TOWSON UNIVERSITY
COLLEGE OF GRADUATE STUDIES AND RESEARCH

ELECTROPHYSIOLOGICAL RESPONSES OF TASTE-RECEPTOR CELLS TO VARIOUS FEEDING STIMULANTS, DETERRENTS, AND THEIR INTERACTIONS IN GYPSY MOTH LARVAE, *LYMANTRIA DISPAR* (L.)

by

Timothy Lee Martin

A thesis

presented to the faculty of

Towson University

in partial fulfillment

of the requirements for the degree

Master of Science

May, 2011

Towson University
Towson, Maryland 21252

TOWSON UNIVERSITY
COLLEGE OF GRADUATE STUDIES AND RESEARCH
THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Timothy Martin

Entitled Electrophysiological Responses of Taste-Receptor Cells to Various Feeding Stimulants, Deterrents, and Their Interactions in Gypsy Moth Larvae, *Lymantria dispar* (L.)

has been approved by the thesis committee as satisfactorily completing the thesis requirements for the degree Master of Science.

Chair, Thesis Committee	Date 5/12/2011
Committee Member	Date 5/12/2011
Committee Member	Date 5/12/2011
Committee Member	Date
Committee Member	Date
Committee Member	Date
Associate Dean, College of Graduate Studies and Research	Date 19 May 2011

ACKKNOWLEDGEMENTS

This work was supported by NIH grants 1R15DC007609-01 and 3R15DC007609-01S1 to V.D.S. and grants from FCSM and TU to T.M. I gratefully thank Dr. Vonnie Shields for her generous support, leadership, patience, and for providing numerous opportunities through the years. I also gratefully thank Dr. James Saunders for his continuous encouragement, guidance, and for also providing many invaluable opportunities. I appreciatively acknowledge Dr. Joseph Dickens, for critically reviewing the manuscript, Jeff Klupt, Bob Kuta, Ming Chen, Jillian Sanford, and Katelyn Beattie for technical assistance. Many thanks to Howard Kaplon for help with the statistical analyses and Robert Bennett (USDA, Beltsville, MD) for kindly supplying us with egg masses. Versions of both chapters have been submitted to the Journal of Insect Physiology for publication.

ABSTRACT

ELECTROPHYSIOLOGICAL RESPONSES OF TASTE-RECEPTOR CELLS TO VARIOUS FEEDING STIMULANTS, DETERRENTS, AND THEIR INTERACTIONS IN GYPSY MOTH LARVAE, *LYMANTRIA DISPAR* (L.)

Timothy Lee Martin

Gypsy moth larvae are highly polyphagous feeders. They possess taste sensory organs, the medial and lateral galeal styloconic sensilla, which play an important role in host-plant selection through the detection of phytochemicals, such as alkaloids. The styloconic sensilla each house four taste receptor cells, including a sugar, salt, deterrent, and inositol cell. Using a single cell electrophysiological tip-recording method, my aim was to characterize the temporal firing patterns and sensitivities of the receptor cells within each sensillum when exposed to a selected phytochemicals. These results revealed that one or more cells responded to selected alkaloids, sugars, sugar alcohols, and salt. The deterrent cell exhibited a robust temporal firing pattern and displayed varying sensitivity to alkaloid stimulation. I also examined the effects of mixture interactions of these phytochemicals on food palatability. This study offers insights into the role of phytochemicals, especially alkaloids, in the taste physiology of this larval insect.

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CHAPTER ONE

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF TASTE RECEPTOR CELLS ON THE MOUTHPARTS OF GYPSY MOTH LARVAE, *LYMANTRIA DISPAR* (L.)

A version of this chapter has been submitted for publication to the Journal of Insect Physiology

1. Introduction

Caterpillars are ideal models for both neural and behavioral studies of feeding. Lepidoptera possess gustatory sensilla on the maxillae, which play an important role in host-plant selection. Three taste cells occur in each epipharyngeal sensillum, three to five taste cells in each of five sensilla at the tip of each maxillary palp, and four cells in each medial and lateral styloloconic sensillum (Schoonhoven and Dethier 1966). Food plant recognition is thought to be primarily mediated by the input from each bilateral pair of the styloconic sensilla (Schoonhoven and Dethier, 1966; de Boer et al., 1977; de Boer and Hanson, 1987), which play a major role in feeding (de Boer and Hanson, 1987; de Boer, 1991). Interestingly, Waldbauer and Fraenkel (1961) and de Boer and Hanson (1987) showed that ablating the styloconic sensilla of the tobacco hornworm, *Manduca sexta* resulted in widening of its host range and permitted *M. sexta* larvae to feed on plants that are not normally members of the host range of this insect. Frazier (1986) reported that rejection of plants was thought to be mediated by either the medial or lateral styloconic sensillum, but both were involved in the rejection behavior to different substances.

Insect gustatory receptors transduce the quality and quantity of complex plant chemistry into a neural code of action potentials. Complex stimuli e.g., plant saps often evoke action potentials in several receptor cells innervating one or more sensilla (Schoonhoven et al., 1998). The absolute frequency and temporal distribution of action potentials in a spike train contain information about the stimulus. This information is transmitted by axonal processes to the subesophageal ganglion without synapses (Kent and Hildebrand, 1987). Unraveling the sensory code can be achieved by stimulating

specific sensilla and electrophysiologically quantifying the trains of action potentials (input), as well as quantifying the behavior (output) on the basis of how much food is consumed (Bernays and Chapman, 1994).In this study, electrophysiological responses of gypsy moth larvae to four alkaloids and two sugars, which are known phagodeterrent and phagostimulatory compounds, respectively, were examined.

Gypsy moth larvae, *Lymantria dispar* (L.) are major pest defoliators in much of the United States and destroy millions of hectares of trees annually. The larvae are highly polyphagous and display a wide host plant preference, feeding on the foliage of hundreds of plants including forest, fruit, shade, and ornamental trees (Mosher, 1915; Shields et al., 2003), but favor leaves of deciduous hardwood trees, such as oak, maple, and sweet gum (Mosher, 1915; Liebhold et al., 1995; Shields et al., 2003). This preference is thought to be due to the predominance of phagostimulatory compounds, such as sugars, and absence of phagodeterrent compounds, such as alkaloids (Barbosa and Krischik, 1987; Miller and Hanson, 1989; Barbosa et al., 1990; Shields et al., 2003). Previous feeding behavioral bioassays using gypsy moth larvae found nine alkaloids that act as feeding deterrents (Shields et al., 2006; Shields et al., 2008). In addition, alkaloids can behave as growth inhibitors and/or toxins (Saunders et al., 1992). Unfortunately due to the experimental design of many feeding behavioral bioassays, it has been impossible to determine among several mechanisms, such as, gustation, olfaction, somatosensory, and viscerosensory (e.g., habituation, pre- and post-ingestive feedback mechanisms, and/or toxicity)(e.g., Glendinning et al., 2001) and their contribution to the underlying neural mechanisms of alkaloid feeding deterrency. Here we investigate the role of contact chemoreceptors housed within each of the lateral and medial styloconic sensilla

of gypsy moth larvae in the detection of phagodeterrent secondary plant compounds (i.e., alkaloids: caffeine, aristolochic acid, nicotine, and strychnine), as well as phagostimulantory sugars (i.e., sucrose and inositol).

2. Materials and Methods

2.1 Insects

L. dispar (New Jersey strain) were obtained as eggs from the USDA-APHIS, Otis Air National Guard Base (Falmouth, Massachusetts, USA). The colony was reared on a high wheat germ artificial diet (Bio-Serv, Frenchtown, New Jersey; MP Biomedicals, Solon, Ohio, USA) at 25 ± 2º, 60% relative humidity, and a 12 h light:12 h dark photoperiod regimen (Shields et al., 2003; Shields et al., 2006). Fifth instar larvae that were 12-18 h postmolt, randomly selected, and 24 h food deprived were used for all experiments. Late instar larvae were chosen for experiments, since they typically perform most of the feeding in the field (Leonard, 1974, 1984; Lance and Barbosa, 1982).

2.2 Electrophysiology

The sensory responses from taste receptor cells within the lateral and medial styloconic sensilla were recorded using the tip-recording method (after Hodgson et al., 1955). The head of the caterpillar was excised and mounted on a saline-filled (see below) glass micropipette containing a silver wire (indifferent ground electrode). Placing the electrode into the head in this fashion typically caused the separation and eversion of each styloconic sensillum for easier access during recording. A minimal amount of dental wax was used to seal the cut end of the head to the glass micropipette. A similar

micropipette (tip diameter, 10-30 um) containing a stimulating solution (stimulating electrode; see below) was placed over the tip of the styloconic sensilllum. The electrode was connected to the input of the preamplifier with a gain of 10x (Syntech Taste Probe, Hilversum, The Netherlands). At least three minutes were allowed between stimulations to allow for recovery of the receptor cells. For each experiment, the caterpillars were naïve to the compounds prior to testing and were selected at random, as was selection of right or left sensillum. The caterpillars were used more only once, with the exception of those used for dose-response experiments. The order in which a stimulating solutions was presented to the caterpillar was randomized to prevent any bias, with the exception of those used for dose-response experiments (see below). All recordings were made between 0900 and 1700 hours during the light segment of the photoperiod.

Electrical activity from individual styloconic sensilla were amplified and conditioned (bandpass filter set at 100-1200 Hz) prior to being digitized by a 16-bit analog-to-digital interface (IDAC-4, Syntech). Spike activity was analyzed off-line using Autospike (Syntech, version 3.8). For each electrophysiological recording, we stimulated a sensillum for total of 3 sec. Action potentials generated 50 ms after contact with the sensillum were quantified using the "spike-counter" tool in the Autospike software, which allowed the number of spikes to be placed in 100 ms bins.

2.3 Taste stimuli

For all experiments, stimulus compounds were dissolved in 30 mM KCl (Fisher Scientific) in distilled water to improve the signal-to-noise ratio. The latter solution, alone, was also used when filling the indifferent electrodes. This concentration of KCl

did not stimulate the deterrent cell. To facilitate the dilution of the alkaloid compounds, 10% ethanol was added to the electrolyte solution. Ethanol, at this concentration, had no discernible effect on the activity of either of the salt receptor cells responding to KCl (two-tailed paired t-tests KCl versus KCl + ethanol, lateral sensillum, n = 10: small cell, P = 0.3605; large cell, P = 0.2031; medial sensillum, n = 10: small cell, P= 0.2933; large cell, P = 0.3260). All solutions were tested at room temperature (25 ± 2º,C).

For dose-response experiments, stimulating solutions included various concentrations of each of the four alkaloids tested [nicotine ((-)-nicotine), caffeine, aristolochic acid (sodium salt), and strychnine (hemisulfate) (MP Biomedicals, Solon, OH]. These concentrations were similar to results obtained previously from feeding experiments and correlated well with what is likely to occur in plants (Shields et al., 2006; Shields et al., 2008). Sucrose and inositol [sucrose, J.T. Baker; inositol (myo-inositol) Sigma] were tested at only 100 mM, as these principal sugars often occur at higher concentrations in plants. Dose response curves for each alkaloid were obtained by presenting a series of concentrations starting from lowest to highest (0.00001-10 mM). To ensure a reproducible response for a particular concentration being tested, 1-2 replications of each solution were made. Periodic stimulation with 30 mM KCl, alone, served as a control. For each electrophysiological recording, the number of action potentials generated 0.05 s-1.05 s after the contact artifact were quantified in 0.1s increments.

2.4 Data analysis

The limitations of using the tip-recording technique are that it is possible to stimulate a single sensillum, but not the specific taste receptor cells within it. It was clear that the action potentials generated by the sugar-sensitive cell, in response to sucrose, inositol-sensitive cell, in response to inositol, and deterrent-sensitive cell, in response to the alkaloids, fired in a regular temporal pattern and that the interspike intervals gradually decreased over time in an orderly fashion. By visualizing the shape and temporal firing pattern of the recording and using the "Amplitude Histogram View" feature of Autospike, it was possible to identify and assign spike amplitudes to the taste receptor cells (see also Results section).

For temporal and dose response experiments, a randomized complete block design was run using General Linear Model Analysis of Variance (GLM ANOVA) ($\alpha = 0.05$), to test the null hypothesis that there was no difference in the response among all concentrations of each alkaloid compared with the control. Each sensillum (i.e., individual) was the blocking variable, the dose was the fixed variable, and the firing frequency was the response variable. A Tukey-Kramer multiple comparison test was used to compare responses of the deterrent-sensitive cell to various concentrations of each alkaloid.

For feeding bioassays, a Kruskal Wallis one-way ANOVA and Dunn's multiple comparison test was run ($\alpha = 0.05$).

3. Results

3.1 Lateral and medial styloconic sensilla

Gypsy moth larvae bear two pairs of styloconic sensilla located on the maxillary galea (Figs. 1A, B). Each comprises a small cone, with a terminal pore, which stand erect on a style. (Figs. 1B, C). Each sensillum is innervated by five bipolar neurons. Four of the dendrites terminate near the tip of the sensillum and bear ultrastructural features consistent with contact chemonsensilla, while the fifth terminates near the base of peg and bears features consistent with mechanosensilla (Fig. 1D).

3.2 Characterization of taste receptor cells in the medial styloconic sensillum

3.2.1 Characterization of the KCl-sensitive cell

Two spikes of differing amplitudes likely representing the output of two chemosensory cells respond to 30 mM KCl (one small KCl-sensitive cell and one large KCl-sensitive cell) (Fig. 2A). Both of these cells spiked infrequently, intermittently, and out of phase, as compared with the inositol- or deterrent-sensitive cells housed within the same sensillum (compare Fig. 2A with 2C-G). The large amplitude KCl-sensitive cell fired at a rate of 6.84 impulses/s ± 0.76, whereas the small amplitude KCl-sensitive cell, at a rate of 11.90 ± 0.81 impulses/s (n = 68) (total impulses, 18.74 ± 1.30).

3.2.2 Characterization of the deterrent-sensitive cell

A single cell responded to the four alkaloids tested (i.e., strychnine, nicotine, aristolochic acid and caffeine) (Figs. 2D-G). This cell was termed the "deterrent-sensitive" cell, since previous feeding studies demonstrated that gypsy moth larvae were deterred by these alkaloids (Shields et al., 2006; Shields et al., 2008). A mixture of all four alkaloids (i.e., 0.25 mM each of caffeine, nicotine, aristolochic acid, and strychnine)

was applied to the medial styloconic sensillum to see how many taste-receptor cells were stimulated. If all four alkaloids individually stimulated the same cell within this sensillum (i.e., deterrent-sensitive cell), then the mixture of these alkaloids would activate only a single cell. This stimulating solution elicited the regular and rapid firing pattern of only a single cell bearing a large amplitude spike (Fig. 2H). In addition, the infrequent and intermittent firing of a smaller amplitude KCl-sensitive cell also occurred. There was no deterrent-sensitive cell in the lateral sensillum of gypsy moth that responded to any of the alkaloids tested (Figs. 2 M-P).

3.2.2.1 Deterrent-sensitive cell = KCl-sensitive cell

It was noted that increasing the concentration of the KCl solution from 30 mM (Fig. 3A) to 50mM (Fig. 3B) resulted in the increased firing activity of a single cell bearing the same large, regular, and rapid spike amplitude as the deterrent-sensitive cell.

The firing frequency of the large amplitude KCl-sensitive cell using 30 mM KCl significantly differed to that at 50 mM KCl ($P = 0.002$; $n = 8$ sensilla; one-tailed paired t-test), but did not significantly differ to that of the small amplitude KCl-sensitive cell at either concentration of KCl ($P = 0.224$; $n = 8$ sensilla; one-tailed paired t-test). It was predicted that if the higher concentration salt solution (i.e., 50 mM) and the alkaloids stimulated the same taste receptor cell, then the binary mixture of these two compounds should activate only a single taste receptor cell. This prediction was evaluated by stimulating the sensillum with a binary mixture of 50 mM KCl with a single alkaloid (e.g., 1 mM strychnine) and it was found that this mixture elicited the regular and rapid firing of only a single large amplitude cell, namely the deterrent-sensitive cell (Fig. 3C).

The single small amplitude KCl-sensitive cell also fired intermittently to the 50 mM KCl. Of the four alkaloids tested, strychnine elicited the strongest and most robust response at the concentration tested.

The feeding effect of increasing KCl concentration was tested in a dose dependent manner on gypsy moth larvae by incorporating this inorganic salt into an artificial diet at concentrations ranging from 0.1 mM to 500 mM ($P = 0.026$; $n = 8$-10 larvae) (Fig. 3D). A significant decrease in consumption (deterrency or aversive response) was observed at 500 mM (0.54% relative mean consumption). At lower doses, consumption remained at or near 100% (0.1 mM, 94.1%; 30 mM, 94.6%, 100 mM, 92.2%) relative to the control.

3.2.2.2 Temporal response characteristics of the deterrent-sensitive cell

The instantaneous firing rate (total impulses/100 ms) of the deterrent-sensitive cell to two concentrations, each of four alkaloids (i.e., strychnine, caffeine, aristolochic acid, and nicotine) across 3 sec of stimulation was quantified (Figs. 4A-D). Representative low (i.e., .001 mM) and high (i.e., 1 mM) concentrations were tested. and were compared to that of the solvent, (30 mM KCl), alone. All four alkaloids tested (i.e., strychnine, caffeine, aristolochic acid, and nicotine), elicited a phasic-tonic response from the deterrent-sensitive cell. At 1 mM, strychnine (167.1 ± 9.38 impulses/s), caffeine (98.0 ± 7.08 impulses/s), and nicotine (96.8 ± 14.23 impulses/s) elicited an initial rapid high firing frequency which decreased over time (Figs. 4A-C). It took approximately 100 (caffeine and nicotine) to 200 ms (strychnine) for the deterrent-sensitive cell to reach peak activity (Figs. 4A, B, D), after which it decreased gradually over the next 700 ms, 500 ms, and 1700 ms, respectively (Fig. 4). This was followed by a tonic pattern of

firing (900 ms, caffeine; 700 ms, nicotine; 2000 ms, strychnine) (Fig. 4). Aristolochic acid, on the other hand, elicited a firing frequency (96.3 ± 6.04 impulses/s), which initially accelerated before decelerating (Fig. 4C). It took approximately twice as long (400 ms), as compared with the other alkaloids, for the cell to reach peak activity, after which it decreased gradually over the remaining 1000 ms (Fig. 4C) followed by a tonic pattern of firing (1500 ms). As concentration decreased for all of the alkaloids, the pattern of firing gradually became linear (Fig. 4). At 0.001 mM, the deterrent cell fired at a much lower rate (43.9 ± 3.88, strychnine; 41.3 ± 5.01, caffeine; 48.1 ± 3.66, aristolochic acid, and 52.9 ± 3.70 impulses/s, nicotine) and only a tonic pattern of firing was observed (Fig. 4).

3.2.3 Characterization of the inositol-sensitive cell

A single spike height was elicited by the sugar alcohol, inositol (inositol-sensitive cell) (Fig. 2C). The spike amplitude from this cell was larger than that of the smallest KCl-sensitive cell, but smaller than that of the deterrent-sensitive cell (Fig. 2I). This cell fired regularly and rapidly in response to 100 mM inositol. It was predicted that if inositol and the alkaloid compounds stimulated different taste receptor cells then the binary mixture of these two compounds should activate two taste receptor cells. This prediction was tested by stimulating the sensillum with a binary mixture of 100 mM inositol and one of the alkaloids (1 mM caffeine) and found that this mixture elicited the regular and rapid firing of two taste receptor cells of differing spike amplitudes; a large amplitude cell and a much smaller amplitude cell.

3.2.3.1 Temporal response characteristics of the inositol-sensitive cell

The instantaneous firing rate of the inositol-sensitive cell to 100 mM inositol, was 94.1 ± 4.45 impulses/s (Fig. 5). A phasic-tonic pattern of firing was noted; the tonic pattern of firing began after approximately 1700 ms. Inositol did not elicit a response in the lateral sensillum (Fig. 2L).

3.3 Dose-Response Characteristics of Deterrent-Sensitive Cell to Various Alkaloids

Dose response curves were constructed for responses from the deterrent-sensitive neuron elicited by serial dilutions of the alkaloids: aristolochic acid, strychnine, nicotine, and caffeine (Figs. 6A-D). In all cases, each of the alkaloids was dissolved in the solvent, 30 mM KCl. The deterrent-cell was equally sensitive to each of the alkaloids with a lowest detectable concentration of 0.001 mM for (Figs. 6A-D). Responses of the deterrent-sensitive cell plateaued at 1 mM for strychnine, caffeine, and nicotine (Figs. 6A, B, D), and 0.1 mM, for aristolochic acid (Fig. 6C). The dynamic range (i.e., the steepest portion of the dose-response curve) for aristolochic acid (three log units) was narrower compared with that of the other three alkaloids (four log units).

3.4 Lateral Styloconic Sensillum

3.4.1 Characterization of the KCl-sensitive cell

Two taste receptor cells of differing amplitudes fired in response to 30 mM KCl . As in the medial sensillum, both of these cells fired intermittently, infrequently, and out of phase as compared with that of the sucrose-sensitive cell housed in same sensillum. The large amplitude cell fired at a rate of 8.39 ± 1.03 impulses/s, whereas the small

amplitude cell, at a rate of 11.47 ± 0.84 impulses/s (n = 51) (total impulses, 19.86 ± 1.56) (Fig. 2J).

3.4.2 Characterization of sucrose-sensitive cell

Only a single cell fired in response to sugar and the spike amplitude from this cell was intermedite to the height of KCl-sensitive taste cell and the deterrent-sensitive taste cell (Fig. 2K). This cell fired regularly and rapidly in response to 100 mM sucrose.

3.4.2.1 Temporal response characteristics

At the 100 mM concentration of sucrose, the sucrose-sensitive cell had a high firing rate of 178.3 ± 5.49 impulses/s and, similar to the other taste cells described, above, had a phasic-tonic pattern of firing (Fig. 7). The phasic pattern of firing lasted much longer in the sucrose-sensitive cell, as compared with that of the inositol-sensitive cell, as a tonic pattern of firing did not begin until approximately 1600 ms (Fig. 7). Sucrose did not elicit a response in the medial sensillum (Fig. 2B).

4. Discussion

Lepidopteran larvae, such as the gypsy moth, possess chemosensory sensilla, on the antennae, galeae, maxillary palps, and epipharynx (Schoonhoven and Dethier, 1966). Sensilla located on the maxillae play important roles in host-plant selection (Ma, 1972). Food plant recognition is thought to be primarily attributed to the activity of the styloconic sensilla (Schoonhoven, 1972). The styloconic sensilla are in constant contact with plant sap liberated during feeding. As plant material enters the terminal pore of these sensilla, stimulating phytochemicals enter, diffuse, and interact with the underlying

gustatory neurons. In previous studies, Shields (1996; 2009) found that the galeal styloconic sensilla are each innervated by four neurons and exhibit ultrastructural features consistent with contact chemoreceptors; a fifth neuron exhibits features consistent with mechanoreceptors. The distal dendrites within the styloconic sensilla of *L. dispar* extend into the lumen of the cone of each styloconic sensillum and terminate at various levels below the pore (Shields, 2009).

The primary function of the gustatory receptor system in immature insects is to enable them to recognize food and to signal the presence of nutrients and harmful substances (Bernays and Chapman, 2001). Schoonhoven and Jermy (1977) indicated the relevance of investigating both the sensory physiology and behavior of an insect to determine if the information being processed by taste receptor cells elicits feeding acceptance or deterrence. To this end, Simmonds and Blaney (1990) showed that plant acceptability is determined by the activity levels in the four taste cells in each styloconic sensillum. They avoided such labels, such as 'salt', 'sugar', 'amino acid', and 'deterrent' cell. Bernays and Chapman (2001) also suggested that rather than designate gustatory receptor cells according to the type of compound that stimulates them (i.e., sugar cell, amino acid cell, salt cell), a better approach would be to suggest that these cells be designated according to their function, e.g., compounds signaling nutrient presence (i.e., phagostimulatory cell) versus deterrence (i.e., deterrent cell).

4.1 Responses to deterrent compounds

Shields et al. (2006, 2008) examined the feeding behavioral response of gypsy moth larvae to several alkaloids, including the four tested in this study (i.e., aristolochic

acid, caffeine, nicotine, strychnine). In all cases, when these compounds were applied exogenously to highly favored red oak leaves, feeding was deterred. The same alkaloids have feeding deterrent effects on other lepidopteran larvae, for example: caffeine, in *Pieris brassicae* (Schoonhoven and Jermy, 1977), *M. sexta* (Wrubel and Bernays, 1990; Glendinning, 1996; Glendinning et al., 1999; Glendinning et al., 2001) and *Bombyx mori* (Ishikawa, 1966; Asaoka, 2000); strychnine and nicotine, in *B. mori* (Ishikawa, 1966; Asaoka, 2000), *P. brassicae* (Ma, 1969; 1972), *M. sexta* (Glendinning, 1996) and *Helicoverpa armigera* (Zhou et al., 2010) (strychnine only); and aristolochic acid, in *L. dispar* (Miller and Feeny, 1983); *M. sexta* (Glendinning et al., 1999; Glendinning et al., 2001). Here these four alkaloids stimulated the same deterrent-sensitive cell of the medial styloconic sensillum thus supporting the concept that this cell may be responsible for mediating feeding deterrence.

The temporal firing patterns of caffeine, nicotine, and strychnine were similar to each other, but qualitatively different from those elicited by aristolochic acid. While the deterrent sensitive cell displayed a phasic-tonic firing pattern (initial response began robustly and then decreased with time) to caffeine, nicotine, and strychnine, this was not the case with aristolochic acid. The initial response of the latter alkaloid took several hundred milliseconds to reach its maximal firing rate, upon which the firing rate decreased. Similar temporal firing patterns,were observed in the deterrent-sensitive cell in *M. sexta* with respect to caffeine and aristolochic acid (Glendinning and Hills, 1997). Glendinning and Hills (1997) postulated that while these alkaloids stimulated the same deterrent-sensitive cell, this cell responded through at least two different excitatory transducation pathways: one responding to caffeine, and the other to aristolochic acid.

The behavioral deterrency threshold concentration for caffeine and nicotine was 0.1 mM, whereas that for aristolochic acid and strychnine was 1 mM (Shields et al., 2008). Electrophysiological observations in this study suggest that the lowest detectable concentration of the deterrent-sensitive cell was two logarithmic steps lower for caffeine and nicotine and three logarithmic steps lower for aristolochic acid and strychnine (i.e., 0.001 mM for all the alkaloids tested). This similar electrophysiological detectable concentration for these alkaloids may imply that the deterrent-sensitive cell housed in the medial styloconic sensillum of gypsy moth is a general deterrent-sensitive cell, which responds to alkaloids with a high sensitivity. This statement can be validated by the fact that gypsy moth larvae avoid foliage containing alkaloids (Barbosa and Krischik, 1987; Miller and Hanson, 1989; Barbosa et al., 1990; Shields et al., 2003). A future direction of this study will be to test a broader range of secondary plant compounds, other than alkaloids, to validate if this cell is truly a general deterrent-sensitive cell.

In interpreting the behavioral ED50 values (i.e., approximate concentration that reduced feeding by 50%), Shields et al. (2008) suggested that aristolochic acid and strychnine were among the most potent of the nine alkaloids tested, followed by caffeine and nicotine. Electrophysiological evidence in this study, however, showed that strychnine elicited the highest firing frequency (approximately 1.7 times higher that of caffeine, nicotine, and aristolochic acid), possibly signaling carries a higher potency relative to the other alkaloids. While the firing frequency to aristolochic acid appeared to be lower than strychnine, and similar to that of caffeine and nicotine, electrophysiologically speaking, this may have been attributed to the fact that alkaloids bearing a methylenedioxyphenyl group, such as aristolochic acid, enhance insecticide

chemical toxicity by inhibiting mixed-function oxidase enzymes (MFOs) (Hodgson et al., 1995). MFOs are thought to be able to catabolize and anabolize compounds such as plant toxins (Scott, 1999). It is not clear, however, if aristolochic acid can be detoxified by MFO enzymes (Neal, 1989). If the latter were true in gypsy moth larvae, this could prevent the insect from being able to detoxify this alkaloid and could explain why this alkaloid was ranked as the most potent feeding deterrent (of the four alkaloids tested in this study), possibly attributed to post ingestive effects, since feeding behavioral bioassays span a longer period of time to carry out.

4.2 Responses to inorganic salt (i.e., KCl)

The lateral and the medial styloconic sensilla each house two taste receptor cells which responded to KCl (below 50 mM) (one small KCl-sensitive cell and one large KCl-sensitive cell). Both of these cells fired infrequently, intermittently, and out of phase, as compared with the the inositol-, deterrent-, and sucrose-sensitive cells housed within the same sensillum. In the medial sensillum, concentrations of 50 mM and higher KCl elicited the firing of the deterrent-sensitive cell. This was demonstrated by stimulating the sensillum with a binary mixture of 50 mM KCl with a single alkaloid, strychnine(1 mM) elicited regular, rapid, and robust firing of only the single amplitude cell (i.e., the deterrent-sensitive cell). It is therefore reasonable to conclude that the large amplitude KCl-sensitive cell and the deterrent-sensitive cell are one in the same cell. A similar result, in three caterpillar species, was reported by Dethier (1973), Peterson et al. (1993), and Bernays and Chapman (2001).

Dethier (1977) stated that the ability to perceive inorganic salts is likely to be a protective function in order to avoid ingestion of excess amounts. The effect of increasing KCl concentration on feeding by gypsy moth larvae was tested by incorporating measured amounts of KCL into an artificial diet. A dramatic decrease in feeding at occured 500 mM KCL, an effect not observed with lower concentrations. Interestingly, Dethier (1968) and Hiroi et al. (2004), in flies, and Ma (1972) and Blom (1978), in lepidopteran larvae, found an initial stimulatory or appetitive response at low concentrations (i.e., 5-10 mM) of KCl, followed by an aversive response at high concentrations (> 500 mM). We suggest that KCl at these high concentrations is a behavioral deterrent and the large amplitude KCl-sensitive cell is responsible for mediating this response.

4.3 Responses to inositol and sucrose

The gustatory system of *L. dispar* responds robustly to two carbohydrates (sucrose and inositol). *L. dispar* larvae bear a single taste receptor cell in the medial styloconic sensillum that responds to the sugar alcohol, inositol, and one in the lateral sensillum that responds to the sugar, sucrose. Previous behavioral studies on gypsy moth larvae by Shields et al. (2006) showed that sucrose acted as a larval feeding stimulant. Sugars (e.g., sucrose) and sugar alcohols (e.g., inositol) act as potent phagostimulants in many insects (e.g., Yamamoto and Fraenkel, 1960; Städler and Hanson, 1978; Dethier, 1976; Bernays, 1985; Bernays and Chapman, 1994; Glendinning et al., 2000; Bernays and Chapman, 2001; Schoonhoven and van Loon, 2002). Although some insects, such as dipterans, have taste neurons that respond to both sugars and sugar alcohols,

lepidopterans have separate taste neurons for each (Dethier, 1976; Schoonhoven and van Loon, 2002). Interestingly, Glendinning (2008) points out that most insects bear taste cells that respond selectively to saccharides (e.g., glucose, sucrose, fructose, and trehalose) and/or sugar alcohols (e.g., sorbitol, mannitol, and myo-inositol). In some species, taste receptor cells sensitive to sucrose respond exclusively to sucrose, glucose, or fructose (discussed in Schoonhoven and van Loon, 2002). Interestingly, compounds, such as saccharin, sodium cyclamate, and thaumatin, while perceived to humans as sweet, do not elicit responses from to the sugar sensitive cells in caterpillars (Schoonhoven, 1974; Schoonhoven and van Loon, 2002). Sucrose-sensitive taste cells in caterpillars typically bear a threshold sensitivity of 0.1-1 mM and reach saturation at about 100 times higher concentrations (Chapman, 2003). Known threshold levels for inositol-sensitive cells in lepidopteran larvae typically occur at 0.1 mM, similar to that of sucrose (Ishikawa, 1967; den Otter, 1992; Bernays et al., 1998). Sucrose and inositol levels in plants occur > 1 mM and 0.5-10 mM, respectively (Chapman, 2003; Morré et al., 1990; Nelson and Bernays, 1998), corresponding to the receptive range of the receptor cells. Other lepidopteran larvae that have exhibited a response to sucrose in the lateral styloconic sensillum include: *B. mori* (Ishikawa, 1963; Asaoka, 2000); *P. brassicae* (Ma, 1972), *Choristoneura fumiferana* (Albert and Parisella, 1988), *Spodoptera exempta*, *Maruca testulalis*, *Eldana saccharina*, and *Chilo partellus* (den Otter, 1992), *Heliothis virescens*, *Heliothis subflexa* (Bernays et al., 2000), and *M. sexta* (Schoonhoven, 1972). Other Lepidoptera in which responses were elicited by inositol only in the medial styloconic sensillum include: *Cossus cossus*, *Leucoma salicis*, *Episema caeruleocephala* (Schoonhoven, 1972), *Dendrolimus pini* (Ma, 1972), *Papilio troilus*, *Estigmene acrea*,

Helicoverpa zea (Dethier, 1973), *H. virescens*, *H. subflexa* (Bernays et al., 2000), *Mamestra configurata* (Shields and Mitchell, 1995), and *S. exempta* (Ma, 1977).

To date, there are only sparse accounts of the sensory responses of taste receptor cells present in one or both styloconic sensilla of gypsy moth larvae to various plant phytochemicals. Dethier and Kuch (1971) and Schoonhoven (1972) performed initial surveys of the responses of a number of lepidopteran larvae, including the gypsy moth .Pertaining to the results of this study, these included sucrose, in the lateral sensillum (Dethier and Kuch, 1971; Schoonhoven, 1972), inositol, in both sensilla (Dethier and Kuch, 1971), the presence of salt (NaCl) or "S" cells in both sensilla (Dethier and Kuch, 1971; Schoonhoven, 1972), and a deterrent or "D" cell in the medial sensillum (Schoonhoven, 1972). The results of this study agree with those reported by these authors, with the exception that inositol did not elicit a sensory response in the lateral sensillum. It is also unclear from as to the nature of the inorganic salt or deterrent compound(s) that were used to stimulate the "SALT" and "D" cells, respectively (Schoonhoven, 1972), so a clear comparison with results of our current study is not possible.

The results of this study may be useful in the design of appropriate strategies for crop protection from insect pests, such as gypsy moth larvae. While the present study focused on single-component stimuli, a future study addresses taste-mixture interactions, which will, no doubt, lead to a better understanding of insect-plant interactions, since the majority of the foods that gypsy moth larvae would encounter in the wild would contain a complex mixture of taste stimuli.

Figure 1.

Scanning (SEM) and transmission electron micrographs (TEM) of *L. dispar*. (A) SEM of the ventral mouthparts. The lateral (black arrow) and medial (white arrow) styloconic sensilla are indicated. (B) SEM showing higher magnification of the lateral (l) and medial (m) styloconic sensilla. The white arrows denote the location of the cones. (C) SEM showing higher magnification of the cone and terminal pore of a medial styloconic sensillum. The inset shows a higher magnification of the terminal pore (white arrow). (D) TEM showing four taste and one mechanosensory (asterisk) distal dendrites innervating a styloconic sensillum.

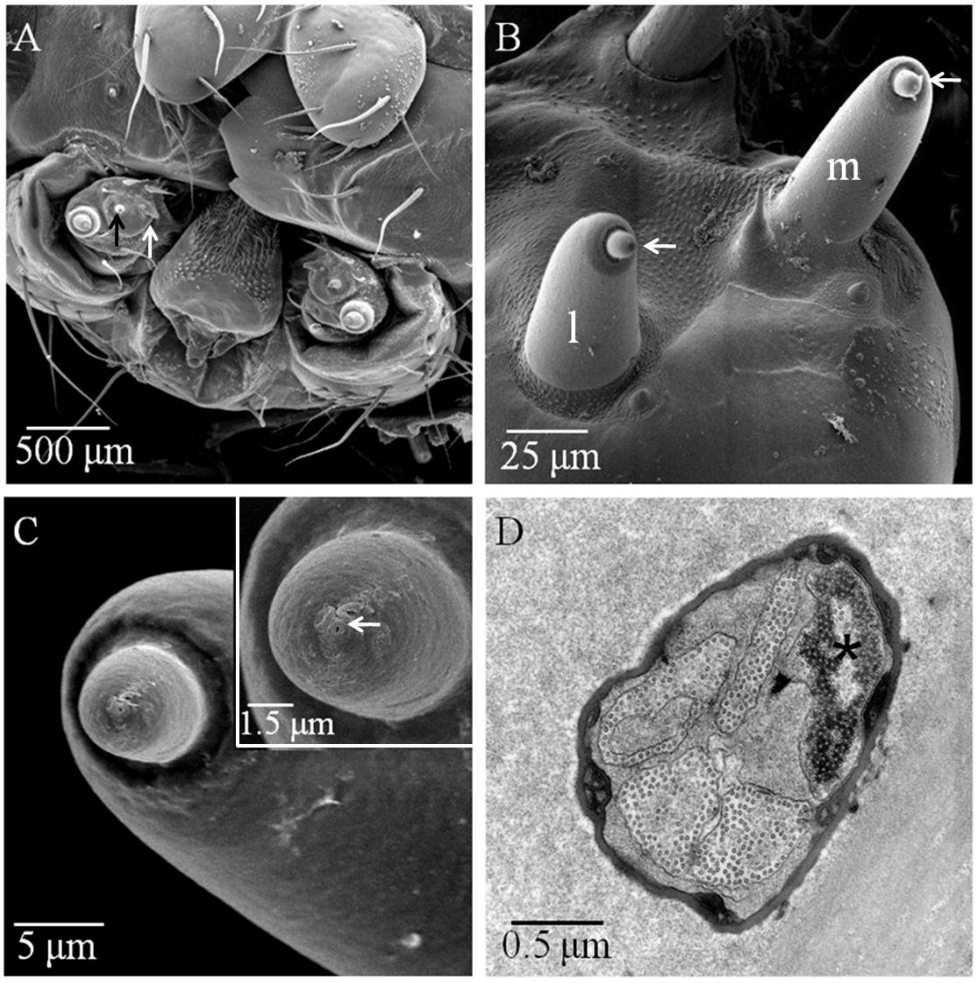


Figure 2.

Representative electrophysiological traces of responses from the taste receptor cells in the medial and lateral styloconic sensilla to various stimuli. (A, J) 30 mM KCl (control), (B, K) 100 mM sucrose, (C, L) 100 mM inositol, (D, M) 1 mM strychnine, (E, N) 1 mM caffeine, (F, O) 1 mM aristolochic acid, (G, P) 1 mM nicotine, (H) a mixture of 0.25 mM concentrations of each of strychnine, caffeine, aristolochic acid and nicotine, and (I) a mixture of 1 mM caffeine and 100 mM inositol. Arrowheads represent the response of the small amplitude KCl-sensitive cell. Asterisks represent the response of the large amplitude KCl-sensitive cell.

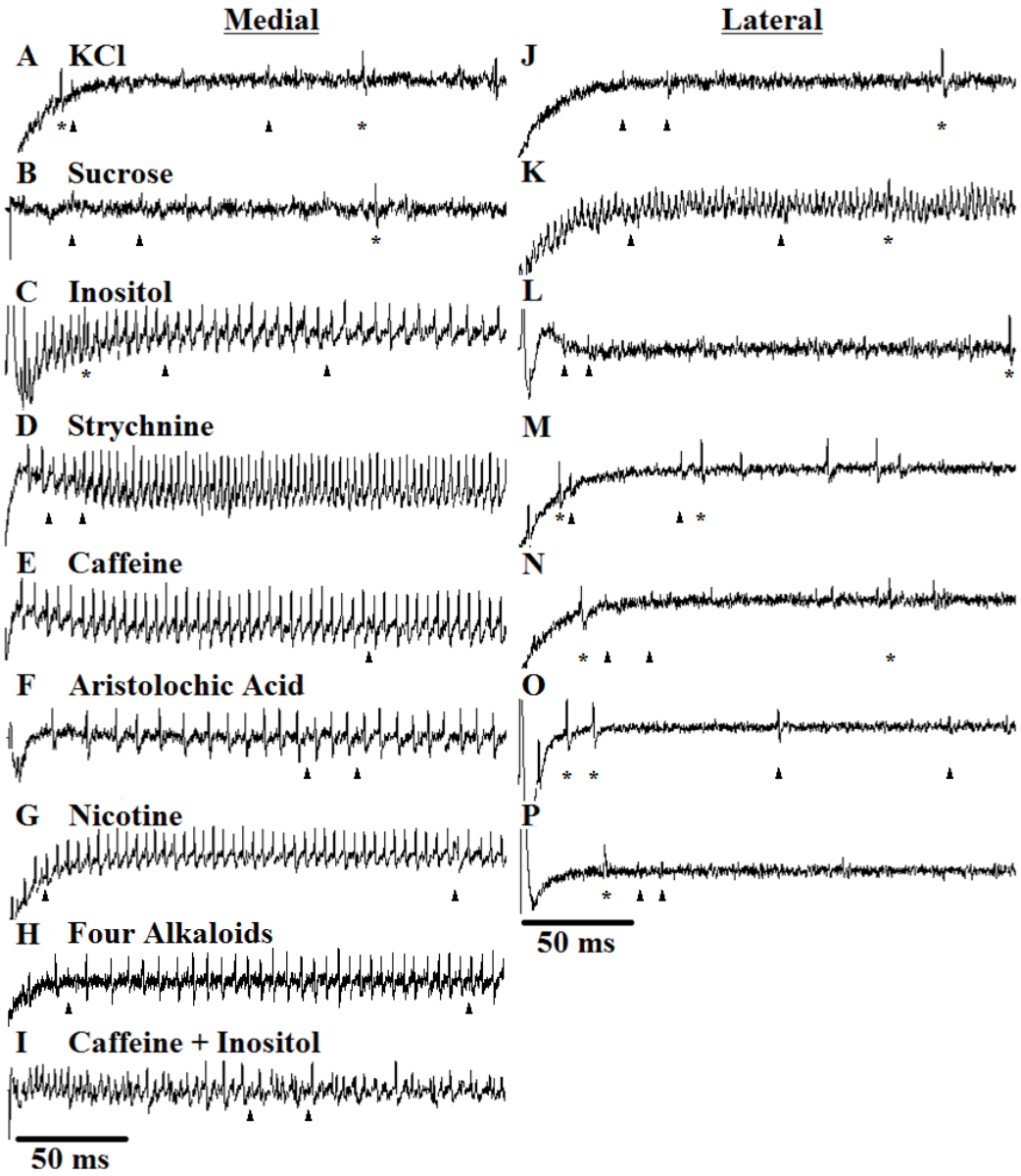


Figure 3.

Representative electrophysiological traces of responses from the KCl-sensitive/deterrent-sensitive cell in the medial styloconic sensilla to (A) 30 mM KCl, (B) 50 mM KCl, and (C) 1 mM strychnine in 50 mM KCl. (D) Dose response (% consumption) to increasing concentrations of KCl in a feeding behavior bioassay. The asterisk denotes the concentration of KCl that significantly decreased feeding.

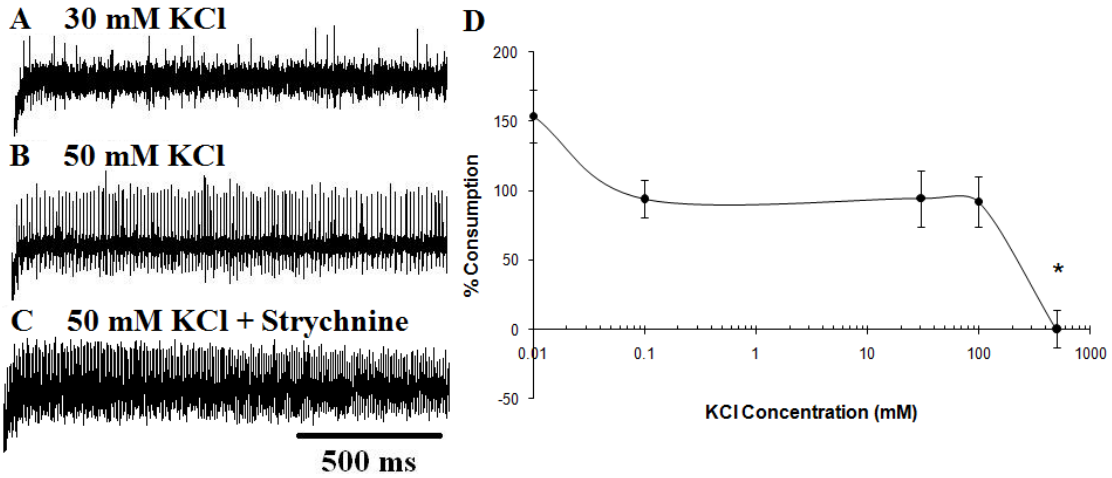


Figure 4.

Temporal pattern of firing (mean number of impulses per 100 ms) by the deterrent-sensitive cell of the medial styloconic sensillum to 0.001 mM and 1 mM concentrations of four alkaloids, including (A) strychnine, (B) caffeine, (C) aristolochic acid, and (D) nicotine. The asterisk denotes the start of the temporal firing pattern for 1 mM concentrations. Results are derived from (A), n = 10, 14 sensilla, respectively; (B), n = 15, 19; (C), n = 15, 18; (D), n = 7, 8. Error bars are S.E.

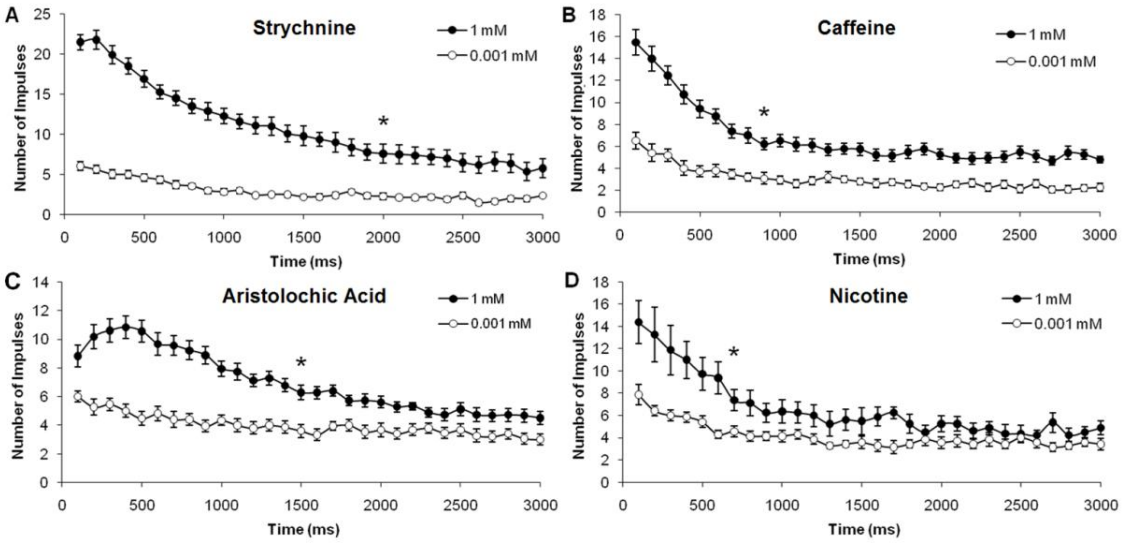


Figure 5.

Temporal pattern of firing (mean number of impulses per 100 ms) by the inositol-sensitive cell of the medial styloconic sensillum to 100 mM inositol. The asterisk denotes the start of the temporal firing pattern for 100 mM inositol. Error bars are S.E. n = 29 sensilla.

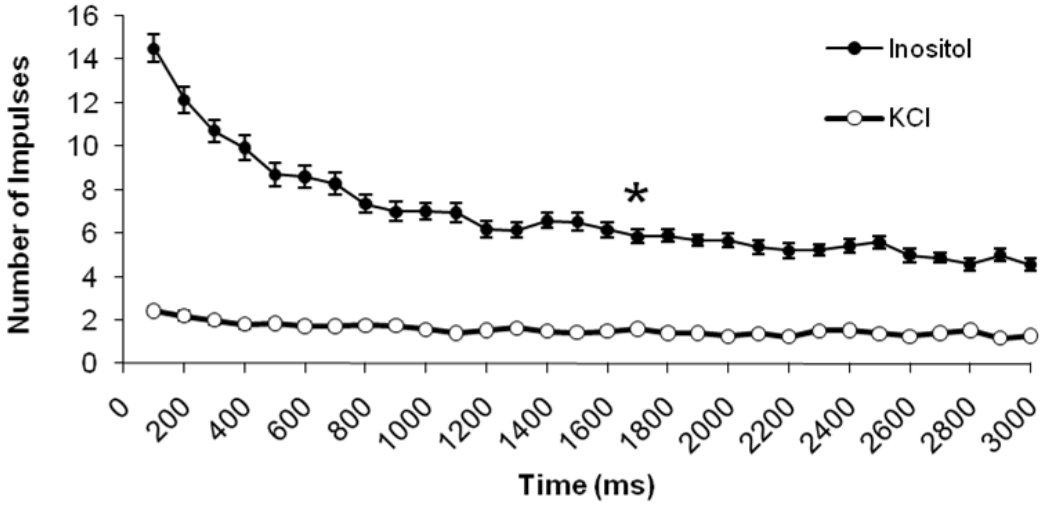


Figure 6.

Dose responses (mean number of impulses per 1 s) of the deterrent-sensitive cell to increasing logarithmic concentrations of (A) strychnine, (B) caffeine, (C) aristolochic acid, and (D) nicotine. The dashed lines represent the number of impulses per 1 s of 30 mM KCl (control). The asterisk denotes the threshold concentration at which a significant response was first detected. The deterrent-sensitive cell reached its response plateau at 1 mM for strychnine, caffeine, and nicotine, but at 0.1 mM, for aristolochic acid. Results are derived from (A), n = 7 – 20 sensilla; (B), n = 7 – 19; (C), n = 8 – 18; (D), n = 5 – 19. Error bars are S.E.

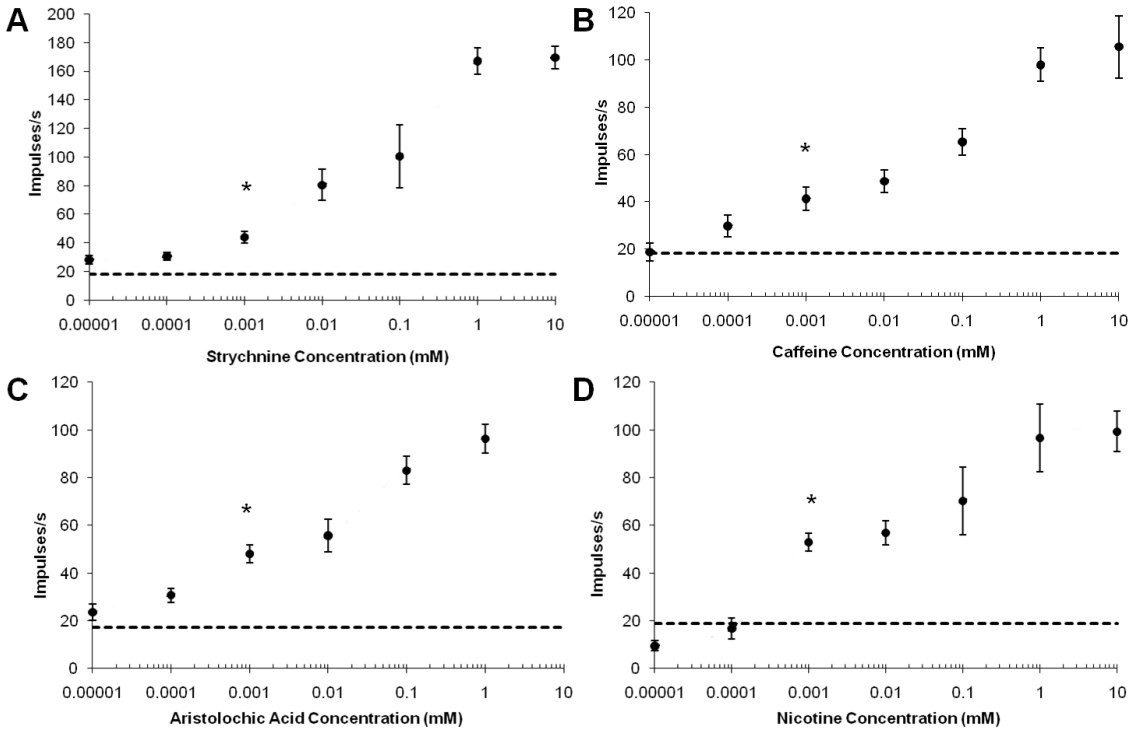
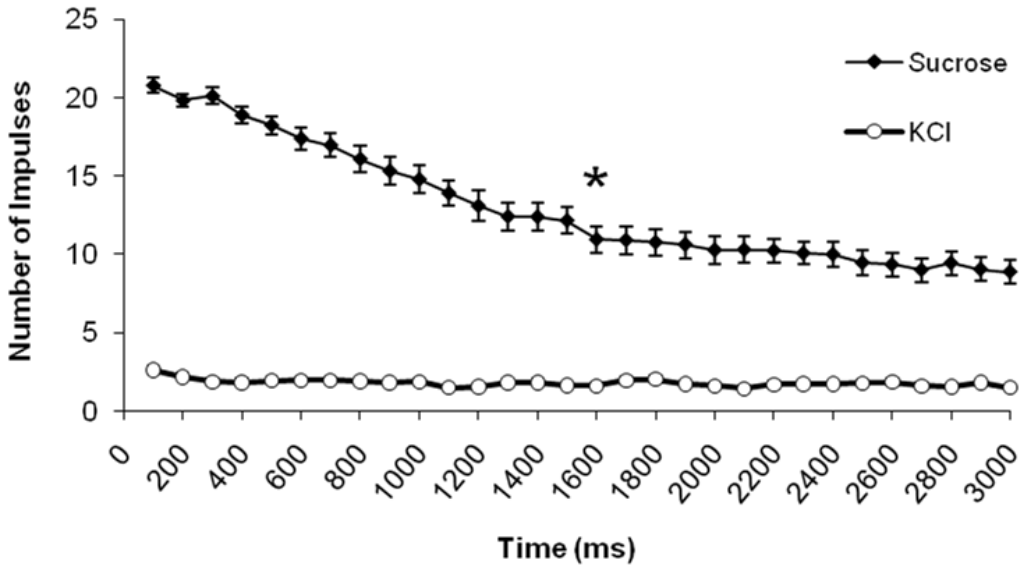


Figure 7.

Temporal pattern of firing (mean number of impulses per 100 ms) by the sugar-sensitive cell of the lateral styloconic sensillum to 100 mM sucrose. The asterisk denotes the start of the temporal firing pattern for 100 mM sucrose. Error bars are S.E. n = 25 sensilla.



CHAPTER TWO

EFFECT OF PHAGOSTIMULANT MIXTURES ON THE RESPONSES OF DETERRENT- AND SUGAR-SENSITIVE CELLS IN GYPSY MOTH LARVAE, *LYMANTRIA DISPAR* (L.)

A version of this chapter has been submitted for publication to the Journal of Insect Physiology

1. Introduction

Food plant recognition and selection is thought to be primarily mediated by input from each bilateral pair of styloconic sensilla located on the galea, which are considered the primary sensory organs involved in feeding (Schoonhoven and Dethier, 1966; Schoonhoven, 1972; de Boer and Hanson, 1987; de Boer, 1991). Previous morphological studies in lepidopteran larvae have demonstrated that the maxillary galeal lateral and medial styloconic sensilla of lepidopteran larvae are each innervated by four gustatory receptor cells (Schoonhoven and Dethier, 1966; Ma, 1972; Schoonhoven, 1972; Shields, 1994; Shields, 2009), which are in constant contact with plant sap liberated during feeding. These cells have been referred to as the salt, sugar, inositol, and deterrent receptors (Schoonhoven, 1972; Schoonhoven et al., 1992), since they respond to salt, sweet, inositol, and bitter compounds, respectively (e.g. Schoonhoven, 1974; Frazier, 1986; Shields and Mitchell, 1995; Glendinning, 1996; Bernays et al., 1998; Glendinning et al., 1999; 2000). Plant material is thought to enter each of these sensilla through a pore at the tip and to interact with the underlying dendrites (Zacharuk, 1985; Zacharuk and Shields, 1991; Shields, 1994), resulting in action potentials being sent to the brain of the insect. The responses of these cells to phytochemicals present in a plant are key in determining which plants are deemed to be acceptable or unacceptable as feeding hosts (Bernays and Chapman, 1994).

Gypsy moth larvae, *Lymantria dispar* (L.) are polyphagous feeders and are major pest defoliators in most of the United States destroying millions of hectares of trees annually (Liebhold, 1995). The larvae feed on the foliage of hundreds of plants, but favor leaves of deciduous hardwood trees, such as oak, maple, and sweet gum (Mosher,

1915; Liebhold et al., 1995; Shields et al., 2003). In the previous study, the response properties of taste receptor cells housed in the medial and lateral styloconic sensilla to alkaloids and sugars, found previously to be feeding deterrents and stimulants, respectively (Shields et al., 2006; Shields et al., 2008) were investigated. More specifically, it was determined if there were functional differences between the two sensilla, such that one sensillum encoded a different subset of compounds than the other sensillum. In addition, the sensitivity and temporal coding properties of individual receptor cells in each sensillum to some of these compounds were determined (e.g. Schoonhoven, 1982; Hanson and Peterson, 1990; Glendinning and Hills, 1997).

In the present study, the effects of mixtures of phagodeterrent and phagostimulatory mixtures of alkaloids and sugars on the responses of the deterrent-sensitive, inositol-sensitive, and sugar-sensitive cells were investigated, since taste receptor cells in nature are exposed typically to complex mixtures.

2. Materials and Methods

2.1 Insects

L. dispar eggs (New Jersey strain) were obtained from USDA-APHIS, Otis Air National Guard Base (Falmouth, Massachusetts, USA). The caterpillars were reared on a high wheat germ-based artificial diet (Bio-Serv, Frenchtown, New Jersey; MP Biomedicals, Solon, Ohio, USA) and maintained at 25 ± 2ºC, 60% relative humidity, and a 12 h light:12 h dark photoperiod regimen (Shields et al., 2003; Shields et al., 2006). The selection of 12-18 h postmolt, 24 h food deprived fifth instar larvae was randomized for all experiments. The larvae were naïve to the test compounds (see below) prior to testing.

2.2 Electrophysiology

Electrical responses from taste receptor cells within the lateral and medial styloconic sensilla were recorded using the tip-recording method (after Hodgson et al., 1955) as described in more detail in the previous study. In brief, the recording procedure involved mounting the head of the caterpillar on a saline-filled micropipette containing a silver wire to serve as the indifferent ground electrode, and placing a stimulating electrode over the tip of a styloconic sensillum. At least three minutes were allowed to elapse between each successive stimulation in order to minimize carry-over effects. Whenever possible, selection of right or left sensilla was randomized. The order in which a stimulating solution was presented to the caterpillar was randomized to prevent bias. All recordings were made between 0900 and 1700 hours during light of the photoperiod.

The extracellular AC signals were recorded from individual styloconic sensilla, amplified by a gain of 10 X and run through a bandpass filter set at 100-1200 Hz (Syntech, Hilversum, The Netherlands). The recordings were digitized by a 16-bit analog-to-digital interface (IDAC-4, Syntech) and analyzed off-line with Autospike software (version 3.8) (Syntech). For each electrophysiological recording, a single sensillum was stimulated for a total of 3 sec, but only the action-potentials for the first 1000 ms were quantified, starting at 50 ms after contact with the sensillum by using the "spike-counter" tool in the Autospike software. The number of impulses was counted in 100 ms increments.

2.3 Taste stimuli

Stimulus compounds dissolved in 30 mM KCl (Fisher Scientific) in distilled water to improve the signal-to-noise ratio in solution were tested at room temperature and

comprised: a sugar, sucrose (J.T. Baker); a sugar alcohol, inositol (myo-inositol) (Sigma); and the alkaloids, strychnine (hemisulfate) and caffeine (MP Biomedicals, Solon, OH). The solvent solution, alone, was used as a control stimulus and to fill the indifferent electrodes. To fully dissolve the alkaloids into solution, 10% ethanol was added to the 30 mM KCl solution. At this concentration, ethanol had no discernable effect on the electrical activity of the taste-receptor cells (see previous chapter).

For all experiments, 1 mM concentrations, well within the range of concentrations of alkaloids found in plants, were used (Shields et al., 2006; Shields et al., 2008). Sucrose and inositol were used in 100 mM concentrations as these principal sugars often occur at higher concentrations in plants. To ensure a reproducible response for a particular solution being tested, 1-2 replications of each solution were made. Periodic stimulation with 30 mM KCl alone was carried out to obtain a baseline reference throughout each experiment (Figs. 1A, 1I, 3A, 3I).

2.4 Data analysis

Only those recordings with a good signal-to-noise ratio were analyzed. The shape and temporal firing pattern of each recording was visualized using the "Amplitude Histogram View" feature of Autospike, where it was possible to identify and assign spike amplitudes to the taste receptor cells.

For all experiments, a randomized complete block design was run using GLM ANOVA ($\alpha = 0.05$) to test the null hypothesis that there was no difference in the response among all mixtures compared with the control. Each sensillum (i.e., individual) was the blocking variable, the solution (single or mixture) was the fixed variable, and the firing

frequency was the response variable. A Tukey-Kramer multiple comparison test was used to compare the responses of the taste receptor cells to various mixtures.

3. Results

3.1 Effect of sugars on the deterrent-sensitive cell

The alkaloid feeding deterrents, strychnine or caffeine, elicited robust, long-lived, phasic-tonic responses from a single large taste receptor cell with a large amplitude cell housed in the medial styloconic sensillum (Figs. 1E, 3E). The lateral sensillum elicited no response to these alkaloids in the lateral sensillum (Figs. 1M, 3M; see also Figs. 1O, 3O). The effect of the sugar, sucrose, (S) (Figs. 1J, 3J), sugar alcohol, inositol (I) (Figs. 1C, 3C), or their combination (IS) (Figs. 1D, 3D) was tested on the response of the deterrent sensitive cell (D) to strychnine (Fig. 1E) and caffeine (Fig. 3E). Inositol (DI) (80.0 ± 6.36 impulses/s) (Fig. 1G) and sucrose (DS) (76.8 ± 8.07 impulses/s) (Fig. 1F) were equally effective in significantly suppressing the response of the deterrent cell to strychnine (D) (165.9 ± 9.95 impulses/s) (Fig. 2A). The combination of sucrose and inositol (DIS) (Fig. 1H) resulted in the best suppression of the response of the deterrent-sensitive cell (23.4 ± 3.00 impulses/s) (Figs. 1H, 2A). Similarly, inositol (DI) (43.4 ± 6.69 impulses/s) (Fig. 3G) and sucrose (DS) (40.3 ± 2.06 impulses/s) (Fig. 3F) were equally effective in significantly suppressing the response of the deterrent-sensitive cell to caffeine (D) (119.2 ± 8.27 impulses/s), but the combination of inositol and sucrose (DIS) (8.6 ± 2.13 impulses/s) proved to be the most effective (Figs. 3H, 4A).

3. 2 Effect of the deterrents on the phagostimulatory cells

The effect of the addition of either strychnine (I, alone: 89.6 ± 6.69 impulses/s) (Fig. 2B) or caffeine (I, alone: 108.1 ± 6.00 impulses/s) (Fig. 4B) was tested on the response of the inositol-sensitive cell (I) (Figs. 1C, 3C, respectively). The addition of strychnine (ID) (52.6 ± 4.97 impulses/s) or caffeine (ID) (57.9 ± 3.83 impulses/s) significantly reduced the response of the inositol-sensitive cell (Figs. 2B, 4B, respectively). The addition of sucrose to either of the deterrents (i.e., strychnine, 46.5 ± 4.32 impulses/s or caffeine, 42.1 ± 3.04 impulses/s) (ISD) did not produce a response that was different from that of the application of the deterrent, alone (Figs. 2B, 4B, respectively).

The effect of the addition of either strychnine (S, alone: 167.4 ± 9.42 impulses/s) (Fig. 2C) or caffeine (S, alone: 157.0 ± 12.58 impulses/s) (Fig. 4C) was also tested on the response of the sucrose-sensitive cell (S) (Figs. 1N, 3N, respectively). The addition of either strychnine (SD) (187.1 ± 8.62 impulses/s) or caffeine (SD) (173.7 ± 9.02 impulses/s) was not effective in significantly suppressing the response of the sugar-sensitive cell (Figs. 2C, 4C, respectively). The addition of inositol to either strychnine (SID) (185.4 ± 8.61 impulses/s) (Figs. 1P, 2C) or caffeine (SID) (175.6 ± 7.28 impulses/s) (Figs. 3P, 4C) also did not have any significant effect on the response of the sucrose-sensitive cell.

3. 3 Mixture interactions between the phagostimulatory cells

Inositol exhibited a strong response from a single taste receptor cell with a smaller amplitude than that of the deterrent-sensitive cell housed in the medial sensillum (Figs. 1C, 3C). Inositol did not elicit a response in the lateral sensillum (Figs. 1K, 3K; see also Figs. 1O, 3O). The response of the inositol-sensitive cell (I) (89.6 ± 6.69 impulses/s,

inositol, alone, Fig. 2B; 108.1± 6.00 impulses/s, Fig. 4B) was significantly attenuated with the addition of sucrose (IS) (68.1 ± 7.85 impulses/s, Fig. 2B; 70.0± 6.43 impulses/s, Fig. 4B) (see also Figs. 1D, 3D and figure legend).

Sucrose elicited a vigorous response from a single taste receptor cell with a smaller amplitude than that of the deterrent-sensitive cell housed in the lateral sensillum (Figs. 1J, 3J). Sucrose did not elicit a response in the medial sensillum (Figs. 1B, 3B). Unlike the effect of sucrose addition on the response of the inositol-sensitive cell, the response of the sucrose-sensitive cell to sucrose (S) (167.4 ± 9.42 impulses/s, sucrose, alone, Fig. 2C; 157.0 ± 12.58 impulses/s, Fig. 4C) was not significantly decreased with the addition of inositol (SI) (190.1 ± 8.81 impulses/s, Fig. 2C; 163.8 ± 5.56 impulses/s, Fig. 4C) (see also Figs. 1L, 3L and figure legend).

4. Discussion

The styloconic sensilla play an important role in host-plant selection behavior (Schoonhoven and van Loon, 2002). Lepidopteran larvae bear sensilla with taste receptor cells that are tuned to detect feeding stimulants (e.g., sugars, inositol, amino acids, secondary plant substances occurring in their host plants), as well as cells (e.g., deterrent-sensitive cells and perhaps, salt cells) signaling compounds which deter or inhibit feeding (Schoonhoven and van Loon, 2002). When the insect bites into a leaf, the styloconic sensilla are exposed to a complex mixture of phytochemicals, however the responses of the taste receptor cells housed within these sensilla do not directly represent their responses to the individual compounds alone. It is possible that interactions may occur between stimulating molecules prior to any taste receptor cell being stimulated and/or there may also be some interneuronal interactions (Chapman, 2003).

In this study, the effects of mixtures of phagodeterrent and phagostimulatory mixtures (i.e., alkaloids and sugars) were tested on the responses of the deterrent-sensitive, inositol-sensitive, and sugar-sensitive cells to determine if any mixture interactions were occurring. While inositol (DI) and sucrose (DS) equally suppressed the response of the deterrent-sensitive cell to the feeding deterrents, strychnine and caffeine, their combination (DIS) was the most effective. Shields and Mitchell (1995) found that the activity of the deterrent-sensitive cell responding to the deterrent, sinigrin, was reduced by sucrose or inositol in two caterpillar species. Other studies also demonstrated the suppression of the deterrent-sensitive cells by sugars in lepidopteran larvae (e.g., Simmonds and Blaney, 1983; Mitchell, 1987; Blaney and Simmonds, 1990; Chapman et al., 1991; Schoonhoven et al., 1992; Bernays and Chapman, 2000; Glendinning et al., 2000).

In our current study, strychnine or caffeine inhibited response of the inositol-sensitive cell, but not the response of the sucrose-sensitive cell. In addition, when sucrose was combined with either of the deterrents, the response to the mixture did not differ from that of the response to the deterrent, alone. The addition of inositol to either of the deterrents had no effect on the suppression of the sucrose-sensitive cell. Deterrents have been reported previously to inhibit the response of sugar-sensitive cells in other lepidopteran larvae (e.g., Morita, 1959; Ma, 1977; Dethier, 1982; Simmonds et al., 1990; Frazier, 1986; van Loon, 1990; Hirao and Arai, 1991; Messchendorp et al., 1996), as well as other non-lepidopteran species (Mitchell and Sutcliffe, 1984; Mitchell, 1987). Sucrose suppressed responses of the inositol-sensitive cell, but inositol had no effect on responses of the sucrose-sensitive cell.

Previous studies have shown that inhibitory mixture interactions can occur among taste cells within the same sensillum (see above for references). Evidence of these interactions was seen here with the suppression of the deterrent-sensitive cell by inositol and vice versa as both cells are housed in the medial sensillum. Thus, when strychnine or caffeine was combined with inositol the overall firing rate was lower than the sum of the firing rates from each individual component of the mixture. Suppression of the inositol-sensitive cell by strychnine or caffeine was also noted. In this case, these inhibitory interactions involve the activation of different taste receptor cells housed within the same sensillum. Of course, mixtures of components that stimulate separate cells in a single sensillum can also produce synergism (e.g., Dethier and Kuch, 1971). Possible mechanisms that may explain these inhibitory mixture interaction effects include: (i) direct electrical communication between neurons via the presence of gap junctions, as evidenced in taste receptor cells of flies (Isidoro et al., 1993) and thermo-hygrosensory neurons of a lepidopteran larva (Steinbrecht, 1989) and (ii) one taste receptor cell suppressing the activity in a neighboring cell through indirect interactions (Jefferys, 1995; Bokil et al., 2001). Inhibitory mixture interactions among taste cells within different sensilla were also noted. This was demonstrated by the greatest suppression of the deterrent-sensitive cell by sucrose and its combination with inositol (sucrose-sensitive cell housed in the lateral sensillum; inositol and deterrent-sensitive cells, in the medial sensillum). The mechanism(s) by which sucrose interacted to suppress the deterrent-sensitive cell is unclear.

The presence of certain secondary plant chemicals such as alkaloids, as well as their interactions with other plant components, influence host-plant specificity and host

selection in phytophagous insects, such as gypsy moth larvae (Barbosa and Krischik, 1987; Miller and Hanson, 1989; Barbosa et al., 1990; Shields et al., 2003). Plants containing alkaloids are considered unfavorable or intermediate in acceptability for this insect (Barbosa and Krischik, 1987; Shields et al., 2003). It is clear from this study, as well as that of the previous study, that the feeding deterrency of alkaloids (Shields et al., 2006; Shields et al., 2008) is likely to be mediated by way of the deterrent-sensitive cell in the medial sensillum of gypsy moth larvae. This can be ascertained by the relatively high sensitivity of this deterrent-sensitive cell to various alkaloids (see previous chapter). The high firing rate of the deterrent-sensitive cell in response to alkaloids may be useful in alerting the insect of their presence and potentially harmful effects, and may be mediated by the electrophysiological suppression of the inositol-sensitive cell by the deterrent-sensitive cell. In addition, the electrophysiological suppression of the deterrent-sensitive cell by sucrose and inositol may allow the insect to maintain a broader feeding range by temporarily inhibiting the responsiveness of the deterrent-sensitive cell and rendering its food more acceptable. Interestingly, Schoonhoven (1969) reported that *Manduca sexta* was able to ingest greater quantities of unpalatable plant tissues when inositol is added to them. Therefore, one may question the apparent significance of counteracting the inhibitory effects of some compounds (e.g., alkaloids) on feeding. Glendinning et al. (2000) reported that if *M. sexta* encountered some relatively harmless plant compounds that stimulated the deterrent-sensitive cell while feeding on highly nutritious foliage, it would be clearly beneficial to still ingest this plant. In this case, if toxic plant compounds are ingested unknowingly, these caterpillars possess post-ingestive response mechanisms that will allow them to detoxify these compounds or to

inhibit feeding (e.g., Glendinning, 1996). A somewhat similar scenario may be the case for gypsy moth larvae, where detoxification capacities have been reported for some compounds (Ahmad and Forgash, 1975; Appel and Maines, 1995).

Sugars play a critical role in determining the palatability of food, while inositol may act to enhance its palatability (Glendinning et al., 2000). A previous feeding bioassay, testing the effect of varying alkaloid concentrations on the feeding behavior of gypsy moth larvae (Shields et al., 2008) showed that the lowest concentrations at which gypsy moth larvae were deterred from feeding on alkaloid-laden disks (i.e., deterrency threshold or DT concentration) ranged from 0.1-10 mM. The electrophysiological study in the previous chapter demonstrated that the lowest detectable concentration of the alkaloids, strychnine, caffeine, nicotine, and aristolochic acid was slightly lower (i.e., 0.001 mM). In addition to the behavioral (Shields, 2006; Shields, 2008) and sensory physiological studies (see previous chapter), this study provides strong support that the sensory input from the deterrent, sucrose, and inositol-sensitive cell likely caused the observed feeding responses to alkaloids, sucrose, and inositol.

Figure 1.

Representative electrophysiological traces of responses from the taste receptor cells in the medial and lateral styloconic sensilla to various stimuli. (A, I) 30 mM KCl (control), (B, J) 100 mM sucrose, (C, K) 100 mM inositol, (D, L) a mixture of 100 mM sucrose and 100 mM inositol, (E, M) 1 mM strychnine, (F, N) a mixture of 100 mM sucrose and 1 mM strychnine, (G, O) a mixture of 100 mM inositol and 1 mM strychnine, and (H, P) a mixture of 100 mM sucrose, 100 mM inositol, and 1 mM strychnine. Arrowheads represent the response of the sugar-sensitive cell, circles represent the response of the inositol-sensitive cell, and rectangles represent the response to the deterrent sensitive cell. All recordings were taken from the same animal.

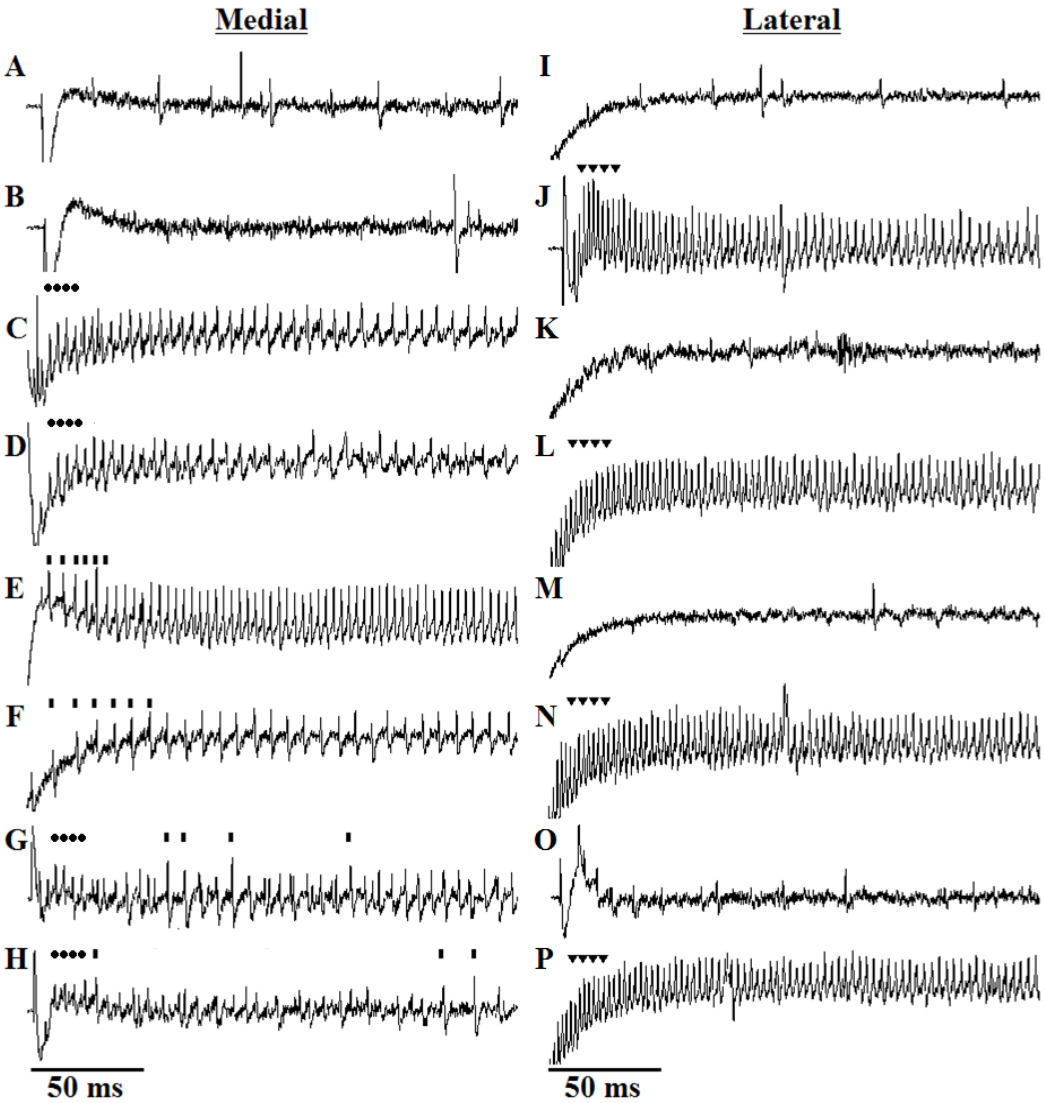


Figure 2.

The effect of various mixtures on the response of different taste receptor cells using the deterrent, strychnine. (A) Mean neural response (impulses/s) of the deterrent-sensitive cell to 1 mM strychnine (D) and mixtures of 1 mM strychnine and 100 mM inositol (DI), 1 mM strychnine and 100 mM sucrose (DS), and 1 mM strychnine, 100 mM inositol, and 100 mM sucrose (DIS). (B) Mean neural response of the inositol-sensitive cell to 100 mM inositol (I) and mixtures of 100 mM inositol and 100 mM sucrose (IS), 100 mM inositol and 1 mM strychnine (ID), and 100 mM inositol, 100 mM sucrose, and 1 mM strychnine (ISD). (C) Mean neural response of the sugar-sensitive cell to 100 mM sucrose (S) and mixtures of 100 mM sucrose and 100 mM inositol (SI), 100 mM sucrose and 1 mM strychnine (SD), and 100 mM sucrose, 100 mM inositol, and 1 mM strychnine (SID). Results are derived from (A), n = 11 – 17 sensilla; (B), n = 11 – 21; (C), n = 8 – 17. Error bars are S.E.

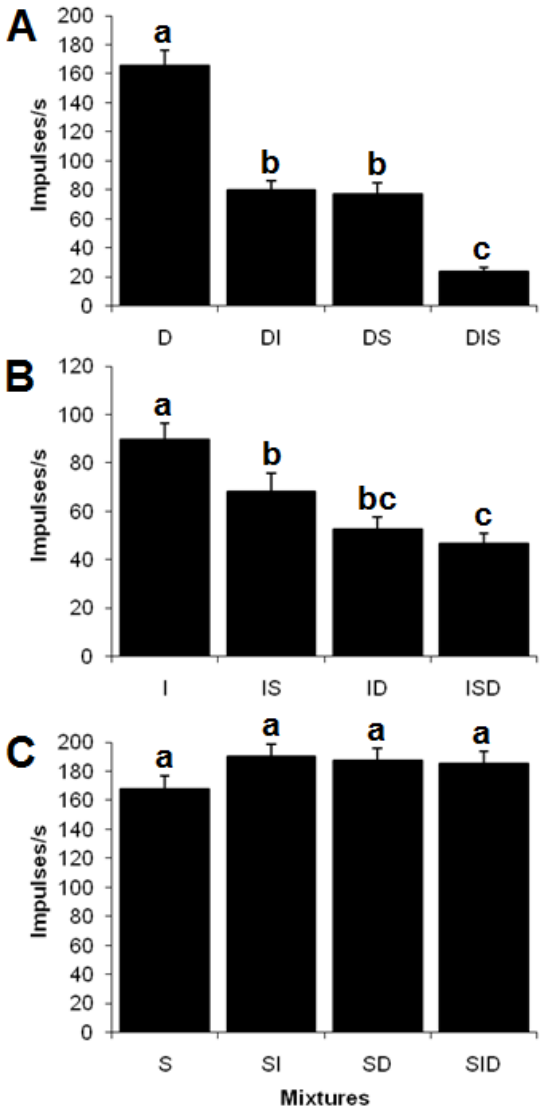


Figure 3.

Representative electrophysiological traces of responses from the taste receptor cells in the medial and lateral styloconic sensilla to various stimuli. (A, I) 30 mM KCl (control), (B, J) 100 mM sucrose, (C, K) 100 mM inositol, (D, L) a mixture of 100 mM sucrose and 100 mM inositol, (E, M) 1 mM caffeine, (F, N) a mixture of 100 mM sucrose and 1 mM caffeine, (G, O) a mixture of 100 mM inositol and 1 mM caffeine, and (H, P) a mixture of 100 mM sucrose, 100 mM inositol, and 1 mM caffeine. Arrowheads represent the response of the sugar-sensitive cell, circles represent the response of the inositol-sensitive cell, rectangles represent the response to the deterrent sensitive cell, and asterisks represent doublets. All recordings were taken from the same animal.

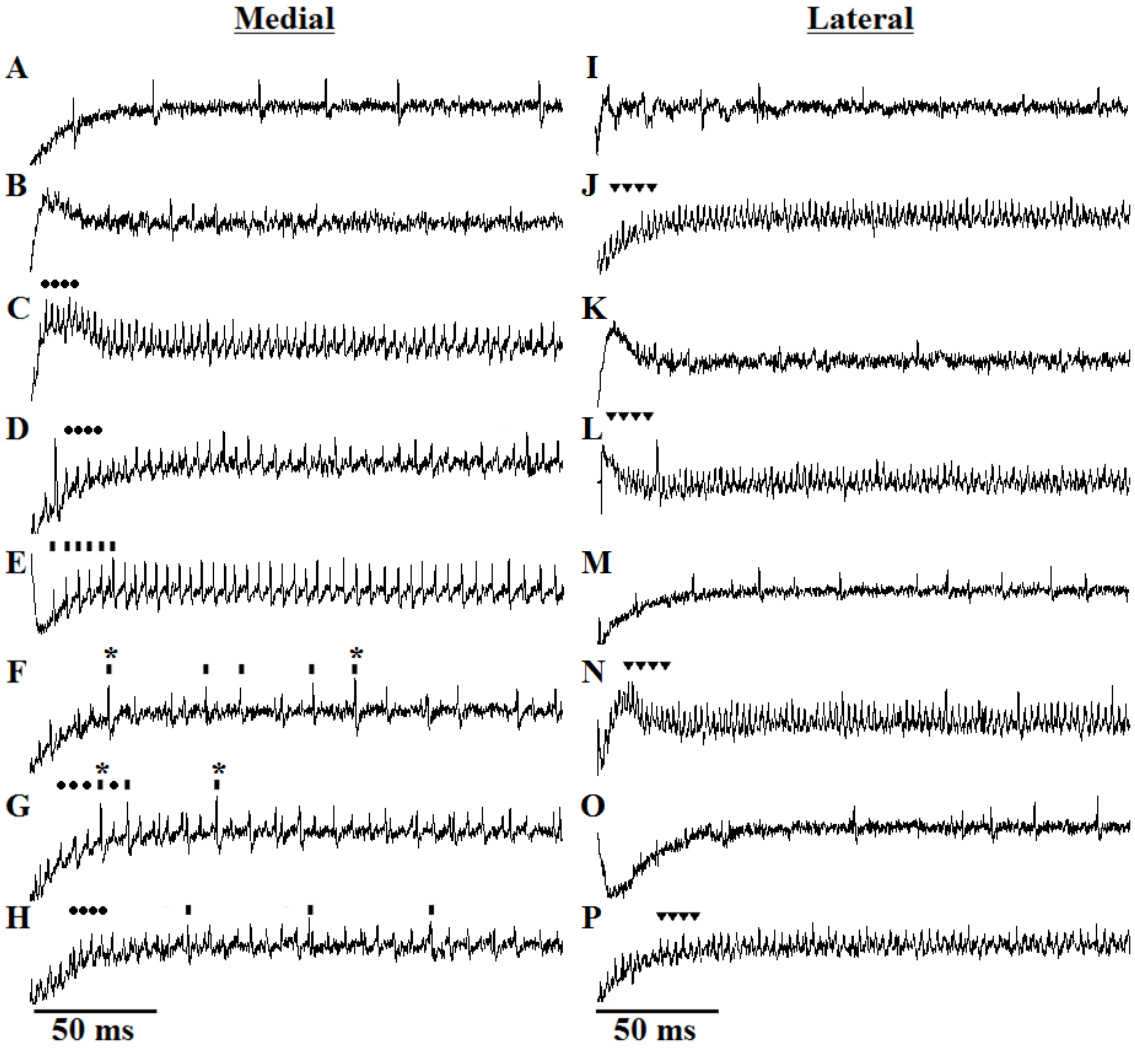
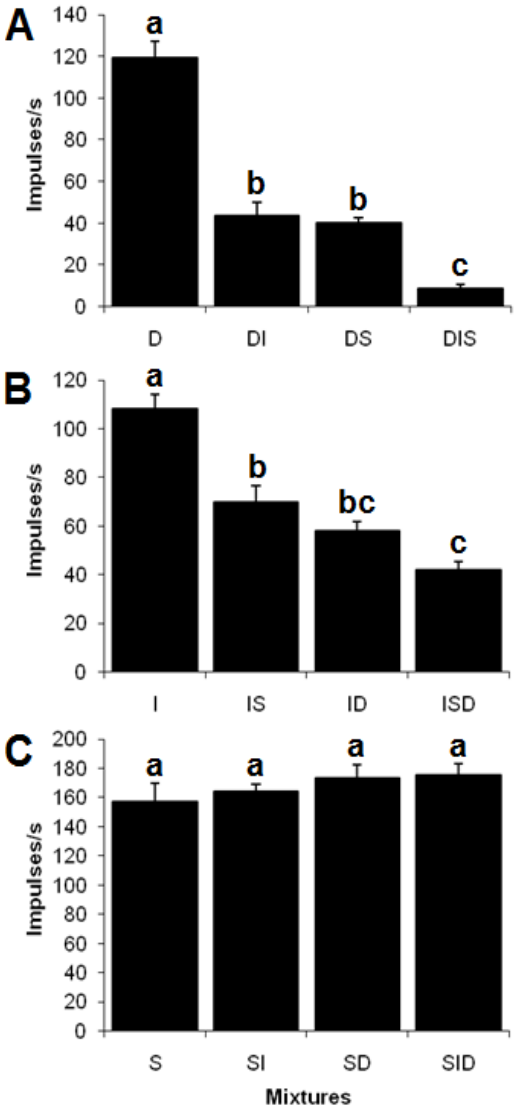


Figure 4.

The effect of various mixtures on the response of different taste receptor cells using the deterrent, caffeine. (A) Mean neural response (impulses/s) of the deterrent-sensitive cell to 1 mM caffeine (D) and mixtures of 1 mM caffeine and 100 mM inositol (DI), 1 mM caffeine and 100 mM sucrose (DS), and 1 mM caffeine, 100 mM inositol, and 100 mM sucrose (DIS). (B) Mean neural response of the inositol-sensitive cell to 100 mM inositol (I) and mixtures of 100 mM inositol and 100 mM sucrose (IS), 100 mM inositol and 1 mM caffeine (ID), and 100 mM inositol, 100 mM sucrose, and 1 mM caffeine (ISD). (C) Mean neural response of the sugar-sensitive cell to 100 mM sucrose (S) and mixtures of 100 mM sucrose and 100 mM inositol (SI), 100 mM sucrose and 1 mM caffeine (SD), and 100 mM sucrose, 100 mM inositol, and 1 mM caffeine (SID). Results are derived from (A), n = 7 – 10 sensilla; (B), n = 6 – 8; (C), n = 5 – 8. Error bars are S.E.



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CURRICULUM VITAE

NAME: Timothy Martin
SEX: Male
BIRTH DATE: November 4, 1987
BIRTH PLACE: Oklahoma City, OK

CURRENT ADDRESS:
Department of Biological Sciences
Towson University
Towson, MD 21252
PHONE: 240-676-8635

INSTITUTIONS ATTENDED	DATES	DEGREE
Towson University Towson, MD	8/2005-5/2009	B.S. in Molecular Biology, Biochemistry and Bioinformatics B.S. in Chemistry *Magna Cum Laude*
Towson University Towson, MD	8/2009-5/2011	M.S. in Biology expected 5/2011

GRADUATE COMMITTEE
Dr. Vonnie Shields – Insect Morphology and Physiology
Dr. James Saunders – Natural Products Chemistry and Molecular Biology
Dr. Elizabeth Vaidya – Behavioral Neuroscience
Dr. Joseph Dickens – Invasive Insect Biocontrol and Behavior

COURSES IN SCIENCE AND RELATED AREAS (U = Undergraduate; G = Graduate)	SEMESTERS
Analytical Chemistry + Lab (U)	1
Biochemistry (U)	1
Biochemistry Lab (U)	1
Biodiversity (U)	1
Bioethics (U)	1
Bioinformatics + Lab (U)	1
Biological Literature (U)	1
Calculus II + Lab (U)	1
Calculus Based Physics + Labs (U)	2
Cell Biology (G)	1
Data Interpretation and Analysis for the Biologist (G)	1
Ecological Biochemistry (U)	1
Exploration of Careers in Science, Technology, Math (U)	2
General Biology + Labs (U)	2
General Chemistry + Labs (U)	2
Genetics (U)	1
Gene Expression and Regulation (G)	1

Genomics + Lab (U)	1
Graduate Seminar in Molecular Neuroscience (G)	1
Immunology (G)	1
Independent Research in Biology (U)	2
Independent Study in Insect Chemical Ecology (G)	1
Intermediate Laboratory (Physical Chemistry) (U)	1
Medical Microbiology + Lab (U)	1
Molecular Biology (U)	1
Molecular Ecology and Evolution (G)	1
Organic Chemistry + Labs (U)	2
Physical Chemistry (U)	1
Professional Aspects in Biology (G)	1
Systematic Biology (G)	1
Using Information Effectively in Science (U)	1

RESEARCH PROJECTS

1/2008-5/2009 – Neurophysiology of gustatory neurons in gypsy moth larvae (*Lymantria dispar*)

6/2009-present – Determining the sensitivity of gustatory neurons in gypsy moth larvae (*Lymantria dispar*) to selected phytochemicals

PUBLICATIONS

MARTIN, T. L., AND V. D. C. SHIELDS. 2011a. Electrophysiological characterization of taste receptor cells of gypsy moth larvae, *Lymantria dispar* (L.). Submitted to Journal of Insect Physiology.

MARTIN, T. L., AND V. D. C. SHIELDS. 2011b. An electrophysiological analysis of the effect of phagostimulant mixtures on the responses of a deterrent-sensitive cell of gypsy moth larvae, *Lymantria dispar* (L.). Submitted to Journal of Insect Physiology.

SHIELDS, V. D. C., AND T. L. MARTIN. 2010. The effect of alkaloids on the feeding behavior and neurophysiology of Lepidopteran larvae. *In* Frank Columbus (ed.), Alkaloids: Properties, Applications and Pharmacological Effects (pp. 109-138). Nova Science Publishers Inc., Hauppauge, New York.

SHIELDS, V. D. C., AND T. L. MARTIN. 2011. The structure and function of taste organs in caterpillars. *In* E. J. Lynch and A. P. Petrov (eds.), Nova Science Publishers Inc., Hauppauge, New York. In press.

ORAL/POSTER PRESENTATIONS

• Towson University 9th Annual Student Research and Scholarship Expo, Towson, Maryland (5/2008)

• Towson University 18th Annual Fisher College Honors Convocation, Towson, Maryland (5/4/2008)

• Society for Neuroscience 2008 Conference, Washington D.C. (11/15/2008)

• Faculty for Undergraduate Neuroscience 2008, Washington D.C. (11/17/2008)

• 8th Annual International Conference on the Science of Botanicals (ICSB), Oxford, Mississippi, (5/6 – 5/10/2009)
• Towson University 19th Annual Fisher College Honors Convocation, Towson, Maryland (4/26/2009)
• Towson University 10th Annual Student Research and Scholarship Expo, Towson, Maryland (5/5/2009)
• 49th Annual Phytochemical Society of North America Meeting, Towson, Maryland (8/8 – 8/12/2009)
• Towson University 11th Annual Student Research and Scholarship Expo, Towson, Maryland (4/21/2010)
• ICE 10 PhD Course in Insect Chemical Ecology, Pennsylvania State University, State College, Pennsylvania (6/1 – 6/15/2010)
• 22nd USDA Interagency Research Forum on Invasive Species, Annapolis, Maryland (1/11 – 1/14/2011)
• Experimental Biology 2011, Washington, DC (4/12/2011)
• Towson University 12th Annual Student Research and Scholarship Expo, Towson, Maryland (4/21/2011)

AWARDS
• Towson University Provost Scholarship (8/2005 – 5/2009)
• Maryland State Senatorial Scholarship (8/2005 – 5/2009)
• Maryland State Delegate Scholarship (8/2005 – 5/2009)
• CoSMIC Scholars Scholarship (8/2008 – 5/2009)
• Towson University Dean's List (8/2005 – 5/2006, 1/2008 – 5/2009)
• Best Undergraduate Student Poster Award at the 8th Annual International Conference on the Science of Botanicals (5/6 – 5/10/2009)
• Wilfred B. Hathaway Award for Outstanding Graduate Student in the Biological Sciences (5/1/2011)

GRANTS
• General Henry H. Arnold Education Grant (08/2005 – 05/2006, 08/2008 – 05/2009)
• Fisher College of Science and Mathematics (FCSM) Undergraduate Research Grant (05/2008 – 05/2009)
• FCSM Undergraduate Travel Grant (08/2008 – 05/2009)

PROFESSIONAL SOCIETIES
American Chemical Society

EXPERIENCE
• Student Researcher – Dept. of Biology, Towson University (1/2008 – 5/2009)
• Teaching Assistant for Medical Microbiology – Dept. of Biology, Towson University (8/2009 – present)
• Research Assistant – Dept. of Biology, Towson University (1/2010 – present)

EXTRACURRICULAR ACTIVITIES

• Active member of the Molecular Biology, Biochemistry, and Bioinformatics (MB3) club (8/2007 – 5/2009)
    - Science, Technology, Engineering, and Mathematics representative for MB3 club (8/2008 – 1/2009)
        - Volunteered to organize Saturday Morning Science program with the Hackerman Academy of Mathematics and Science for K-12 students
        - Organized Science and Technology Day for high school students (11/6/2008)
    - Event Coordinator for MB3 club (1/2009 – 5/2009)
        - Continued organizing Saturday Morning Science program
        - Student representative for MB3 program at Destination Towson, a program/seminar for incoming freshman to get familiar with the different majors
    - MB3 club was awarded the Hoke L. Smith Outstanding Student Organization Award
• Mentor for the MB3 club (6/2009 – present)
    - MB3 club was awarded the Outstanding Community Service Organization Award
• Active member of Beta Beta Beta Biological Honor Society (1/2009 – 5/2009)
• Member of the organizing committee for the 49th Annual Phytochemical Society of North America Meeting, Towson University, Towson, MD (8/8 – 8/12/2009)
• Member of the organizing committee for the 26th Annual Mid Atlantic Plant Molecular Biology Society Meeting, Patuxent National Wildlife Refuge, Beltsville, MD (8/20 – 8/21/2009)
• Member (graduate student representative) of the College Curriculum Committee for the Fisher College of Science and Mathematics (1/2010 – 1/2011)

REFERENCES

Dr. Vonnie Shields – Dept. of Biology, Towson University, Towson, MD 21252
Dr. James Saunders – Molecular Biology, Biochemistry, and Bioinformatics Program, Towson University, Towson, MD 21252
Dr. Don Thomas – Hackerman Academy of Mathematics and Science, Towson University, Towson, MD 21252