificial diet. Three sets of operated larvae; those lacking both styloconic sensilla (lateral and medial), those lacking the lateral sensilla styloconica, and those lacking the medial sensilla styloconica, and an unoperated control group, were tested on four non-host plants. It was found that removal of either the medial or the lateral styloconic sensilla caused a dramatic rise in feeding. It was concluded that the styloconic sensilla were crucial for host-plant recognition.

The relative function of the antennae and the maxillary palps was elucidated in another study by de Boer (1992). He found that diet experience of *Manduca sexta* larvae influenced the effect of input from the styloconic sensilla. The weighted chemosensory contribution from the antennae and the maxillary palps was dominant over the input from the styloconic sensilla on larvae reared on an acceptable non-host plant. For larvae reared on a host plant, information via the lateral maxillary sensilla styloconica proved to be critical to detect feeding deterrent chemicals in the non-host plant. Thus, it appears that the antennae and the maxillary palps are important in mediating the process of feeding induction in lepidopterous larvae, but the maxillary styloconic sensilla maintain their predominant position in the hierarchy governing feeding for larvae on their natural host plants.

Because feeding behaviour in insects appears to be markedly dependent on chemosensory input, much work has concentrated on analysis of the function, sensitivities, and specificities of insect chemoreceptors. In most insects there exists a positive relationship between the rate of response from gustatory chemoreceptors tested with phagostimulants and feeding behaviour (Blom, 1978). For example, Albert & Parisella (1988a) used electrophysiological techniques to test the sucrose sensitivity of lateral sensilla styloconica on the galea of sixth-instar spruce budworm. They found that the physiological threshold for sucrose (<0.5 mM/l) corresponded well with the established behavioural feeding threshold of between 0.1 and 1.0 mM/l (Albert et al., 1982). This indicates that sensory input from the lateral sensilla styloconica parallels behavioural responses. Experimental results suggest that chemoreception at the level of the taste sensilla is subject to modification. In *Manduca sexta*, *Spodoptera exempta*, and *S. littoralis,* the maxillary chemoreceptors are strongly influenced by the type of diet on which they were reared (Städler & Hanson, 1976; Schoonhoven, 1976; Schoonhoven et al., 1987). Simpson et al. (1991) showed that the immediate nutritional state of the insect affects peripheral chemosensory input. In this experiment, locusts were fed artificial diets lacking either protein or carbohydrates. Locusts pretreated on diets containing no protein had increased chemosensory responses to an amino acid mixture, while insects fed a protein diet containing no carbohydrates showed increased chemosensitivity to sucrose.

The hormonal status of the insect may also play a role in modulating sensory responses. Chemosensitivity and ovarian cycles in the blowfly *Phormia regina* are both believed to be modified by juvenile hormone (Angioy et al., 1983).

Collectively, these reports imply that peripheral taste receptors have a certain degree of plasticity. The mechanisms involved in governing this phenomenon are presently uncertain. However, evidence supports the hypothesis that the heamolymph plays a major role in controlling the sensitivities of gustatory sensilla. A haemolymph-borne factor reduces the sensitivity of lactic acid receptors in *Aedes aegypti,* and this reduction correlates with changes in host-seeking behaviour after a blood meal (Davis, 1984). In blowflies, the sensitivity of two receptor cell types correlates with the ionic composition of the haemolymph (Jackmann et al., 1982). In locusts, injections of amino acids into the haemolymph cause a subsequent reduction in sensitivity of receptor neurons associated with the maxillary styloconic sensilla toward amino acids (Abisgold & Simpson, 1988). A mechanism responsible for transporting substances from the haemolymph was demonstrated by Phillips and Vande Berg in 1976. Thus, it seems probable that constituents of the heamolymph exert a controlling effect on the dendrites of the gustatory sensilla. The mechanisms controlling this have yet to be elucidated.

PROJECT GOALS AND OBJECTIVES

In a recently published paper (Albert & Bauce, 1994), fourth- and sixth-instar larvae of *Choristoneura fumiferana* are particular with respect to which part of host conifer buds they prefer to feed upon. Aqueous extracts from terminal and lateral shoots of young and old balsam fir trees were tested against a water disc control on fourth- and sixth-instar budworm larvae. Fourth-instar larvae had a preference for the terminal shoots of both young and old trees. Sixth-instar larvae preferred extracts from lateral shoots of young trees. On old trees, the sixth-instars exhibited a trend towards preferring lateral shoot extracts, however, this was not statistically significant. The feeding rate on lateral *versus* terminal shoots showed a significant difference for the fourth-instar larvae on both young and old trees. For the sixth-instar larvae the feeding rate was enhanced significantly on lateral shoots of old trees, but showed no difference between the terminal and lateral shoots of young trees.

This project undertook to correlate the behavioural feeding response of *Choristoneura fumiferana* with neurophysiological responses recorded from insects stimulated with the same extracts. Because the sensilla styloconica of the galea appear to be critical in the formulation of feeding decisions, electrophysiological tests were conducted on the lateral and the medial sensilla styloconica on the galea of fourth-and sixth-instar larvae.

A preliminary experiment was undertaken to characterize the response of the fourth-instar larvae to molar solutions of sucrose previously tested on much larger sixth-instar larvae (Albert & Parisella, 1988a). This was done both to elucidate whether the two instars differ in their sensitivity to sucrose, and to provide a control solution for electrophysiological tests on fourth instars with extracts.

In addition, subsequent to the experiments with extracts, tests were done using those amino acids known to be feeding stimulants (Albert & Parisella, 1988b). Responses of fourth- and sixth-instar larvae were measured and compared. Lastly, an experiment on sixth-instar larvae was completed in order to characterize the response of the styloconic sensilla to the feeding deterrent, valine.

While the main objective of this investigation was to study the relationship between behaviour and neurophysiology, a secondary goal was to investigate the respective functions of the lateral and the medial sensilla styloconica in this species. Studies have indicated that receptor neuron types present in the lateral and medial sensilla styloconica vary from species to species in Lepidoptera. For example, a neuron in the lateral maxillary sensilla styloconica in *Bombyx mori* (Bombycidae) is sensitive to D-mannose. This sensitivity is not present in the lateral styloconica of *Pieris brassicae* (Pieridae), but is present in the medial styloconica of *Dendrolimus pini* (Lasiocampidae) Even closely related species such as *Pieris rapae* (Pieridae) and *Pieris brassicae* (Pieridae) have been shown to differ in their chemoreceptive repertoire. Sensitivities to inositol and salicin are present in *P. rapae,* but absent in *P. brassicae* (Schoonhoven, 1987). In many lepidopterans, the medial maxillary sensilla styloconica, in particular, are involved with perception of deterrent chemicals. In *Eldana saccharina,* a group of chemicals called limonoids are known feeding deterrents. When the maxillary styloconica are stimulated with one of these compounds, pedonin, receptor neurons in the lateral sensilla styloconica are inhibited, while the receptor neurons in the medial sensilla styloconica display a higher frequency of firing. This implies that altering of the afferent messages transmitted by receptor neurons within the two sensilla styloconica is responsible for the feeding effect noted, and that the medial sensilla styloconica have receptors neurons sensitive to the deterrent chemical (Waladde et al., 1988).

In this study, the chemosensory domains of the lateral and medial maxillary sensilla styloconica towards the substances tested were explored in both age groups of spruce budworm larvae.

CHAPTER 1

Fourth- and Sixth-Instar *Choristoneura fumiferana* Larvae Differ in Their Chemosensory Response to Sucrose and Proline

A. Abstract

Receptor neurons associated with the styloconic sensilla on the galea of fourth-and sixth-instar spruce budworm larvae were tested using electrophysiological techniques. Fourth-instar larvae are more sensitive to lower sucrose concentrations than previously tested sixth-instars which are more sensitive to higher sucrose concentrations. Responses to four phagostimulatory amino acids: proline, lysine, serine, and alanine were measured. A proline-sensitive neuron, believed to be the water receptor neuron, was found in the medial sensilla styloconica of both larval subsets. Fourth-instar larvae are significantly more sensitive to proline than are sixth-instars. In the medial styloconic sensilla, the fourth-instar larvae showed a trend towards greater sensitivity to serine compared to sixth-instars. Sixth-instar larvae tested with the feeding deterrent, valine, indicated the presence of deterrent receptor sites on a cell in the lateral sensilla styloconica.

B. Introduction

Ingestion of adequate levels of protein and carbohydrates is essential for optimal growth and survival of young insects. Accordingly, carbohydrates and amino acids have repeatedly been shown to be strong phagostimulants (Albert *et al.*, 1982; Albert & Parisella, 1985; Bernays &

Simpson, 1982; Cook, 1977; Schiff *et al.*, 1989).

Evidence indicates that the relative nutritional need for protein and carbohydrates changes throughout the growth and development phase in insects. For example, ingestion of dietary nitrogen shortens development time and affects future fecundity of first-instar larvae in the lepidopteran *Samea multiplicalis* (Pyralidae). In *Lymantria dispar* (Lymantriidae), nitrogen consumption and utilization decreased in larvae as they matured (Taylor, 1984; Montgomery, 1982). Chyb and Simpson (1990) showed that the proportion of carbohydrate consumed increased during somatic growth in adult locusts, and that this feeding change corresponded well to fluctuations known to occur in the protein and carbohydrate requirements of the insect during this period. These studies suggest that protein content is particularly important for young insects, and that a shift away from protein towards carbohydrate consumption commences as insects age.

A subsequent study on adult locusts by Simpson *et al.* (1990) demonstrated that changes in chemosensitivity of the maxillary palps to sucrose and amino acids paralleled relative changes in carbohydrate and protein consumption. This indicates that the chemosensory system undergoes modifications throughout the insect's life span that reflect concurrent changes in nutritional requirements.

The object of the present study is to determine if peripheral chemosensory perception of sucrose and amino acids changes in spruce budworm larvae as they mature.

A previous study by Albert and Parisella (1988a) characterized the responses of a sucrose-sensitive neuron in the lateral sensilla styloconica on the galea of sixth-instar larvae. This neuron displayed a rising rate of activity (impulses/s) over a range of increasing molar concentrations of sucrose. In the present study, fourth-instar larvae were tested over the same range of sucrose concentrations and results were compared to those previously obtained with the sixth-instars.

In a behavioural study, Albert and Parisella (1988b) tested a series of fourteen amino acids at molar concentrations representative of those found in balsam fir foliage. They found that the amino acids proline, serine, lysine, and alanine stimulated, and valine deterred, feeding in sixth-instar spruce budworm larvae. We thus also tested the four identified phagostimulatory amino acids on the styloconic sensilla of the maxillary galea of fourth- and sixth-instar larvae. In addition, a separate experiment on sixth-instar larvae was undertaken to characterize the response of receptor neurons associated with the styloconic sensilla to the feeding deterrent valine.

C. Materials and methods

Insects. Second-instar larvae were supplied by the Forest Pest Management Institute, Sault Ste. Marie, Ontario. They were reared in an incubator at 27°C and 70% humidity under a 16L:8D photoperiod and fed an artificial diet (Grisdale, 1984). Age of the larvae was determined by head capsule width (McGugan, 1954). Fourth- and sixth-instar larvae were selected for electrophysiological tests. Care was taken in choosing young representatives from both sets of instars. Newly-moulted unsclerotized larvae, and large bloated sixth-instars that appeared to be at the prepupal stage, were avoided.

Solutions. Sucrose solutions in concentrations of 0.5, 1, 5, 10, 50, and 100 mM/l were dissolved in a 50 mM/l KCl solution in order to provide good conductivity. Individual amino acid solutions were made by dissolving proline, serine, lysine in 50 mM/l KCl to obtain a 25 mM/l solution of the amino acid, or alanine in 50 mM/l KCl to obtain a 50 mM/l solution of the amino acid. Valine was used as a 25 mM/l solution in 50 mM/l KCl.

Electrophysiology. Fourth- and sixth-instar larvae were decapitated and a micropipette filled with Ringer solution (Schnuch & Hansen, 1989) was inserted into the neck opening. The pipette was subsequently fitted onto a Ag/AgCl reference electrode and the head viewed under a compound light microscope. Styloconic sensilla were stimulated using micropipettes filled with test solutions fitted onto a Ag/AgCl recording electrode. Neural impulses from the styloconic chemosensilla were recorded on digital audio tape and then digitized using the Sapid Tools computer program (Smith *et al.*, 1990). The digitized traces representing the response from the first 1 s of stimulation were analyzed visually, or with the aid of the computer program.

Experimental protocol. Sucrose tests on the fourth-instars were done by applying the sucrose solutions to the sensilla styloconica in a random order. Experimental design for the experiments with amino acids consisted of a preliminary stimulation with the 50 mM/l KCl solution followed by consecutive stimulations with the four stimulatory amino acids applied in random succession. The experiment with valine was done in a similar manner with stimulations of valine, proline, and lysine following an initial stimulation with KCl. In all experiments, a two-minute interval between stimulations was allowed in order to facilitate disadaptation of the chemosensilla to the test solution.

Statistics. Data were analyzed using the Number Cruncher Statistical System (J.L. Hintze 865 East North, Kaysville, UT 84037, USA). Due to unequal sample sizes, data were analyzed with the non-parametric

Mann-Whitney test to determine probability values.

D. Results

Fourth-instar larvae: sucrose response. An increased firing rate (impulses/s) in response to increasing molar concentrations of sucrose was displayed by one receptor neuron (Cell 1) in the lateral sensilla styloconica of fourth-instar larvae. A peak response for Cell 1 was reached at 50 mM/l sucrose (Fig. 1.1). A second neuron (Cell 2) did not show an increased response as sucrose concentrations were increased. Cell 2 was probably responding to the 50 mM/l KCL present in the solution, and therefore represents the salt-sensitive cell present in the lateral sensilla styloconica. The activity of Cell 2 decreased with sucrose concentrations above 0.5 mM/l indicating that when a critical level of sucrose is present, this salt cell is suppressed.

Cells in the medial styloconic sensilla did not increase their rate of firing in response to increasing sucrose concentrations (Fig. 1.2). These cells probably represent salt cells. One of these showed a decrease in response rate as the sucrose concentration was elevated. Here again, this signifies that as sucrose concentration rises, the activity of this salt cell is suppressed.

Fourth- and sixth-instar larvae: amino acid response. Cell 1 responses in the medial styloconic sensilla did not differ significantly when alanine and lysine were used as stimuli (Table 1.1; Fig. 1.3). Cell 1 in the medial styloconic sensilla in fourth-instar larvae showed a trend towards greater sensitivity to serine than did Cell 1 in the medial styloconic sensilla of the sixth-instars. However, this difference was not significant (Table 1.1).

A separate neuron (Cell 3) in the medial styloconica of both larval age groups showed a strong response when stimulated with proline (Fig. 1.3). The wide monophasic nature of this neuron is consistent with what is observed to be the water-sensitive cell in the medial styloconic sensilla. Figure 1.4 shows a representative response of this cell to stimulation with proline in a fourth-instar larva along with comparative traces from stimulations with the remaining amino acids and KCl. Figure 1.5 shows the same comparative traces for a sixth-instar larva. This neuron was observed on rare occasions in traces resulting from 50 mM/l KCl stimulations. On very rare occasions, it was observed firing at a low rate in response to stimulations with the other amino acids (Fig. 1.3). This neuron was significantly more sensitive to proline in fourth-instars than in sixth-instars (Table 1.1). The total response to proline in the sixth-instar larva appears higher than that of the fourth-instar larva due to greater activity of Cells 1 and 2 in the sixth-instars (Fig. 1.3).

No significant activity was found in response to any of the phagostimulatory amino acids tested on the lateral sensilla styloconica of both larval age groups. A cell in the lateral styloconica of sixth-instar larvae responded strongly to stimulation with the feeding deterrent valine (Table 1.2, Fig. 1.6). This cell appears identical to the cell responding to solutions containing only 50 mM/l KCl, and is probably a salt-sensitive neuron. Although there was considerable variability between insects, the ratio between the salt response and the valine response was consistent for nine of ten animals tested. One insect showed an uncharacteristically low and erratic response to KCl. This was representative of a "poor hit" and not included with the KCl responses.

Table 1.1. Mann-Whitney test comparisons between fourth- and sixth- instar responses (mean impulses/s) of medial styloconic sensilla to stimulation with amino acids

Stimulus	p value	Comparison
Serine (Cell 1)	0.0910	4th ≥ 6th
Lysine (Cell 1)	0.7751	4th = 6th
Alanine (Cell 1)	0.5310	4th = 6th
Proline (Cell 1)	0.0993	6th ≥ 4th
Proline (Cell 3)	0.0076	4th > 6th

Table 1.2. Mean impulses/s (± S.D.) from lateral styloconic sensilla of sixth-instar larvae to stimulation with valine and KCl

Stimulus	Mean impulses/s	S.D.
KCl	79.0	11
Valine	129.2	31

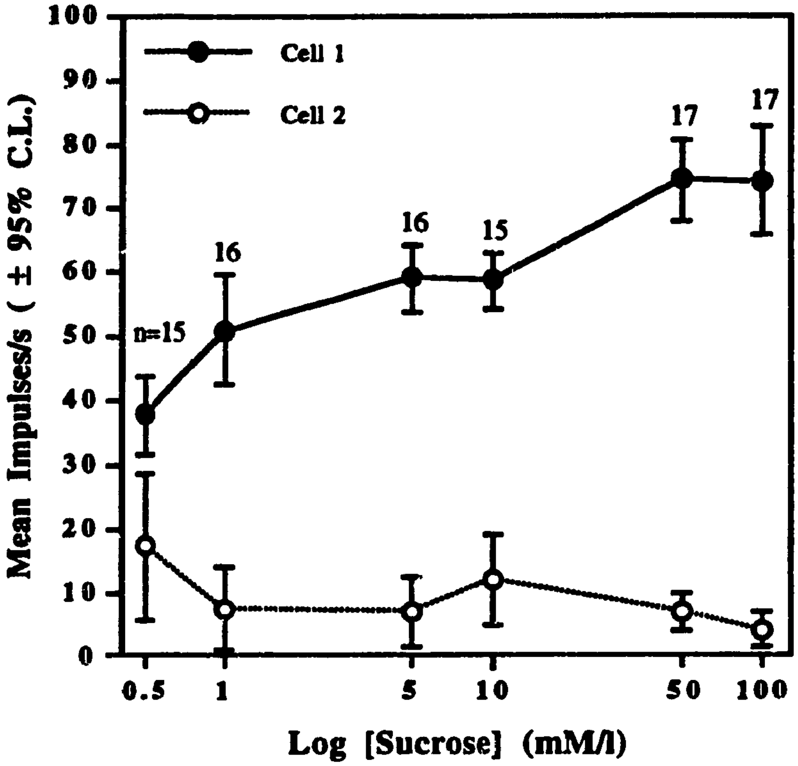

Figure 1.1. Mean impulses/s (± 95% C.L.) from Cells 1 and 2 of lateral styloconic sensilla of fourth-instar larvae stimulated with various concentrations of sucrose in 50 mM/l KCl.

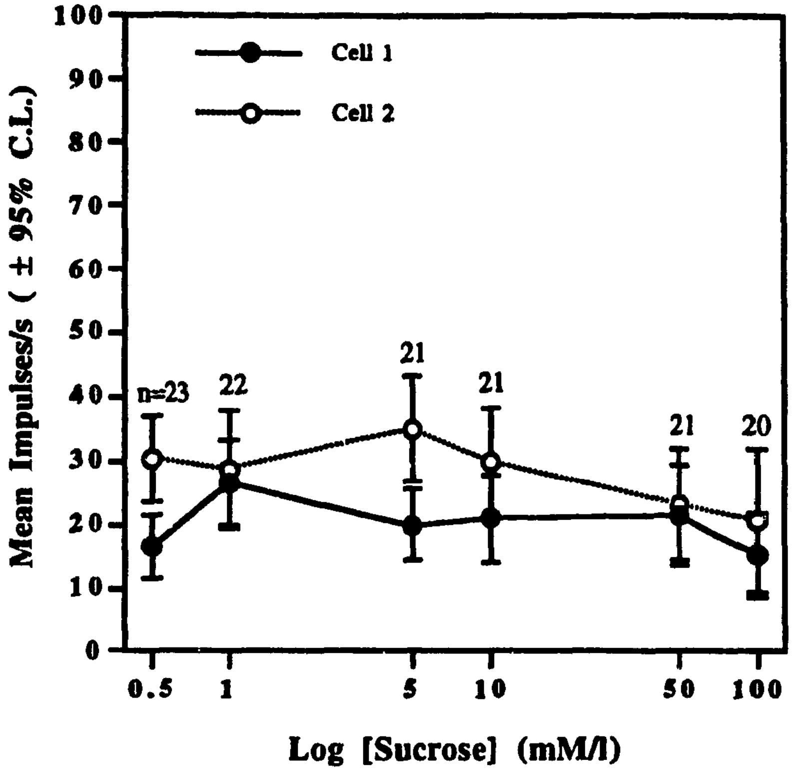

Figure 1.2. Mean impulses/s (± 95% C.L.) from Cells 1 and 2 of medial styloconic sensilla of fourth-instar larvae stimulated with various concentrations of sucrose in 50 mM/l KCl.

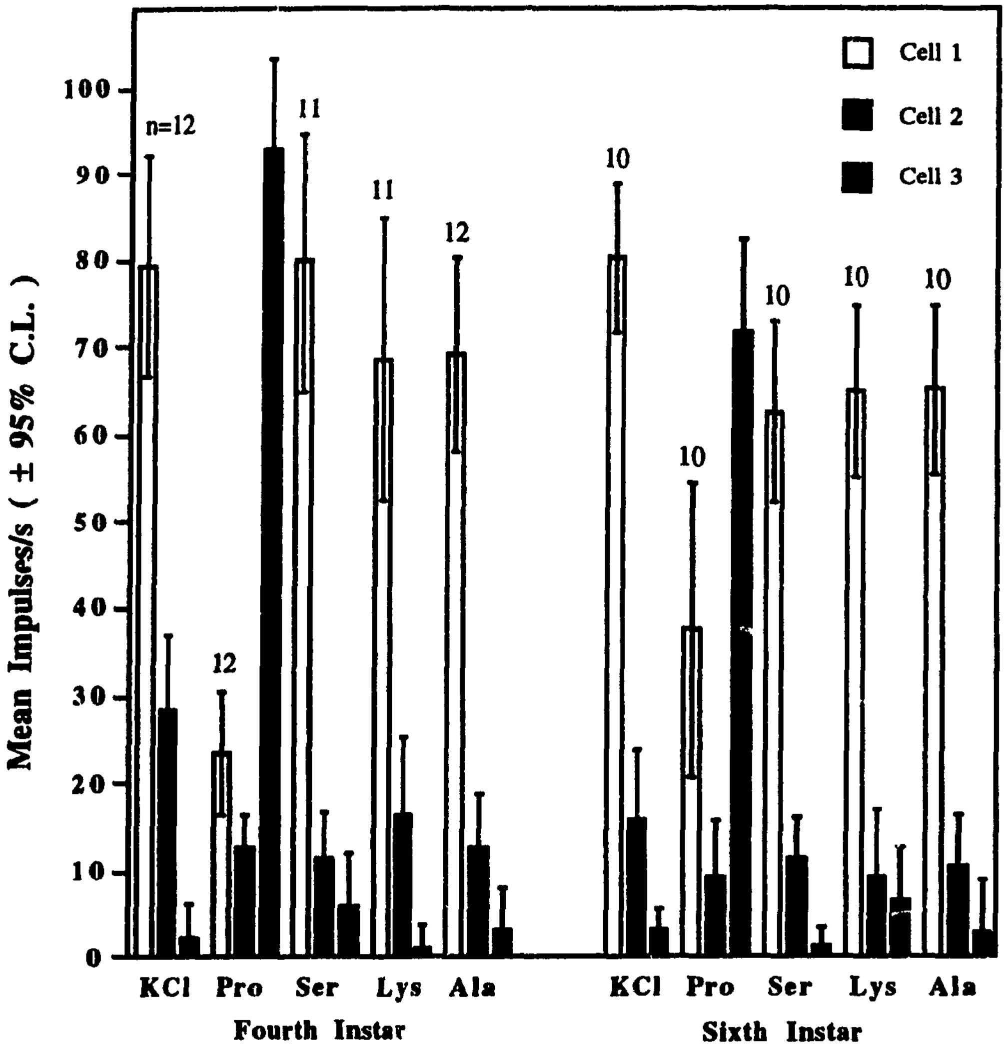

Figure 1.3. Mean impulses/s (± 95% C.L.) from Cells 1, 2, and 3 of lateral styloconic sensilla of fourth- and sixth-instar larvae stimulated with amino acids in 50 mM/l KCl.

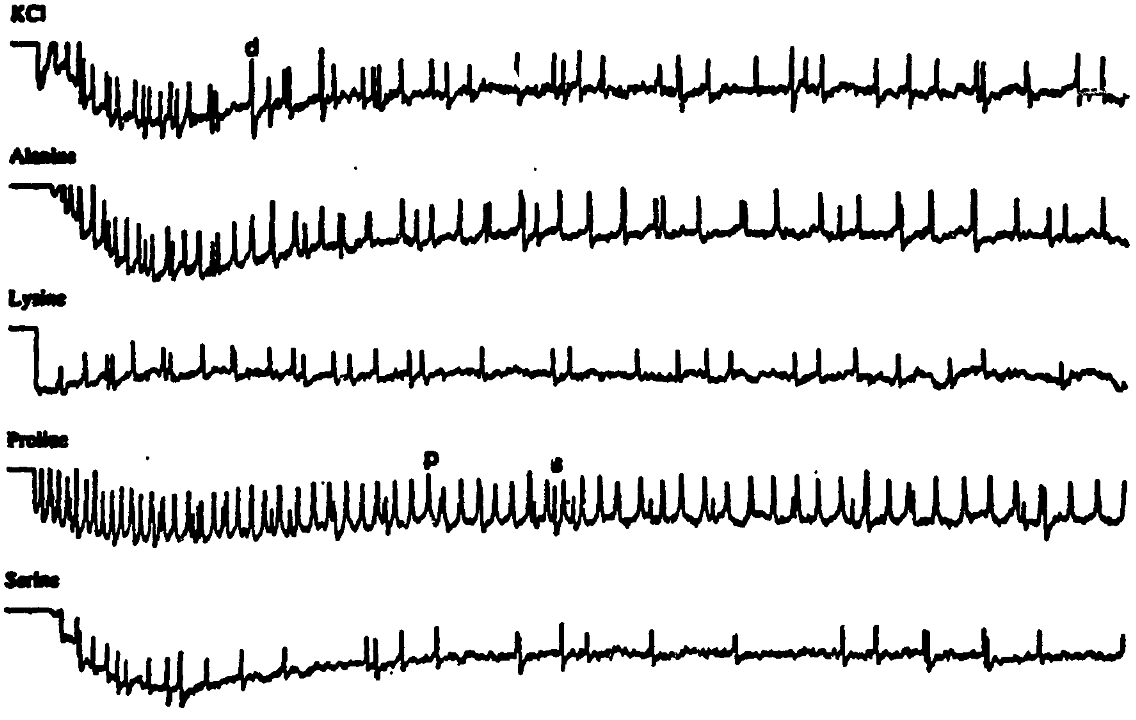

Figure 1.4. Representative traces showing responses of lateral styloconic sensilla of fourth-instar larvae to stimulation with 50 mM/l KCl and amino acids in 50 mM/l KCl. All traces represent the first 0.5 s of the response. p, proline cell; s, salt cell; d, double spike.

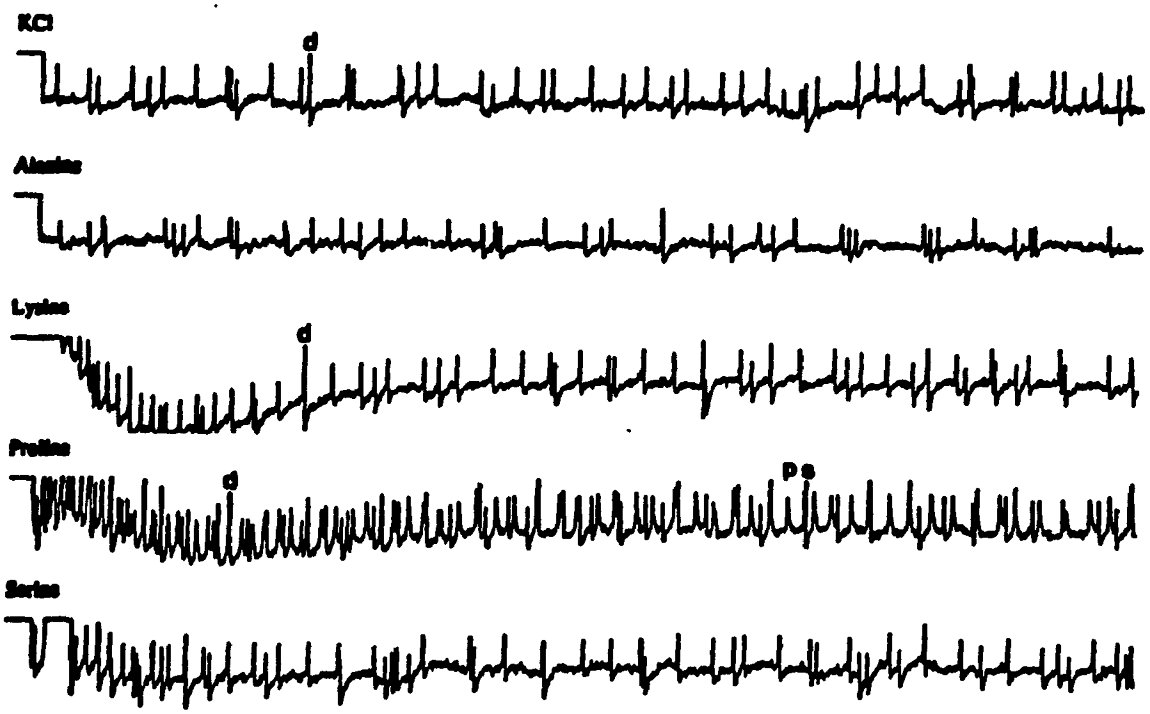

Figure 1.5. Representative traces showing responses of lateral styloconic sensilla of sixth-instar larvae to stimulation with 50 mM/l KCl and amino acids in 50 mM/l KCl. All traces represent the first 0.5 s of response. p, proline cell; s, salt cell; d, double spike.

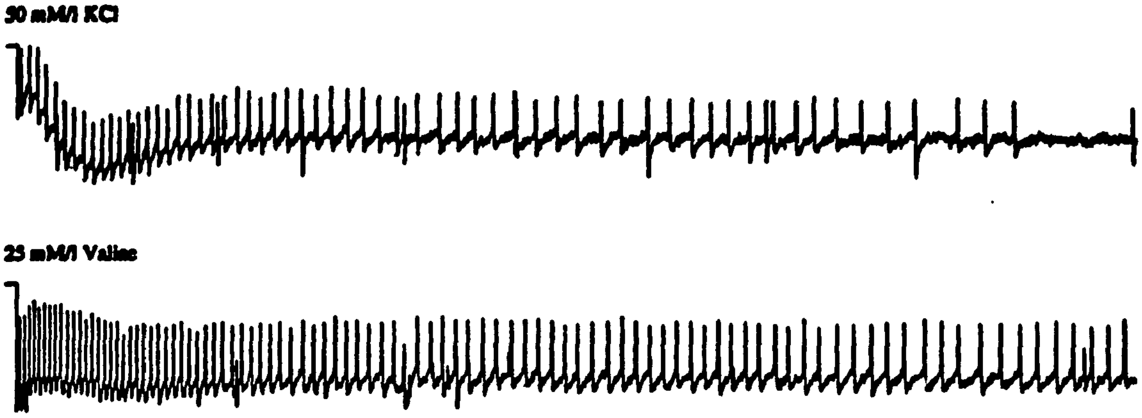

Figure 1.6. Representative traces showing responses of lateral styloconic sensilla of sixth-instar larvae to stimulation with 50 mM/l Kcl and 25 mM/l valine in 50 mM/l KCl. Both traces represent 1 s of response.

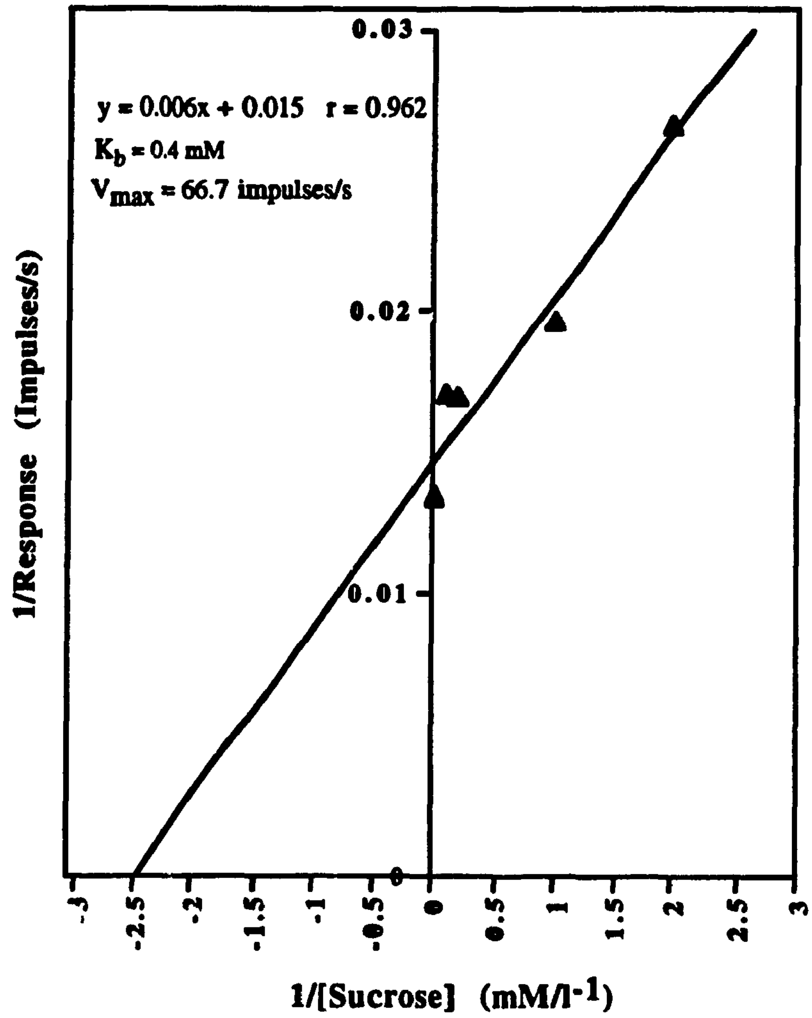

Figure 1.7. Double-reciprocal plot of responses from Cell 1 of lateral styloconic sensilla of fourth-instar larvae to stimulation with various concentrations of sucrose in 50 mM KCl.

E. Discussion

A comparison of the sucrose response from the lateral and medial styloconic sensilla of fourth-instar larvae indicates that a sucrose-sensitive cell is present only in the lateral sensilla styloconica of spruce budworm larvae. It is interesting to note that both the lateral and medial sensilla styloconica contain salt-sensitive cells with firing rates that are subdued as sucrose concentration rises. A high rate of response from a salt cell is thought to act as a feeding deterrent, while a low to moderate response rate is believed to enhance phagostimulation in calliphorid flies (Schnuch & Hansen, 1990). An example of the former can be seen here in the response of the salt-sensitive cell in the lateral styloconic sensilla of the sixth-instars to valine, an amino acid that has been demonstrated to be a feeding deterrent in spruce budworms (Albert & Parisella, 1988b). The effect of a reduction in response rate noted in the salt-cells of the lateral and medial styloconica when sucrose is elevated could be to tone down any deterrent input when a strong feeding stimulant is present. Such an "over-ride" system would ensure that the stimulatory effect of sucrose was perceived by the larvae.

A previous study on the lateral styloconic sensilla of the sixth-instar spruce budworm produced a Michealis-Menton double reciprocal plot with a K_b value of 1.5, and a V_{max} of 200 impulses/s for 1 s of response (Albert & Parisella, 1988a). Comparison of these data with ours from the fourth-instar larvae (Fig. 1.7) show a lower K_b value for the fourth-instars compared to the sixth, and an increased V_{max} for the sixth-instars over the fourths. This suggests that the fourth-instar larvae have an increased chemosensitivity compared to the sixth-instars to low concentrations of sucrose, and that the sixth-instars have a greater chemosensitivity than the fourth-instars to higher concentrations of sucrose. This difference in sensitivity might be an adaptation by the spruce budworm to the naturally-occurring differences in foliage sucrose concentration normally available to fourth- and sixth-instar larvae at the time when they are present on their host trees. Little (1970) showed that the dry weight of sucrose in current needles of balsam fir was 38 mg/g dry wt around June 10 when spruce budworms are in their fourth instar, and increased to 45 mg around June 19 when budworms were entering their sixth instar. Thus, a greater sensitivity to lower sucrose concentrations would be beneficial to the younger larvae. Another possibility is that larvae of different stadia differ in their nutritional requirements, and are choosing their food accordingly. Albert and Bauce (1994) have shown that within-host feeding preferences of fourth- and sixth-instar spruce budworm larvae can be correlated to the nitrogen and sucrose content of the host foliage. The feeding preferences of the fourth-instar larvae are correlated to the nitrogen concentration, and the feeding preferences of the sixth-instars are correlated to the sucrose concentration. Younger insects must concentrate on building up body mass, and consuming protein for developing tissues and organs. Since nitrogen concentration reflects protein content, it is possible that the young caterpillars are choosing foliage that supplies them with the largest source of protein. When the larvae reach their final stage, they have attained their greatest body length, and are preparing for metamorphosis, a process that requires abundant stores of energy. In this scenario, the differing sucrose sensitivities between the two larval age groups is a manifestation of their relative developmental differences.

Results from the amino acid tests completed in this study further substantiate the hypothesis. Of all the amino acids studied in this aforementioned investigation only proline had receptors present for it on a separate neuron (in the medial sensilla styloconica). In addition, this proline-sensitive neuron was more chemosensitive in the fourth-instar larvae. It has been demonstrated that specific amino acids can function as feeding cues in insects. Grasshoppers have the ability to detect and preferentially feed on grass that has been treated with proline and valine (Haglund, 1980). In locusts, the amino acid lysine is involved in signalling protein satiation (Zanotto *et al.*, 1994). The existence of a receptor site which, based on our limited testing, appears to be specific for proline on a separate cell in spruce budworm larvae implies that proline is especially important to these insects. Proline might function as the signal that a protein source is available. If so, then an increased sensitivity for the fourth-instars suggests that they may be intrinsically "programmed" to respond with a higher degree of acuity towards any protein they encounter.

A particular sensitivity to proline could also act to benefit budworm larvae. A study on aphid-infested barley seedlings found that proline concentrations rise significantly on plants infested with greater than 18 aphids. This increase in proline has been tied to disruptions of the plant's water potential as a result of the infestation (Cabrera *et al.*, 1994). It has been observed that several warm dry summers act to predispose host conifers to spruce budworm outbreaks. Coincidentally, it has been noted that drought stress causes an increase in proline concentration in foliage (Rhoades, 1983). A specific sensitivity to proline could stimulate larvae to increase consumption of trees whose defences have been compromised, and thus less likely to mount an effective counter-attack.

CHAPTER 2

Age-Related Responses From the Maxillary Sensilla Styloconica of *Choristoneura fumiferana* Larvae to Foliage Extracts From Balsam Fir Hosts

A. Abstract

An electrophysiological study on the sensilla styloconica of the galea in *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae) larvae showed a differential response between the fourth- and sixth-instar larvae towards extracts from the lateral and terminal shoot foliage of balsam fir. Larvae raised on artificial diet were stimulated with the water soluble fraction of tree extracts obtained from terminal and lateral foliage of 30- and 70-yr-old balsam fir trees. An extract sensitive neuron was found in the lateral styloconic sensilla of both larval instars. The lateral sensilla styloconica in fourth-instar larvae were stimulated to a greater extent by extracts from terminal shoot foliage of both young and old trees. The lateral styloconica in the sixth-instar larvae displayed a greater response to lateral shoot foliage of old trees. Results are discussed with respect to their relationship to feeding preferences and feeding rates observed in a previous behavioural study.

B. Introduction

Many investigations have endeavoured to relate an insect's feeding behaviour with its underlying physiology (Ma, 1972; Schoonhoven & Blom, 1988; Timmins & Reynolds, 1992). Difficulties have arisen in the interpretation of results because the variability of the physiological response is often greater than the behavioural variation (Schoonhoven et al., 1991). This is particularly true when attempts are made to directly correlate feeding behaviour to neurophysiological stimulation from unadulterated food sources (Blaney et al., 1986), including plant saps (Schoonhoven, 1987). In order to preclude problems of this sort, much work has focused on obtaining a neurophysiological response from taste sensilla stimulated with a simple phagostimulant such as sucrose, while concurrently measuring the feeding behaviour. Simmonds et al. (1991) found that the responsiveness of the gustatory sensilla of *Spodoptera littoralis* (Lepidoptera: Noctuidae) to sucrose concentrations varied within the final larval stadium in correlation with larval weight gain and amount ingested. Subsequently, it was shown that changing the artificial diet of *S. littoralis* affected the response of the taste sensilla to sucrose (Simmonds et al., 1992). These results suggest that the relationship between neural sensitivity and feeding behaviour is not spurious, and that a dynamic interaction is taking place. The mechanism of this interplay might well be adaptive, changing throughout the maturation process of the larvae. Thus, age-dependent variation in chemoreception could reflect the intrinsic nutritional requirements of a particular developmental stage.

This investigation follows a behavioural study (Albert & Bauce, 1994) which demonstrated that fourth- and sixth-instar larvae of the spruce budworm have different in-host preferences. The sixth instars exhibited a strong preference for extracts from lateral shoots of young (30 yr) balsam fir trees. Similarly, sixth instars showed a trend towards preferring the lateral shoots of old (70 yr) balsam fir trees. The much smaller fourth instars preferred the terminal shoots of both young and old trees. Curiously, the feeding rates (mm^2/h) were generally higher on the less preferred extracts.

The feeding preference of the fourth-instar larvae was correlated with the nitrogen content of the terminal tip foliage and that of the sixth-instar larvae was correlated with the sucrose content in the lateral tip foliage. Variations in feeding preferences between larvae of different stadia could represent age-dependent changes in taste cell receptor sensitivities. The correlation with nitrogen content for the fourth instars, and sucrose content for the sixth instars indicates that the larvae are responding to specific components of the host plant which are important to the respective larval stages. The present study examines the chemosensory basis underlying this behaviour.

The maxillary galea with its two styloconic sensilla is critically involved in feeding decisions in lepidopterous larvae (Dethier, 1993; Flowers & Yamamoto, 1992; de Boer, 1991). In this electrophysiological investigation, extracts employed in the behavioural study were tested on the sensilla styloconica of fourth- and sixth-instar larvae.

C. Materials and Methods

Insects. Second-instar larvae were obtained from the Forest Pest Management Institute, Sault Ste. Marie, Ontario. They were fed an artificial diet (Grisdale, 1984), and reared in an incubator at 27°C and 70% humidity under a 16L:8D photoperiod. Age of the larvae was determined by head capsule width (McGugan, 1954). Fourth- and sixth-instar females were selected for electrophysiological tests. Care was taken in choosing young representatives from both sets of instars. Newly-moulted unsclerotized insects were avoided for both subsets. Large bloated sixth-instars that appeared to be at the prepupal stage were also avoided.

Extracts. For the original behavioural tests (Albert & Bauce, 1994) conducted in 1991 and 1992, balsam fir trees were randomly selected from compartment 20 of the Forêt Montmorency experimental forest of Université Laval. Half of the foliage was collected during the period when insects were in the fourth instar (June 10). The remaining foliage was gathered when the budworms were in the sixth instar (June 19). This was done in order to duplicate the type of foliage normally available to each instar. Foliage was collected from the mid-crown level of the trees. Extracts were made using 12:5:3 Methanol:Chloroform:Water (Dickson, 1979). The water fraction containing soluble sugars, amino acids, tannins, and phenols was removed and dehydrated in a vacuum. These samples were rehydrated with a known volume of glass-distilled water, such that the final concentration of the sample was equivalent to that which existed in the host tree at the time of collection (ie. 1X). The extracts chosen for this investigation were those which had elicited the extremes in preference in the behavioural tests. KCl was added to each extract solution to bring the final concentration to 50 mM KCl/l for the electrophysiological stimuli to provide adequate conductivity.

Electrophysiology. Fourth- and sixth-instar larvae were decapitated and a micropipette filled with Ringer solution (Schnuch & Hansen, 1989) was inserted into the neck opening. The pipette was then fitted onto a Ag/AgCl reference electrode and the head viewed under a compound light microscope. Styloconic sensilla were stimulated using micropipettes filled with 50 mM/l sucrose dissolved in 50 mM/l KCl as a control solution, or with the host extract solution. Neural impulses from the styloconic chemosensilla were recorded on digital audio tape and subsequently digitized using the Sapid Tools computer program (Smith et al., 1990). The digitized traces representing the first 1 s of response were analyzed visually to determine the number of different cells responding and the number of impulses/s of each cell.

Experimental protocol. An initial stimulation with 50 mM/l sucrose in 50 mM/l KCl solution was followed by separate stimulations with each of the extracts applied in random order. A two-minute waiting period between successive stimulations allowed for receptor disadaptation. Four experiments were completed: fourth-instars on old tree extracts, fourth-instars on young tree extracts, sixth-instars on old tree extracts, and sixth-instars on young tree extracts. Experiments were always done in the morning between 0800h and 1200h.

Statistics. Data were analyzed using the Number Cruncher Statistical System (J. L. Hintze, 865 East North, Kaysville, UT 84037, USA). Because of unequal sample sizes, we used the non-parametric Mann-Whitney test to determine probability values. Within each experiment, the two lateral extracts were compared to each other (ie. H_0: mean number of impulses/s are equal between lateral extract one, and lateral extract two), and the two terminal extracts were compared with each other. There was no statistical difference between the two responses and the data were thus grouped together for subsequent analyses. Similarly, there was no statistical difference between the terminal extracts and they were also combined. The combined firing rate (mean impulses/s) for lateral extracts was then compared statistically to the combined firing rate for terminal extracts.

D. Results

The presence of a neuron (Cell 1) with a strong sensitivity to aqueous balsam fir extracts was found in the lateral styloconic sensilla of all larval subsets. This "extract" neuron constituted the main cell responding in the lateral styloconica, and showed an age-related difference in response to lateral and terminal tip foliage (Table 2.1). Occasionally a second fast-firing neuron was noted (Cell 2). The digital profile and the quick firing rate of Cell 2 suggest that it is a salt-sensitive cell responding to electrolytes present in the extract solutions.

Two cells responded in the medial sensilla styloconica of all larval groups. Cells of the medial styloconic sensilla displayed no difference in response to foliage from young or old trees or from terminal or lateral foliage, and the firing frequency was very low in all cases.

Fourth-instar larvae tested on lateral and terminal foliage extracts from young (Fig. 2.1) and old trees (Fig. 2.2) showed a significant increase in the firing rate of the extract-sensitive neuron (Cell 1) of the lateral styloconica when stimulated with terminal tip extracts (Table 2.1). The first 100 ms of Cell 1 response in young trees was 20.2 impulses/s with terminal extracts compared to 16 impulses/s when stimulated with extracts from lateral shoots (Fig. 2.3). The initial firing rate of Cell 1 to terminal extracts of old trees was 17.7 impulses/s for the first 100 ms of stimulation, and 13.8 impulses/s when stimulated with lateral extracts for the same duration (Fig. 2.4).

Tests on sixth-instar larvae with extracts from young trees showed no significant difference between the firing rate of Cell 1 when stimulated by lateral or terminal shoot extracts (Table 2.1; Fig. 2.5). The first 100 ms of response showed only 0.3 impulses/s difference between lateral and terminal extracts for Cell 1 (Fig. 2.7).

The extract cell in the lateral styloconica of sixth instars tested with lateral and terminal extracts from old trees displayed a significant increase in firing rate when stimulated with lateral shoot extract (Table 2.1; Fig 2.6). For the first 100 ms of stimulation with lateral tip extract Cell 1 responded with a firing rate of 19.0 impulses/s compared to 14.9 impulses/s for the first 100 ms of stimulation with terminal extract (Fig. 2.8).

Table 2.1. Comparison of electrophysiological and behavioural results for fourth- and sixth-instar larvae stimulated with extracts from terminal and lateral tip foliage of young and old balsam fir hosts.

Experiment	Results	p value[1]
Electrophysiology		
4th instars: Young trees	Terminal > Lateral	0.0099
4th instars: Old trees	Terminal > Lateral	0.0079
6th instars: Young trees	Lateral = Terminal	0.8497
6th instars: Old trees	Lateral > Terminal	0.0030
Behaviour (Feeding Preference)[2]		
4th instars: Young trees	Terminal > Lateral	0.0193
4th instars: Old trees	Terminal > Lateral	<0.0001
6th instars: Young trees	Lateral > Terminal	0.0001
6th instars: Old trees	Lateral ≥ Terminal	0.1920
Behaviour (Feeding Rate)[2]		
4th instars: Young trees	Lateral > Terminal	0.0614
4th instars: Old trees	Lateral > Terminal	0.0076
6th instars: Young trees	Lateral = Terminal	0.5459
6th instars: Old trees	Terminal > Lateral	0.0001

[1] Mann-Whitney tests

[2] Data from Albert & Bauce (1994)

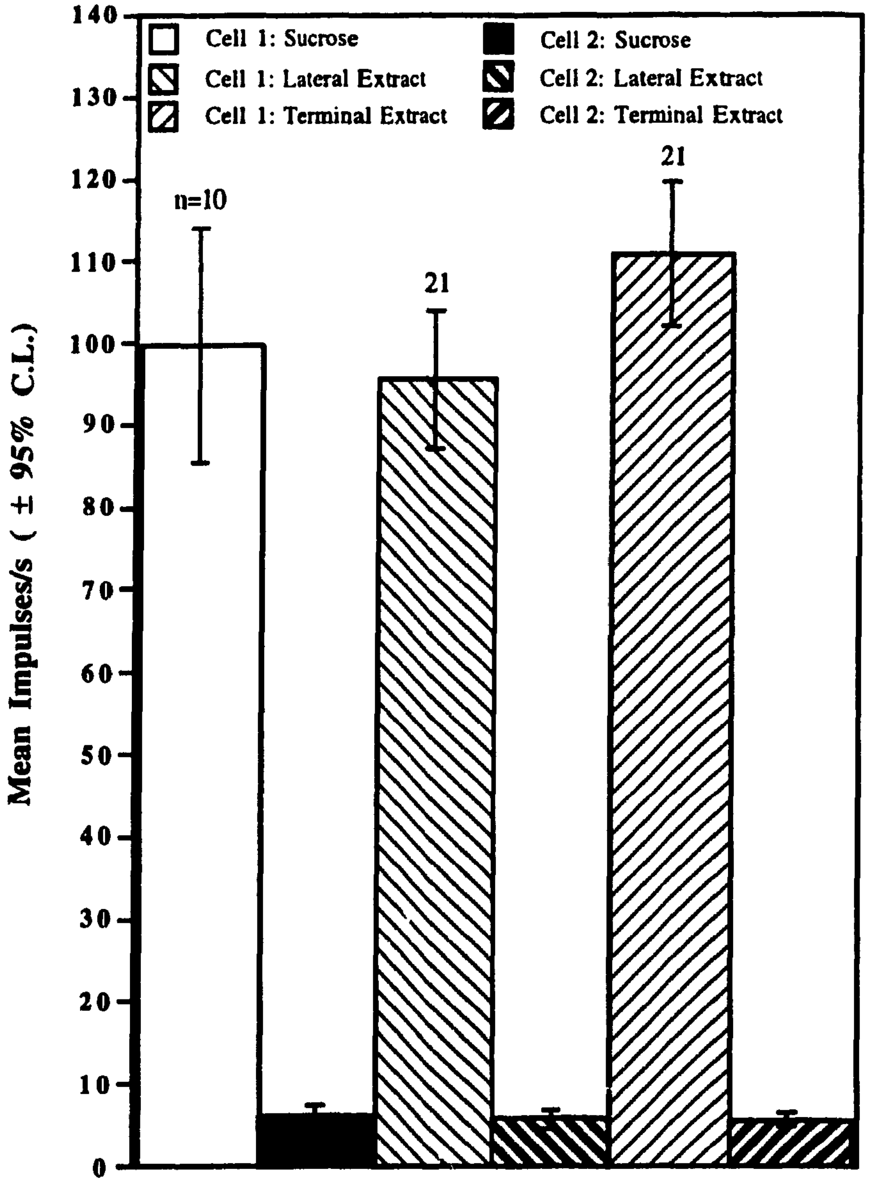

Figure 2.1. Mean impulses/s (± 95% C.L.) of Cells 1 and 2 in lateral styloconic sensilla of fourth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of young balsam fir trees.

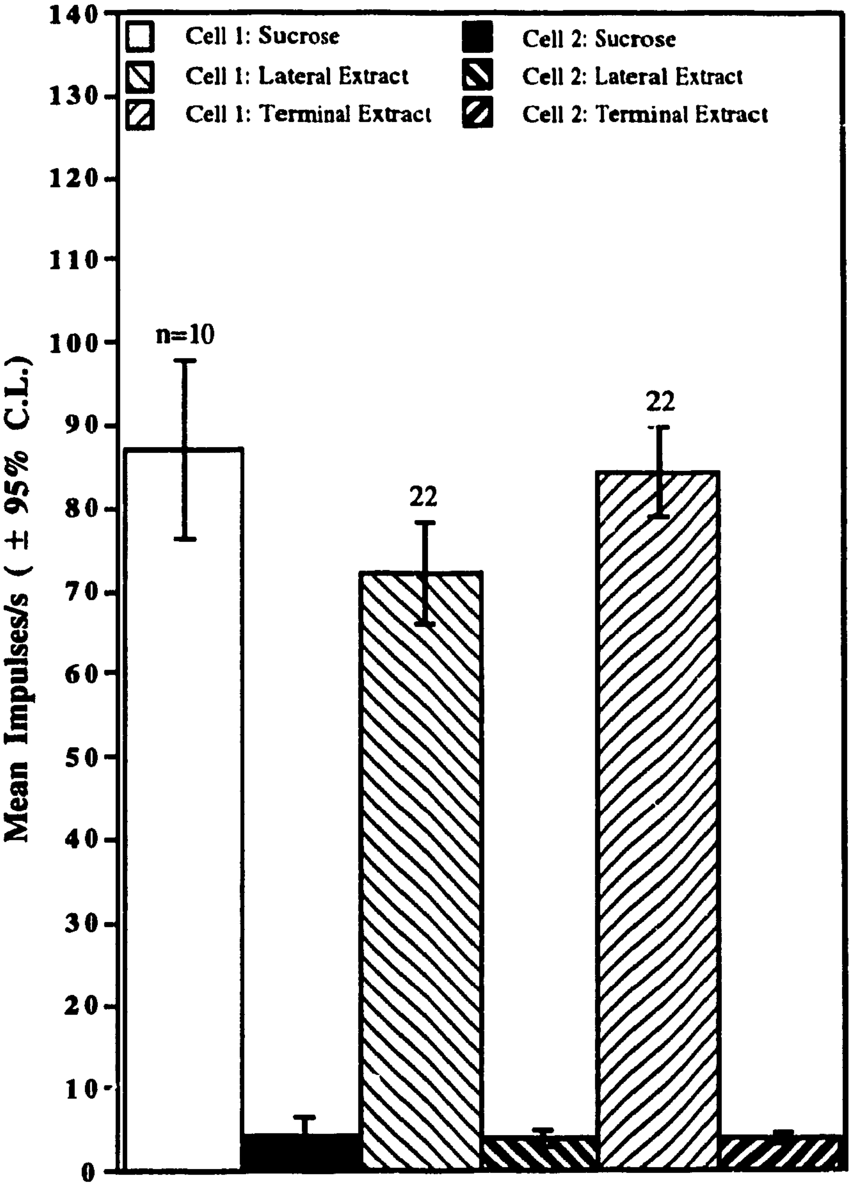

Figure 2.2. Mean impulses/s (± 95% C.L.) of Cells 1 and 2 in lateral styloconic sensilla of fourth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of old balsam fir trees.

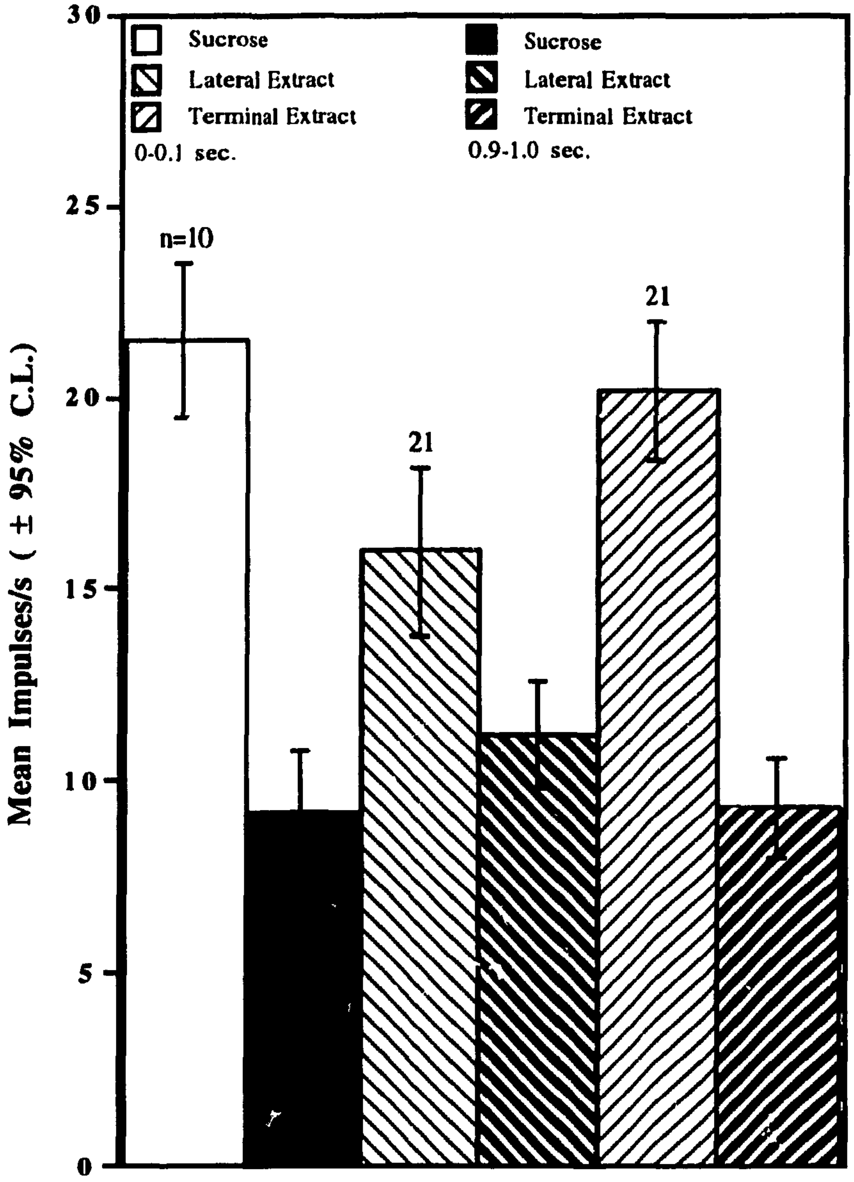

Figure 3.3. Mean impulses/s (± 95% C.L.) of Cell 1 in lateral styloconic sensilla of fourth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of young balsam fir trees. Data are separated into the first and the last 100 ms segments of the 1 s response.

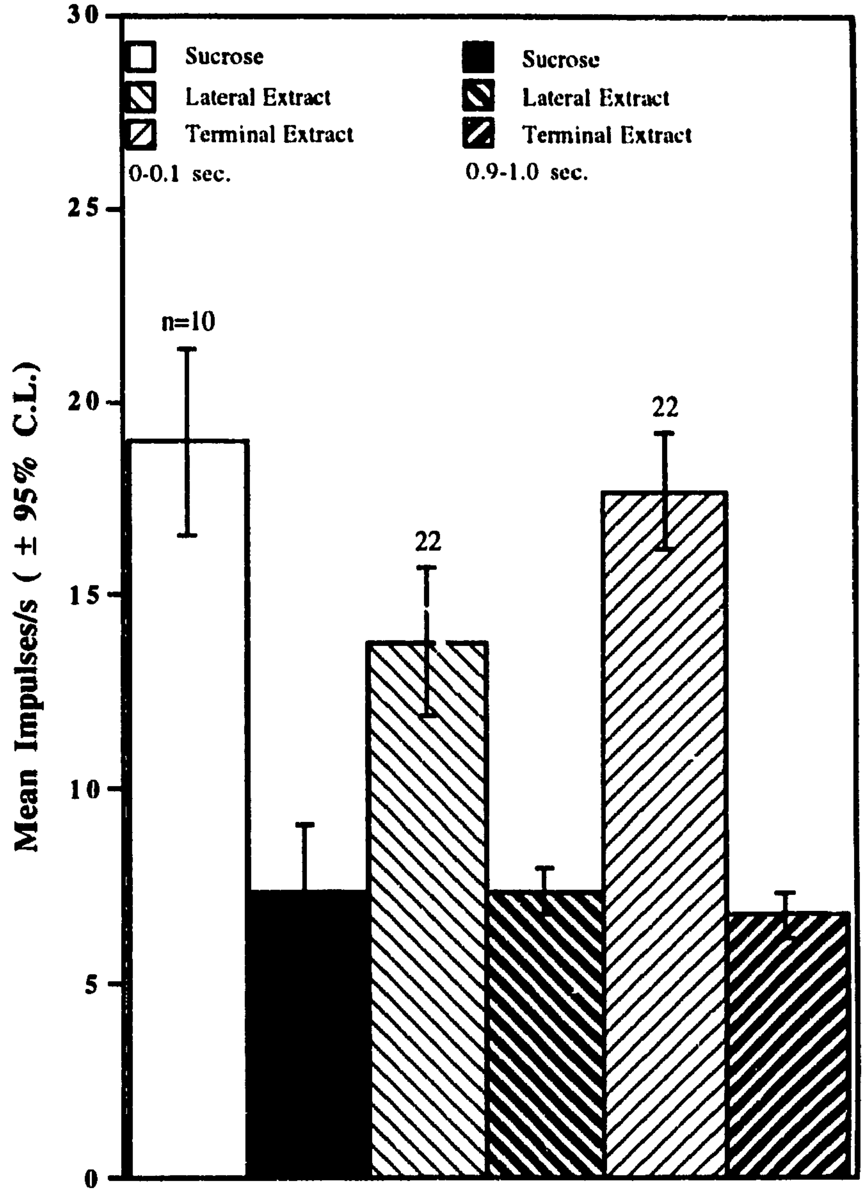

Figure 2.4. Mean impulses/s (± 95% C.L.) of Cell 1 in lateral styloconic sensilla of fourth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of old balsam fir trees. Data are separated into the first and the last 100 ms segments of the 1 s response.

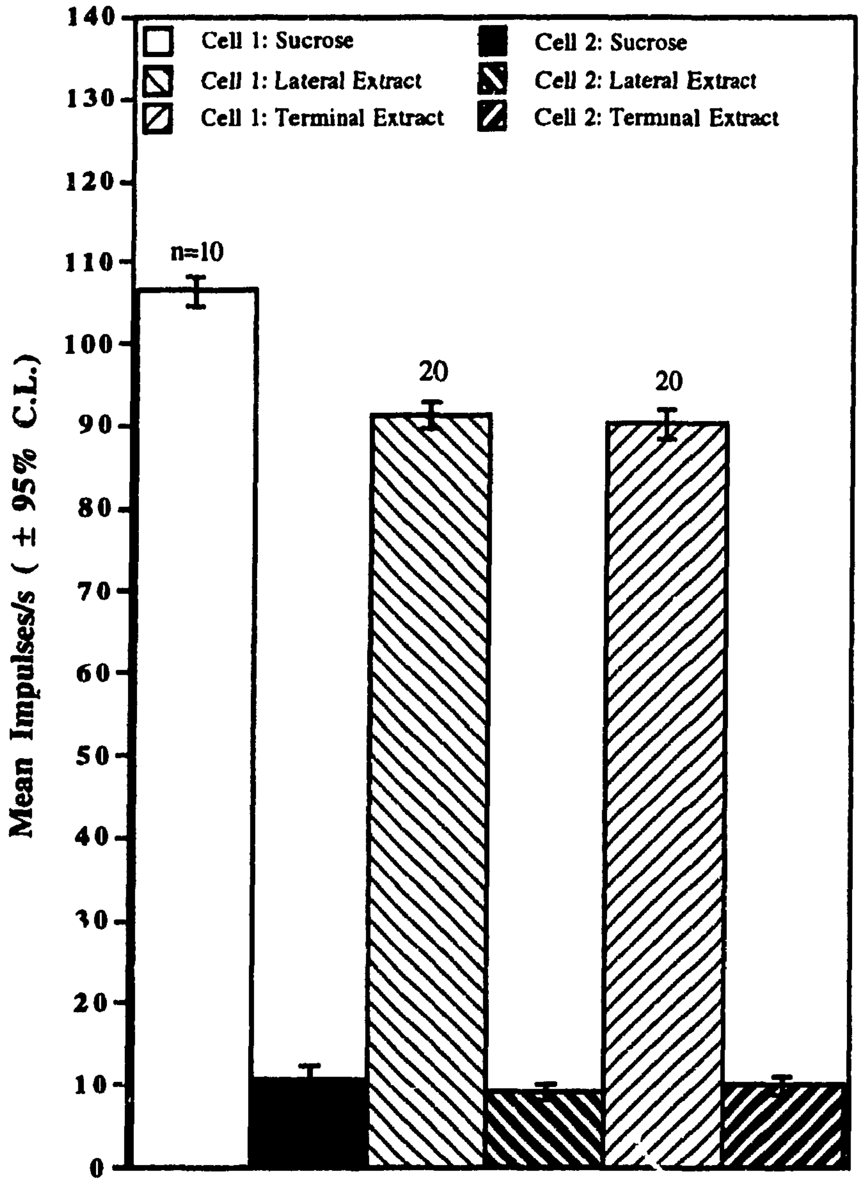

Figure 2.5. Mean impulses/s (± 95% C.L.) of Cells 1 and 2 in lateral styloconic sensilla of sixth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of young balsam fir trees.

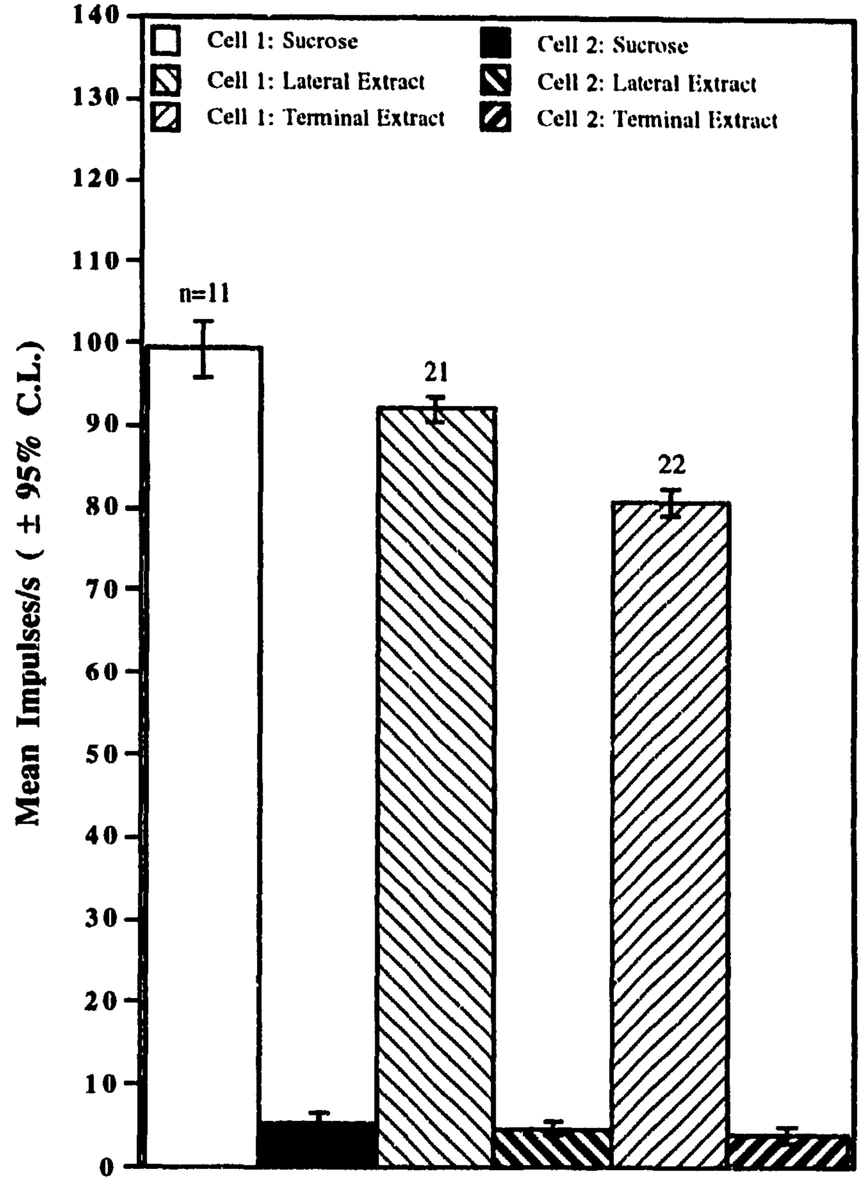

Figure 2.6. Mean impulses/s (± 95% C.L.) of Cells 1 and 2 in lateral styloconic sensilla of sixth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of old balsam fir trees.

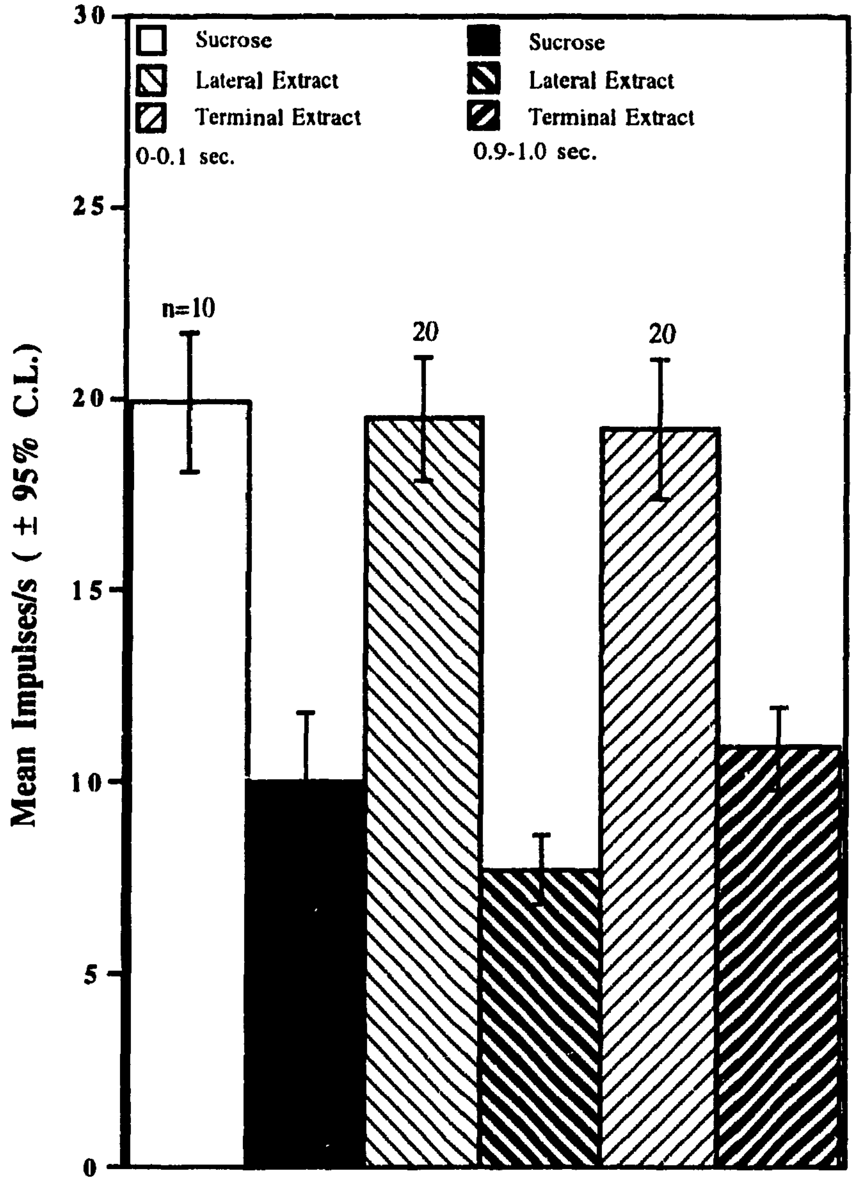

Figure 2.7. Mean impulses/s (± 95% C.L.) of Cell 1 in lateral styloconic sensilla of sixth-instar larvae in response to stimulation with 50 mM/l sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of young balsam fir trees. Data are separated into the first and last 100 ms segments of the 1 s response.

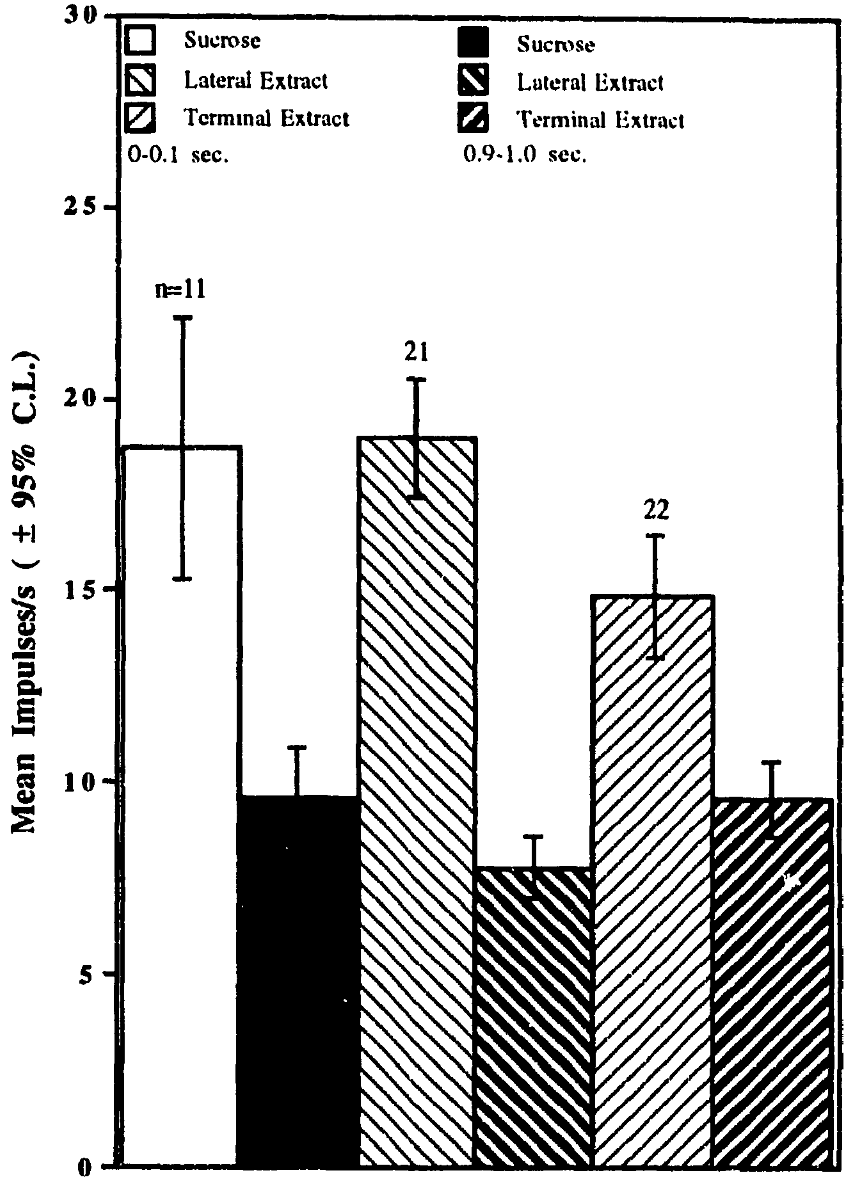

Figure 2.8. Mean impulses/s (± 95% C.L.) of Cell 1 in lateral styloconic sensilla of sixth-instar larvae in response to stimulation with sucrose in 50 mM/l KCl and with lateral and terminal shoot extracts of old balsam fir trees. Data are separated into the first and the last 100 ms segments of the 1 s response.

E. Discussion

A single neuron present in the lateral styloconic sensilla of both fourth- and sixth-instar larvae exhibited a strong response to balsam fir extracts. Results indicate that this neuron shows an age-dependent sensitivity that varies over the two different stadia of spruce budworm larvae.

The initial stimulation experienced by the fourth instars is significantly greater when feeding on terminal shoot foliage. Sixth instars receive greater stimulation when feeding on the foliage from the lateral shoots of old trees. These results correlate well with observed feeding preferences (Albert & Bauce, 1994), and indicate that these larvae have an inherent fine-tuning of their sensory system that allows them to perceive nutrient variations in their host plant.

Several other facets of feeding neurophysiology are implicit from the experimental results. For example, it is interesting to note that feeding rates (Albert & Bauce, 1994) are generally higher on foliage that is providing the least chemosensory stimulation. A possible explanation for this phenomenon is that larvae feeding on less suitable foliage are consuming at a faster rate in order to guarantee that enough will be eaten to provide necessary nutrients. If this were the case, then it could logically be assumed that a greater overall consumption would occur. However, this was not evident in the feeding tests of Albert & Bauce (1994). A second explanation is that the higher feeding rate is an attempt on the part of the larvae to increase incoming peripheral chemosensory stimulation. Spruce budworms have been observed to continuously dip their maxillae into plant saps during feeding (Albert & Parisella, 1988). The actual contact with the plant sap is brief (100 ms). A similar behaviour has been observed in locusts (Blaney & Duckett, 1975). The function of the continuous palpation could be to supply the central nervous system with constant information about food quality. Weakened chemostimulation from less suitable food could result in a compensatory increase in feeding rate.

Indications that an additional mechanism for mediating feeding behavior is present in *Choristoneura fumiferana* become evident when the results from sixth instars tested on extracts from young trees are examined. In the behavioural study by Albert & Bauce (1994), sixth instars showed a strong preference for extracts from the lateral shoots (p=0.0001). Electrophysiological results show that the stimulation elicited by the lateral shoot extracts is not significantly different from that of the terminal shoot extracts (p=0.8497). The chemosensory input is the same, yet a great deal more of the lateral shoot extracts were consumed. The existence of a feedback mechanism with positive (chemosensitivity is maintained, or upgraded), and negative (chemosensitivity is downgraded) components can be hypothesized to account for these results. When larvae are feeding on foliage that is meeting their immediate intrinsic requirements, then a positive reinforcement maintains the feeding response. This reinforcement could involve changes in ionic composition of the dendritic liquor brought about by changes in nutrient concentrations occurring in the haemolymph. This type of mechanism has been implicated in feedback loops believed to exist in other insects (Abisgold & Simpson, 1987; Schoonhoven et al., 1991; Simpson et al., 1990). Similarly, when present nutritional needs are not being met, negative control is exerted to reduce consumption. Considering that spruce budworms are oligophagic insects with a limited developmental time, the existence of a negative component in a feedback control system could be adaptive. If the larvae are not obtaining immediate benefits from feeding on a particular part of the host plant, then it profits them to move to that part of the host which delivers the required nutritional payload. Wagner et al. (1990) showed that foliar nutrients fluctuate throughout the growing season, and it has been demonstrated that these changes affect western spruce budworm larval performance (Clancy et al., 1988). Humeral factors acting to change chemoreception during the course of a meal could be advantageous in this regard, allowing the insect to monitor changes in the host constituents and react accordingly.

In summary, we postulate that there are two major components involved in establishing within-host feeding behaviour for *Choristoneura fumiferana*. First, an age-related chemosensitivity exists which predisposes fourth-instar larvae toward preferring foliage from terminal shoots of balsam fir. Since fourth instars precede the sixth, this is probably a very important predisposition. Likewise, sixth-instar larvae are more chemosensitive to foliage from lateral shoots of old trees. Secondly, a putative feedback mechanism exists that reflects the immediate nutritional status of the developing larvae, and acts to modulate behaviour during the course of feeding. Both these mechanisms work in tandem to ensure that budworm larvae find and ingest the food which their growth and development require.

CONCLUSION

The styloconic sensilla on the maxillary galea of spruce budworm larvae displayed an age-related chemosensitivity. Fourth-instar larvae are more sensitive to low sucrose concentrations than are sixth-instar larvae. This increased sensitivity might allow the younger larvae to perceive the low concentrations of sucrose that are present in the host tree at the time that budworm larvae are in their early stadia. A neuron with marked sensitivity to aqueous extracts of balsam fir foliage was found in the lateral sensilla styloconica of both larval stages. Fourth-instar larvae are more chemosensitive to terminal foliage of young and old balsam fir trees, while sixth-instar larvae are more chemosensitive to lateral shoot foliage from old trees. This age- dependent chemosensitivity could be adaptive allowing budworm larvae to locate that portion of the host tree which will provide them with the most suitable nutrition. A proline-sensitive cell was found in the medial styloconic sensilla of both larval age groups. The wide monophasic nature of this neuron indicates that it probably represents the water cell. Fourth-instar larvae have significantly greater sensitivity towards proline than do sixth-instar larvae. Receptors activated by the feeding deterrent, valine, were found on a neuron in the lateral sensilla styloconica of sixth-instar larvae. The digital signature of this neuron suggests that it is one of the salt-sensitive cells present in the lateral styloconica. The firing rate of this receptor neuron when stimulated with valine implies that the deterrent effect is elicited by an elevated salt-cell response.

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