Differential gurmarin suppression of sweet taste responses in rat solitary nucleus neurons

Christian H. Lemon, Toshiaki Imoto and David V. Smith

1Department of Anatomy and Neurobiology
University of Tennessee College of Medicine
855 Monroe Ave., Suite 515, Memphis, TN 38163

2Div. Integrative Physiology
Dept. Functional, Morphological and Regulation Science
Faculty of Medicine, Tottori University
86 Nishimachi, Yonago 683-0826
Japan

Running Head: Gurmarin suppresses medullary sweet taste responses

Number of text pages: 24
Number of figures: 8
Number of tables: 1
Number of words in abstract: 249

Corresponding author:
Christian H. Lemon
Department of Anatomy and Neurobiology
University of Tennessee College of Medicine
855 Monroe Ave., Suite 515, Memphis, TN 38163
e-mail: chris@utmmed.edu
phone: (901) 448-1139
fax: (901) 448-1272
Abstract

We examined the effect of the sweet transduction blocker gurmarin on taste responses recorded from neurons in the rat solitary nucleus (NST) to determine how gurmarin sensitivity is distributed across neuronal type. Initially, responses evoked by washing the anterior tongue and palate with 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl and 0.01 M quinine-HCl were recorded from 35 neurons. For some cells, responses to a sucrose concentration series (0.01 - 1.0 M) or an array of sweet-tasting compounds were also measured. Gurmarin (10 µg/ml, 2 – 4 ml) was then applied to the tongue and palate. Stimuli were reapplied after 10 - 15 min. Neurons were segregated into groups based on similarities among their initial response profiles using hierarchical cluster analysis (HCA). Results indicated that sucrose responses recorded from neurons representative of each HCA-defined class were suppressed by gurmarin. However, a disproportionate percentage of cells in each group displayed sucrose responses that were substantially attenuated following gurmarin treatment. Post-gurmarin sucrose responses recorded from neurons that composed 57% of class S, 40% of class N, and 33% of class H were suppressed by at least 50% relative to control. On average, attenuation was statistically significant only in class S and N neurons. Although the magnitude of gurmarin-induced response suppression did not differ across sucrose concentration, responses to different sweet-tasting compounds were differentially affected. Responses to NaCl, HCl or quinine were not suppressed by gurmarin. Results suggest that information from gurmarin-sensitive and -insensitive receptor processes converges onto single NST neurons.
Introduction

Substances that selectively modify specific physiological functions have proven useful for exploring the neural representation of sensory information. For taste, lingual application of gurmarin, a protein isolated from the plant Gymnema sylvestre (Imoto et al. 1991), significantly attenuates integrated chorda tympani (CT) nerve responses to sweet-tasting substances in rats (Imoto et al. 1991; Miyasaka and Imoto 1995) and C57BL mice (Ninomiya and Imoto 1995; Ninomiya et al. 1997, 1998). Additionally, palatal gurmarin treatment suppresses integrated responses to sugars, sodium saccharin and sweet-tasting amino acids recorded from the greater superficial petrosal (GSP) nerve in rats (Harada and Kasahara 2000). Gurmarin does not affect responses to non-sweet stimuli representative of other basic taste quality classes (e.g., NaCl, HCl and quinine) in the CT (Imoto et al. 1991; Miyasaka and Imoto 1995) and GSP (Harada and Kasahara 2000) nerves in rats and the CT nerve in mice (Ninomiya and Imoto 1995; Ninomiya et al. 1997, 1998).

Lingual gurmarin treatment does not affect integrated responses to sweeteners recorded from the glossopharyngeal nerve of C57BL mice (Ninomiya et al. 1997). Data obtained from this species suggest the existence of two different types of receptors for sweets, gurmarin-sensitive and -insensitive, that are differentially distributed across the tongue. Moreover, a recent extension of these findings shows that gurmarin application inhibits responses to sucrose in only a subset of sugar-best CT fibers in this strain of mouse (Ninomiya et al. 1999). Those fibers that are affected exhibit absolute or near absolute suppression, with responses to 0.5 M sucrose suppressed to ~ 10% of control on average. This differential effect is somewhat analogous to the influence of amiloride on the neural processing of salt information, as lingual application of amiloride suppresses responses to salts only in NaCl-best CT fibers (Hettinger and
Frank 1990; Ninomiya and Funakoshi 1988) and NaCl- (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996) and sucrose-best (Smith et al. 1996) neurons in the nucleus of the solitary tract (NST). Therefore, it is possible that input arising from gurmarin-sensitive and -insensitive sweet transduction mechanisms is segregated to particular classes of taste-responsive neurons in the brain stem.

To test the hypothesis that gurmarin sensitivity is differentially distributed across gustatory neuron types in the rat NST, we recorded trains of actions potentials evoked by various taste stimuli, including sucrose and other sweeteners, from single NST neurons prior to and following lingual/palatal application of gurmarin. These experiments attempt to relate specific transduction mechanisms to the organization of gustatory neural circuitry within the central nervous system (CNS).
Method

Animals and surgery

Thirty-four adult male Sprague Dawley rats, weighing 210 to 550 g, were used as subjects. Rats were housed individually in a vivarium, which maintained a 12-hour light/dark schedule and ambient temperature of ~ 23 °C. Food and water were available ad libitum. Subjects were deeply anesthetized with urethane (1.5 g/kg, ip) and prepared for electrophysiological recording. Each rat was tracheotomized and secured in a nontraumatic head holder that deflected its snout ~ 27° downward: this configuration served to minimize brain stem movements associated with breathing. The occipital bone was removed and parts of the cerebellum were gently aspirated to expose the brain stem and allow access to the NST. Body temperature was maintained at ~ 37 °C by a heating pad.

Single-unit electrophysiology

Etched tungsten microelectrodes, insulated except for the tip (impedance = 0.5 to 8 MΩ @ 1 kHz, FHC Inc., Bowdoinham, ME), were used to record extracellular action potentials from single NST neurons. For each preparation, the area of the brain stem where the rostral pole of the solitary tract resided was visually located using vascular landmarks present on the dorsal surface of the exposed tissue. A hydraulic micromanipulator was then used to slowly advance the microelectrode through the brain stem. The portion of the NST that contained neurons responsive to lingual stimulation was initially identified by a change in neural activity associated with the passage of anodal current (10 μA/500 ms) across the anterior tongue; cells were then verified as taste-driven by application of various gustatory stimuli (see below). The gustatory-responsive portion of the NST was located ~ 1 mm ventral to the brain stem surface.
Electrophysiological activity was band-pass filtered (bandwidth $\approx 0.3$ to $10$ kHz), differentially amplified (Grass P511 with high impedance probe) and subsequently routed to various monitors and analytic devices. Spikes that arose from single neurons were identified based on waveform consistency, which was continuously observed throughout each recording session using a storage oscilloscope and, following analog to digital conversion (sampling rate = 25 kHz), a template-matching algorithm (Power 1401 RISC acquisition interface coupled with Spike 2 software, CED, Cambridge, UK). Well-isolated neurons with robust responses to 0.5 M sucrose were used for experimentation. Trains of action potentials that arose during recording sessions were pulse-code modulated and stored, along with voice and trial marker cues, on VHS tape. Digital records of aggregate electrophysiological activity, including template-matched spikes, were downloaded to storage media for offline quantitative analysis.

*Taste stimuli*

Generally, neurons were tested with two out of three groups of taste stimuli. Tastants within each group were presented individually to, and subsequently rinsed from, the oral cavity of each preparation under normal (i.e., control) conditions and following oral application of gurmarin. Stimulus trials of interest were replicated as many times as possible. Every neuron was tested with a set of stimuli that consisted of representatives of the four basic taste qualities (herein referred to as the basic stimulus set), which were presented in random order. Some cells were then subjected to a half-logarithmic step ascending concentration series of sucrose whereas others were tested using a randomized array of various other sweet-tasting compounds (see Table 1).
Tastants were made from reagent grade stock dissolved in deionized water. Solutions were delivered at room temperature to the anterior tongue and palate via a gravity flow system at a rate of ~ 2.5 ml/s. A three-way solenoid fluid valve, which was controlled by the acquisition system, regulated solution delivery. A curved, polyethylene tube that extended from the output port of this valve was directed towards the palate of each subject. Visual inspection revealed that this configuration allowed solutions to effectively bathe both the palate and anterior tongue, as solutions were deflected downward upon encountering the palate. Moreover, a test using methylene blue dye was performed on one preparation to verify that this method of taste stimulus delivery adequately bathed both the anterior tongue and palate. Using the described flow system, dye was delivered to the oral cavity for 10 s. The distribution of stain was then assessed with a dissecting microscope, which revealed that the entire anterior tongue and soft palate were dyed. A subsequent test verified that the nasoincisor ducts (NIDs) were also stained.

During data acquisition, taste stimuli were presented to each subject using a specific protocol. The tongue and palate were first rinsed with deionized water for 10 s, followed immediately by a taste stimulus for 10 s. The tongue and palate were then rinsed with at least 50 ml of deionized water and greater than two min were allowed to elapse between trials. The stimulus delivery system was thoroughly rinsed with deionized water between presentation trials.
Experimental protocol and gurmarin application

Once applied, gurmarin is a difficult substance to remove from gustatory epithelia. Integrated CT responses to sucrose required more than four hours for complete recovery following gurmarin treatment despite repetitive distilled water rinses of the tongue (Miyasaka and Imoto 1995). Although methods do exist by which gurmarin removal may be chemically facilitated (Ninomiya et al. 1998), only slight recovery of mouse CT fiber responses to sucrose was observed at 10 min post anti-gurmarin treatment (Ninomiya et al. 1999). Moreover, repeated anti-gurmarin reactions, which required up to 100 min, were necessary for complete recovery of CT sucrose responses to baseline levels (Miyasaka and Imoto 1995). Therefore, we chose a simple pre-/post-gurmarin design, assuming that responses to non-sweet stimuli would serve as controls for the viability of the neurons from which we recorded, as responses to NaCl, HCl and quinine in the CT (Imoto et al. 1991; Miyasaka and Imoto 1995) and GSP (Harada and Kasahara 2000) nerves in rats and the CT nerve in mice (Ninomiya and Imoto 1995; Ninomiya et al. 1997, 1998) are not altered by gurmarin treatment.

Once single-unit responses evoked during control stimulus presentations were recorded, 10 µg/ml gurmarin (dissolved in deionized water; ~ 2.4 µM) was applied to both the tongue and palate of each subject using a blunt-tipped syringe (2 to 4 ml total volume). For each preparation, the application of gurmarin to the tongue and palate was visually verified. Moreover, a test using methylene blue dye was performed on one preparation to verify that our gurmarin application procedure did indeed adequately bathe both the anterior tongue and palate, including the NIDs.

Treatment of the tongue with 10 µg/ml gurmarin has been shown to produce significant and near maximal attenuation of integrated CT nerve responses to 0.5 M sucrose in the rat
(Miyasaka and Imoto 1995). When applied to the rat palate, 10 µg/ml gurmarin yielded significant and substantial suppression of integrated greater superficial petrosal nerve responses to sucrose and other compounds described as sweet tasting by humans (Harada and Kasahara 2000). However, the effect of gurmarin is not instantaneous following application, with 4.8 µM gurmarin requiring roughly five minutes to produce maximal suppression of sucrose responses recorded from single mouse CT fibers (Ninomiya et al. 1999). Therefore, we allowed 10 to 15 min to elapse following gurmarin treatment before proceeding with experimentation. After this time, each component of the stimulus set used under control conditions was presented as previously described and evoked responses recorded. Following completion of this phase, an additional experiment was conducted on some neurons using a stronger concentration of gurmarin to determine if effects, or lack thereof, observed at the standard concentration were indeed limits. For these cells, we doubled the concentration of gurmarin (20 µg/ml; ~ 4.8 µM), applied it as previously described and retested the stimulus set 10 – 15 min post-treatment.

**Data analysis**

The basic metric used to quantify gustatory responses in NST neurons was net response magnitude, expressed in spikes/s. This measure was calculated as the average number of spikes that occurred each s during the first 5 s of taste stimulus presentation minus the average firing rate per s during the 5 s water rinse period that immediately preceded this epoch (i.e., spontaneous discharge). These data served as input to all subsequent statistical analyses. For each neuron, responses evoked by chemical stimuli were considered significant if the net response exceeded the mean plus 2.54 standard deviations of the spontaneous discharge rate.
To describe the breadth of tuning of each neuron, a measure of response profile entropy (Shannon and Weaver 1949; Smith and Travers 1979) was calculated using net responses to each component of the basic stimulus set. Entropy is defined as:

\[
H = -K \left( \sum_{i=1}^{n} P_i \log P_i \right),
\]

where \( P_i \) represents the response to the \( i \)th stimulus expressed as a proportion of the total response to \( n \) stimuli and \( K \) is a scaling constant. For four stimuli \( K = 1.661 \), which results in \( H \) ranging from a minimum of 0 (i.e., neuron responds to only one stimulus) to a maximum of 1 (i.e., neuron responds equally well to all stimuli). Due to the dominance of excitatory responses in our data set (134 out of 140 responses used for entropy calculations were greater than zero) and the observance that no inhibitory responses were found to be significantly less than average spontaneous discharge, \( P_i \log P_i \) was set to zero during the calculation of \( H \) if the response evoked by the \( i \)th stimulus was less than or equal to zero.

Cells were categorized into types based on the stimulus within the basic set that evoked the maximal net response (i.e., their best stimulus) prior to gurmarin treatment. Additionally, hierarchical cluster analysis (HCA; conducted using Statistica, StatSoft Inc., Tulsa, OK) was performed to quantitatively and objectively identify sets of neurons with pre-gurmarin tuning profiles (i.e., net responses to only basic set stimuli) that were most similar. Input to HCA consisted of a distance matrix representing pairwise neuronal tuning profile similarity/dissimilarity, where 1 - Pearson’s product-moment correlation (\( r \)) served as the distance metric. The unweighted pair-group average amalgamation schedule was used.

The effect of gurmarin on taste responses was statistically evaluated by using appropriate analyses of variance (ANOVA). Significant interactions were sometimes explored using planned
interaction comparisons as our general hypothesis dictated \textit{a priori} predictions with regard to the outcome of specific experiments (i.e., post-gurmarin stimulus responses were compared to only their respective controls). For each ANOVA, degrees of freedom and \( P \) values for within-subject tests were corrected using the Greenhouse-Geisser adjustment to protect against violations of sphericity. Although these corrections were made prior to establishing \( P \) levels, only the uncorrected degrees of freedom and \( P \) values are reported. \textit{Post hoc} comparisons among within-subject level means were accomplished through the use of paired samples \( t \) tests in which each observed score was evaluated using a Dunn critical value. This sort of multiple comparison procedure (MCP) is the only sort of \textit{post hoc} test for repeated level means that has adequate control of \( \alpha \) for all pairwise comparisons (Toothaker 1991).
Results

General response characteristics

Trains of action potentials were recorded from 35 NST neurons with robust responses to 0.5 M sucrose (mean response to 0.5 M sucrose across all neurons = 10.5 net spikes/s ± 1.0 SEM). Our conservative statistical criterion indicated that 34 of these cells significantly responded to 0.5 M sucrose. Two neurons in our sample were recorded simultaneously from one preparation. Eleven neurons were found to be sucrose-best, 18 were classified as NaCl-best and six were typed as HCl-best. No quinine-best units were observed in our sample. Overall, these neurons were broadly responsive to the basic stimuli (H = 0.79 ± 0.02 SEM). Nineteen neurons significantly responded to all components of the basic stimulus set, 13 significantly responded to only three of these stimuli, two significantly responded to only two tastants and one cell significantly responded to only one stimulus. Figure 1 displays the across neuron pattern of response evoked by each basic stimulus and the average spontaneous discharge observed for each neuron prior to gurmarin treatment.

-------------------

Insert Figure 1 about here

-------------------

HCA was used to objectively classify neurons into heterogeneous types based on similarities/dissimilarities among their tuning profiles measured in response to the control presentation of each component of the basic stimulus array. The final HCA solution is represented graphically by the dendrogram in Figure 2. HCA suggested three classes of neurons. All sucrose-best neurons were linked into class S (n = 14; H = 0.77 ± 0.03 SEM); three NaCl-
best units with robust responses to sucrose were also bound to this cluster. Class N \((n = 15; \bar{H} = 0.78 \pm 0.04 \text{ SEM})\) was composed almost entirely of NaCl-best neurons, the exceptions being a pair of HCl-best cells with strong NaCl responses. Class H \((n = 6; \bar{H} = 0.89 \pm 0.02 \text{ SEM})\) neurons responded strongly to HCl, NaCl and quinine.

Effects of gurmarin on neuronal responses to sucrose

Trains of action potentials that were evoked by presentation of each component of the basic stimulus set were recorded prior to and following gurmarin (10 µg/ml) application from all 35 neurons in our sample. Gurmarin treatment was found to attenuate sucrose-evoked spike discharge for the majority of these cells, although the degree of suppression varied across affected neurons. Many cells displayed residual responses to sucrose following gurmarin treatment; post-gurmarin responses to sucrose remained significantly greater than average spontaneous discharge for 22 neurons. Complete gurmarin-induced inhibition (i.e., zero net spikes/s) of a response to 0.5 M sucrose was observed during one trial for one neuron within our sample. Gurmarin treatment did not activate NST neurons, implying that 10 µg/ml gurmarin does not produce a gustatory quality sensation in the rat. Figure 3 shows digital oscilloscope records for two NST neurons in which the magnitude of the post-gurmarin response to 0.5 M sucrose was either almost completely (Figure 3A) or partly (Figure 3B) suppressed relative to within-neuron control (i.e., unadulterated sucrose response). For the neuron in Figure 3A, the response to sucrose was almost fully inhibited by gurmarin (96% suppression). For the unit in
Figure 3B, the magnitude of the post-gurmarin response to sucrose was attenuated by 68%. For both neurons, the magnitude of the response evoked by 0.1 M NaCl was not altered by gurmarin treatment.

-----------------------------
Insert Figure 3 about here
-----------------------------

The orotopic receptive field of the neuron depicted in Figure 3B was at least partially composed of the fungiform papillae, as this cell was driven by anodal stimulation of the anterior tongue. As methylene blue dye tests indicated that our gurmarin application procedure effectively bathed both the anterior tongue and palate, and taste stimulus delivery was limited to these same regions, the partial effect observed for this neuron could not be attributed to inadequate gurmarin application. Moreover, we acquired data from five neurons that clearly received input from the fungiform papillae but for which gurmarin treatment failed to attenuate responses to 0.5 M sucrose (criterion = at least 35% suppression relative to within-neuron control; mean suppression = 0% ± 3.42 SEM; maximum suppression = 11%).

Assuming a criterion of at least 35% attenuation relative to within-neuron control, gurmarin application suppressed responses to 0.5 M sucrose for 22 (63%) neurons in our sample. If the criterion was raised to 50% attenuation, 16 (45%) cells were affected. For three (9%) neurons, post-gurmarin sucrose responses were attenuated by 90% relative to control. These descriptors are summarized in Figure 4, which displays across neuron patterns of response to 0.5 M sucrose measured under control conditions (Figure 4A) and after oral application of 10 µg/ml gurmarin (Figure 4B). The difference between these patterns is shown in Figure 4C. Moreover, these data indicate that sucrose responses recorded from neurons representative of each HCA-
determined neuronal class were affected by gurmarin. However, a disproportionate percentage of cells in each class displayed sucrose responses that were substantially attenuated following gurmarin treatment. Eight (57%) class S, six (40%) class N and two (33%) class H neurons exhibited post-gurmarin responses to sucrose that were suppressed by at least 50% relative to control. Figure 4C further describes the magnitude of the effect as observed across neuronal type.

The null hypothesis that gurmarin application did not differentially affect basic taste response magnitudes across neuronal class was statistically evaluated using a neuron (35 cases) by neuronal group (3 levels; each HCA-defined neuronal class served as a level) by gurmarin treatment (2 levels) by stimulus (4 levels) mixed ANOVA. This hypothesis was not accepted as a significant neuronal group by gurmarin treatment by stimulus interaction was found ($F_{6, 96} = 3.34, P = 0.005$). Planned interaction comparisons revealed that responses to sucrose were significantly attenuated following oral application of gurmarin in class S ($F_{1, 32} = 18.12, P = 0.0002$) and N ($F_{1, 32} = 12.14, P = 0.001$) neurons whereas sucrose responses in class H cells were unaffected ($\alpha = 0.01$). For each neuronal class, gurmarin treatment did not affect responses to NaCl, HCl or quinine relative to control ($\alpha = 0.01$). Figure 5 graphically summarizes these data.
To determine if the observed effects, or lack thereof, of 10 µg/ml gurmarin approximated limits, taste responses were recorded from three neurons following oral application of 20 µg/ml gurmarin. Data regarding responses to sucrose that were recorded from these cells are graphed in Figure 6. For the neuron depicted in Figure 6A, gurmarin suppressed responses to 0.1 M, 0.32 M, 0.5 M and 1.0 M sucrose, although concentration-response functions measured following oral application of 10 and 20 µg/ml gurmarin were not different. Moreover, the response evoked by 0.5 M sucrose following 10 µg/ml gurmarin treatment was nearly identical to that measured following application of 20 µg/ml gurmarin (difference = 1 net spike / 5 s), which indicated that 10 µg/ml gurmarin produced a maximal effect. As seen in Figure 6B, 10 or 20 µg/ml gurmarin did not affect responses to 0.5 M sucrose measured from two other neurons. Both of these cells received verified input from the fungiform papillae, but were unaffected by gurmarin treatment. As observed with the standard concentration, oral application of 20 µg/ml gurmarin did not activate NST neurons.

Sucrose concentration-response functions

To determine if the effect of gurmarin was differential across sucrose concentrations, a half-logarithmic step ascending concentration series of sucrose, which ranged from 0.01 to 1.0 M, was presented to 19 neurons (class S: n = 7; class N: n = 10; class H: n = 2) both prior to and following gurmarin application. A neuron by gurmarin treatment (2 levels) by sucrose
concentration (5 levels) mixed ANOVA revealed that sucrose responses recorded from these cells were significantly influenced by gurmarin application and stimulus concentration (gurmarin by sucrose concentration interaction: $F_{4, 60} = 7.74, P = 0.00004$). Planned interaction comparisons indicated that post-gurmarin responses to 0.1 M ($F_{1, 15} = 9.58, P = 0.007$), 0.32 M ($F_{1, 15} = 8.64, P = 0.01$) and 1.0 M ($F_{1, 15} = 10.35, P = 0.006$) sucrose were significantly attenuated relative to control. Although measured responses were negligible, responses to 0.01 and 0.032 M sucrose were not significantly suppressed following gurmarin application ($\alpha = 0.05$). Considering only those concentrations where significant suppression was noted, the amount by which post-gurmarin sucrose responses were attenuated did not significantly differ across sucrose concentration (pairwise comparisons of 0.1 M, 0.32 M and 1.0 M sucrose pre-/post-gurmarin response magnitude differences, Dunn MCP, $\alpha = 0.05$; see Figure 7).

Effects of gurmarin on neuronal responses to various sweet-tasting compounds

The effect of oral application of 10 $\mu$g/ml gurmarin on responses to various sweet-tasting compounds was explored in seven neurons. Given the small $n$ and the sometimes disparate effects observed across the cells, we present data from experiments conducted on individual neurons in Figure 8. For neuron N14, gurmarin treatment attenuated responses to sucrose, fructose, glycine, D-asparagine and D-histidine by at least 50% relative to control. Given the presence of residual post-gurmarin sweet responses, these data suggest that this neuron received input from gurmarin-sensitive and -insensitive receptor mechanisms that were rather broadly
tuned. In contrast, the overall lack of a gurmarin treatment effect noted for neuron N7 implied that sweet responses in this cell were mediated by input derived entirely from gurmarin-insensitive sweet transduction processes. However, data obtained from neurons N15, S5 and H5 were most interesting. For neuron N15, gurmarin treatment did not affect the response to sucrose or fructose. However, post-gurmarin responses to glucose and maltose were suppressed by at least 50%. Moreover, the post-gurmarin response to galactose was fully inhibited. Sweet responses were also differentially affected by gurmarin in neuron H5. For this cell, responses to sucrose and fructose were resistant to gurmarin treatment. However, responses to Na-saccharin, \(\text{D-asparagine}\) and \(\text{D-histidine}\) were suppressed by at least 50% relative to control whereas the post-gurmarin glucose response was fully inhibited. These data suggest that some NST neurons may receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms that are differentially tuned. Data obtained from neuron S5 further exemplify this point. This cell received information regarding the presence of maltose on gustatory epithelia from exclusively gurmarin-sensitive sweet transduction processes, as the response to maltose was completely inhibited following gurmarin treatment. Although the post-gurmarin response to sucrose was attenuated by at least 50% relative to control, the residual indicates that both gurmarin-sensitive and -insensitive receptors contributed to this response. Therefore, the tuning characteristics of the gurmarin-sensitive and -insensitive receptor processes that initiated the transmission of sucrose and maltose information to this neuron differed, as the gurmarin-insensitive component was not responsive to 0.5 M maltose.

-------------------

Insert Figure 8 about here

-------------------
Discussion

We recorded taste responses from 35 individual NST neurons prior to and following oral application of the sweet transduction blocker gurmarin to determine how gurmarin sensitivity is distributed across neuronal type. The majority of NST neurons in our sample received input from gurmarin-sensitive transduction processes. However, many of these neurons exhibited residual post-gurmarin responses to sucrose. As our gurmarin application procedure effectively bathed both the anterior tongue and palate, and taste stimulus delivery was limited to these areas, the presence of residual responses implied that these neurons were driven by both gurmarin-sensitive and -insensitive receptor mechanisms. Sucrose responses recorded from neurons representative of each HCA-defined class were affected by gurmarin, suggesting that information derived from gurmarin-sensitive receptor processes is not restricted to a single NST neuronal type upon arrival at the CNS. However, a differential proportion of cells within each neuronal class exhibited post-gurmarin responses to 0.5 M sucrose that were attenuated by at least 50% relative to control. Based on this criterion, the largest number of affected neurons was found in class S, followed by classes N and H, respectively. On average, post-gurmarin responses to 0.5 M sucrose were found to be significantly suppressed only in class S and N neurons. Additionally, the effect of gurmarin was sometimes differential across different sweet-tasting compounds within individual neurons, which implied that some NST cells may receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms that are differentially tuned to various sweet-tasting ligands. Responses to NaCl, HCl and quinine were not affected by gurmarin treatment.
Information from gurmarin-sensitive receptors is not restricted to a single NST neuronal type

The existence of gurmarin-sensitive and -insensitive receptor mechanisms was first suggested by data concerning the effects of gurmarin on whole-nerve responses to sweeteners recorded from rodents. Although integrated CT (Imoto et al. 1991; Miyasaka and Imoto 1995; Ninomiya and Imoto 1995; Ninomiya et al. 1998) and GSP (Harada and Kasahara 2000) responses to sucrose were found to be significantly suppressed following gurmarin treatment, a residual post-gurmarin response to this stimulus was evident in these recordings. Moreover, the inhibitory effect of gurmarin on CT responses to 0.5 M sucrose became asymptotic, though not absolute, at ~ 5 µM, which produced ~ 80% suppression relative to control; no further suppression was observed even if the tongue was treated with 240 µM gurmarin (Miyasaka and Imoto 1995). For C57BL mice, the effects of gurmarin have been shown to be nerve specific. Whereas integrated CT responses to various sweeteners were suppressed by gurmarin to roughly 50% of control in this species (Ninomiya and Imoto 1995; Ninomiya et al. 1997), those recorded from the glossopharyngeal nerve were recalcitrant to gurmarin treatment (Ninomiya et al. 1997). As gurmarin is believed to act on an apical receptor binding site (Miyasaka and Imoto 1995; Yoshie et al. 1994), these data suggest that at least two receptor processes for sweeteners exist in rodents, classified on the basis of their sensitivity to gurmarin (Ninomiya et al. 1999). Moreover, single sucrose-best CT neurons can be segregated into types on the basis of their susceptibility to lingual gurmarin treatment (Ninomiya et al. 1999), suggesting selective synaptic coupling between taste receptor cells (TRCs) that express gurmarin-sensitive or -insensitive receptors and particular subsets of sucrose-best CT fibers.

Selective coupling between specific types of TRCs and peripheral gustatory neurons has been shown by the effects of lingual amiloride treatment on responses to NaCl in single CT
fibers. Salt responses recorded only from those fibers responding best to NaCl were susceptible to amiloride treatment (Hettinger and Frank 1990; Ninomiya and Funakoshi 1988), suggesting that this fiber type exclusively innervates TRCs that express amiloride-sensitive Na\(^+\) transduction processes. This segregation of amiloride-sensitive salt information to a particular type of CT neuron is similar to the observation that gurmarin-sensitive sweet information is selectively distributed to a particular type of sucrose-best CT fiber (Ninomiya et al. 1999). Because amiloride-sensitive salt information is predominantly relayed to NaCl- (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000) and sucrose-best (Smith et al. 1996; St. John and Smith 2000) NST neurons, it could be hypothesized that gurmarin-sensitive sweet information is also differentially distributed across physiologically-defined NST neuronal types. Such organization may have implications for how sweet information is encoded by neural activity in the CNS, as the arrangement of amiloride-sensitive input contributes to the neural representation of salt information in the NST (Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; St. John and Smith 2000) and gustatory behavioral discrimination between salts (Spector et al. 1996).

To our knowledge, the present study is the first to explore the distribution of gurmarin sensitivity across taste-driven neurons in the CNS. Although gurmarin-sensitive information was not restricted to a particular class of neuron, a disproportionate percentage of cells from each neuronal class exhibited sucrose responses that were substantially attenuated following gurmarin treatment. Over half of the class S neurons exhibited post-gurmarin sucrose responses that were attenuated by at least 50% relative to control; the same could be said for less than half of the N or H class cells. Overall, post-gurmarin responses to sucrose were significantly attenuated only in
class S and N neurons. In some respects, the distribution of gurmarin sensitivity across NST neuronal types relative to those in the periphery (Ninomiya et al. 1999) is similar to that observed for amiloride-sensitive salt input, as the apparent restriction of information derived from a particular receptor process to a specific peripheral neuron class is not absolute in the CNS. However, amiloride is more effective at reducing responses to Na\(^+\) salts in those NST cells that respond maximally to these stimuli relative to the average gurmarin-induced attenuation of responding to sucrose observed in class S cells in the present study (see Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000). Although an amiloride-insensitive component is apparent, salt responses in NaCl-best NST neurons are predominantly derived from amiloride-sensitive salt input (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000) whereas gurmarin-sensitive and -insensitive receptor mechanisms contributed almost equally, on average, to sucrose responses in class S cells (see Figure 5). Although they are somewhat differently organized, the neuronal circuits that underlie amiloride-sensitive salt and gurmarin-sensitive sweet input to the brain distribute information to more than one category of NST neuron.

Convergence of neural information in the gustatory NST has been directly demonstrated (Ogawa et al. 1984; Sweazey and Smith 1987; Travers et al. 1986; Vogt and Mistretta 1990) and implied (Boughter and Smith 1998; Doetsch and Erickson 1970; Hill et al. 1983; Smith et al. 1996; St. John and Smith 2000; Travers and Smith 1979) by a number of studies. Moreover, many NST neurons in the present study appeared to receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms. This was partially suggested by the observation that, for the majority of these cells, residual post-gurmarin responses to 0.5 M sucrose were
found that exceeded statistical threshold whereas the effect of gurmarin on sucrose responses recorded from single mouse CT fibers is purportedly more absolute (Ninomiya et al. 1999); companion rat single fiber data do not presently exist. However, the presence of these residual responses by themselves may not necessarily reflect input derived from gurmarin-insensitive receptor components (Ninomiya et al. 1999), as factors such as inadequate gurmarin concentration and/or treatment could also result in such responses. However, our experimental measures taken to address these possibilities suggest otherwise.

We encountered taste-driven NST neurons during experimentation that did not appreciably respond to sucrose and, thus, were not included in our study. The composition of our neuronal sample reflects this: we recorded from many neurons that were subsequently typed as class S (40% of sample) and N (43% of sample) that responded well to sucrose. However, only 6 (17% of sample) sucrose-responding class H cells were found. Many cells with strong responses to HCl, NaCl and quinine were encountered that did not respond to sucrose, suggesting that some cells may not receive sucrose-mediated input from the anterior tongue and palate, areas known to be populated with TRCs that express sweet receptor mechanisms (Gilbertson et al. 2001). Moreover, this low $n$ may have influenced our findings regarding the effect of gurmarin on responses to sucrose across neuronal class, as failure to observe a significant effect of gurmarin in class H neurons may be attributable to low statistical power. Assuming this caveat to be true and that further investigation would yield a sizable number of sucrose-responsive and gurmarin-sensitive class H NST neurons, our analogy between the differential distribution of gurmarin-sensitive sweet and amiloride-sensitive salt information across NST neuronal types could be rendered less appropriate, although a recent report showed that amiloride
significantly attenuated responses to NaCl in some HCl-best NST neurons (St. John and Smith 2000).

**The effects of gurmarin varied across sweeteners within individual neurons**

Although neurophysiological data are limited, various psychophysical studies have suggested multiple receptor mechanisms for sweet compounds. Intensity matching experiments in humans indicated that concentrations of fructose, glucose and sucrose could be found that rendered these stimuli indiscriminable (Breslin et al. 1996). However, higher concentrations of maltose could not be matched using this procedure, suggesting that maltose activates a separate receptor process. It was recently demonstrated that whole-mouth adaptation to fructose increased discriminability between fructose and glucose in humans, indicating that these two sugars possibly stimulate separate receptor sites (Tharp and Breslin 2002). Other human cross-adaptation experiments have reported similar findings of incomplete cross-adaptation among various sweet stimuli (Faurion et al. 1980; Froloff et al. 1998; Schiffman et al. 1981). Moreover, discrimination experiments, in which intensity was rendered an irrelevant cue (Spector et al. 1997), have shown that rats can discern sucrose from maltose, suggesting independent receptor mechanisms for these stimuli in the rodent. Our data complement these findings by showing that some neurons received input from gurmarin-sensitive and -insensitive receptor processes that responded differentially to sweet compounds (see Figure 8), suggesting that some receptor mechanisms are sensitive to only a subset of, and not all, sweet-tasting ligands. Moreover, our data indicate that some neurons may receive input from TRCs expressing gurmarin-sensitive receptor processes that are unresponsive to sucrose (see Figure 8). Although our low *n* with regard to this type of data warrants further investigation, a complementary differential effect of
gurmarin on responses to a sweetener array has been observed in integrated GSP nerve recordings in rats. Phasic responses to sucrose, fructose, lactose and maltose were significantly inhibited, whereas responses to galactose and glucose were unaffected following palatal gurmarin treatment (Harada and Kasahara 2000). The convergence of peripheral fibers that are driven by differentially tuned gurmarin-sensitive and -insensitive receptor processes onto NST neurons could account for the observed idiosyncratic effects of gurmarin on neuronal responses to various sweeteners.

Recent advances in molecular biology suggest the existence of a single mammalian receptor for sweets, T1R2/T1R3, as this candidate responded to all sweet taste stimuli tested (Li et al. 2002). However, other reports suggest that T1R2/T1R3 is extremely selective, recognizing only a limited range of sweet compounds (Nelson et al. 2001). Moreover, T1R2 is purportedly undetectable in most fungiform taste papillae (Hoon et al. 1999), yet some fungiform TRCs clearly respond to sucrose (Gilbertson et al. 2001). Although our understanding of sweet taste reception is far from complete, it is generally agreed that sweet stimuli activate TRCs through at least two transduction pathways: one involves the generation of cyclic nucleotides, the other modulates levels of inositol triphosphate (Herness and Gilbertson 1999; Lindemann 2001; Lindemann 1996). Receptor and transduction processes are the initial steps towards gustatory perception, which is ultimately a product of neural information processing in the brain. Further understanding of the neural mechanisms underlying sweet perception will necessitate the derivation of the relationships between receptor mechanisms and the physiology of neurons in the CNS.
Implications for gustatory neural information processing

Although scant, data regarding the effects of gurmarin on gustatory behavioral tasks do exist. Gurmarin was found to suppress the avoidance of sucrose for C57BL mice trained in a conditioned taste aversion paradigm (Nakashima et al. 2001). Additionally, rats fed a diet containing Gymnema sylvestre exhibited a transient reduction in preference for sucrose: intake decreased and subsequently recovered several days later at a time when gurmarin binding proteins, which suppress the activity of gurmarin, appeared in the saliva of these subjects (Katsukawa et al. 1999). The present study demonstrated that sucrose responses recorded from class S and N neurons were significantly attenuated following gurmarin treatment, indicating that these cells processed gurmarin-sensitive sweet input. Therefore, activity generated by class S and N neurons possibly contributes to such sucrose-mediated behavioral tasks. This correlate reinforces the notion of a distributed neural code for taste in the CNS, where activity generated by a network of individual cells of different physiologically-defined types underlies the neuronal representation of multiple stimulus qualities and parameters (Erickson 1968; Pfaffmann 1959; Scott and Giza 2000; Smith and St. John 1999).
Acknowledgements

This work was supported in part by National Institute of Deafness and Other Communication Disorders Grants DC005270 to C. H. Lemon and DC00353 to D. V. Smith. The authors would like to thank Dr. John D. Boughter, Jr. for valuable comments on this manuscript. A portion of these results was presented at the 2002 meeting of the Association for Chemoreception Sciences, Sarasota, FL.

Address for reprint requests: C. H. Lemon, Department of Anatomy and Neurobiology, University of Tennessee College of Medicine, 855 Monroe Ave., Suite 515, Memphis, TN 38163.
References


Figure legends

Figure 1. A. Across neuron patterns of response to the basic stimuli. Net response magnitude is represented along the ordinate whereas neurons are segregated from left to right along the abscissa into best-stimulus types (sucrose-best, NaCl-best and HCl-best cells, respectively) and rank ordered within each type according to the magnitude of the response to their best stimulus (e.g., S1 denotes the sucrose-best neuron with the largest response to sucrose among sucrose-best units; N5 indicates the NaCl-best cell with the fifth largest response to NaCl relative to other NaCl-best units). Taste-evoked net response rates were averaged for neurons where stimulus trials were replicated. B. Entropy values for each neuron calculated using net response magnitudes evoked by each component of the basic stimulus set.

Figure 2. Dendrogram depicting the results of hierarchical cluster analysis performed on 35 taste-driven NST neurons. Degree of neuronal correlation is represented along the ordinate whereas individual neurons are denoted along the abscissa using labels described in Figure 1. Labels are offset using arrows to highlight best-stimulus groups relative to neuronal classes suggested by the solution.

Figure 3. Digital oscilloscope records obtained from two taste-driven NST neurons depicting almost complete (neuron A) and partial (neuron B) suppression of the response to 0.5 M sucrose following application of 10 µg/ml gurmarin to both the tongue and palate. For neuron A, the response to sucrose was almost fully inhibited by gurmarin treatment (unadulterated response = 4.6 net spikes/s; following gurmarin = 0.2 net spikes/s) whereas the response to 0.1 M NaCl remained intact (unadulterated response = 4.8 net spikes/s; following gurmarin = 4.4 net
spikes/s). For neuron B, the magnitude of the post-gurmarin response to 0.5 M sucrose was attenuated by 68% relative to control (unadulterated response = 20 net spikes/s; following gurmarin = 6.4 net spikes/s). As observed in neuron A, gurmarin does not alter neuron B’s response to 0.1 M NaCl (unadulterated response = 8.2 net spikes/s; following gurmarin = 9.0 net spikes/s). The waveform templates used to match spikes that arose from each neuron are shown; time scale does not apply to templates. Upward arrows denote stimulus onset.

**Figure 4.** Across neuron patterns of response evoked by 0.5 M sucrose under control conditions (A) and following oral application of 10 µg/ml gurmarin (B). Neurons are segregated according to HCA class, rank ordered within each class according to the net response magnitude evoked by sucrose under control conditions and identified along the abscissa using labels described in Figure 1. If applicable, responses were averaged across multiple sucrose presentation trials recorded from the same neuron within each condition. An averaged response is denoted by an integer, which indicates the number of trials reflected by the mean. C. Across-neuron difference between the control and post-gurmarin across neuron patterns of response evoked by 0.5 M sucrose. The percentage criterion by which the post-gurmarin response to 0.5 M sucrose was attenuated relative to control is indicated for each neuron.

**Figure 5.** The effect of gurmarin on responses to 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl and 0.01 M quinine-HCl as observed across each HCA-derived neuronal class. Oral application of gurmarin significantly attenuated responses to sucrose in class S (mean suppression = 46.5% ± 9.7 SEM) and N (mean suppression = 32.1% ± 12.5 SEM) neurons. Bars indicate mean response plus SEM. Asterisks indicate a statistically significant difference between mean control and
post-gurmarin response magnitudes ($\alpha = 0.01$). Note that the scale for the class S bar graph differs from that of classes N and H.

**Figure 6.** Effects of oral application of 10 and 20 µg/ml gurmarin on sucrose responses recorded from three neurons.  

*Panel A.* Data from neuron N13. Although different from control, responses to 0.5 M sucrose and sucrose concentration-response functions measured following treatment with the standard and doubled concentration of gurmarin were similar.  

*Panel B.* Data from neurons N10 and N15. Responses to 0.5 M sucrose measured under control conditions (trials < 0) and following either 10 (trials 1 and 2) or 20 (trial 3) µg/ml gurmarin treatment were nearly identical.

**Figure 7.** Mean (± SEM) sucrose concentration-response functions obtained from 19 neurons that were tested with an ascending sucrose concentration series both prior to and following application of gurmarin to the tongue and palate.

**Figure 8.** Effect of oral application of gurmarin on responses to various sweet compounds as observed for seven neurons. Gurmarin treatment was sometimes selective for particular stimuli within a cell. A single asterisk denotes post-gurmarin response attenuation of $\geq 50\%$ whereas double asterisks signify $\geq 100\%$ suppression relative to control. See Table 1 for stimulus abbreviations.
**Table 1.** Taste stimuli and abbreviations

<table>
<thead>
<tr>
<th>Stimuli representative of basic taste qualities</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 M sucrose (suc, “sweet”)</td>
</tr>
<tr>
<td>0.1 M sodium chloride (NaCl; “salty”)</td>
</tr>
<tr>
<td>0.01 M hydrochloric acid (HCl; “sour”)</td>
</tr>
<tr>
<td>0.01 M quinine hydrochloride (quinine; “bitter”)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration series</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 M sucrose</td>
</tr>
<tr>
<td>0.032 M sucrose</td>
</tr>
<tr>
<td>0.1 M sucrose</td>
</tr>
<tr>
<td>0.32 M sucrose</td>
</tr>
<tr>
<td>1.0 M sucrose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other sweet compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 M fructose (fru)</td>
</tr>
<tr>
<td>0.3 M galactose (gal)</td>
</tr>
<tr>
<td>0.5 M glucose (glu)</td>
</tr>
<tr>
<td>0.3 M glycine (gly)</td>
</tr>
<tr>
<td>0.5 M maltose (mal)</td>
</tr>
<tr>
<td>0.01 M sodium saccharin (sac)</td>
</tr>
<tr>
<td>0.1 M d-asparagine (asp)</td>
</tr>
<tr>
<td>0.1 M d-histidine (his)</td>
</tr>
</tbody>
</table>
Figure 1

A

sucrose 0.5 M

NaCl 0.1 M

HCl 0.01 M

quinine 0.01 M

spontaneous discharge

B

entropy

H

S1, S5, S10, N1, N5, N10, N15, H1, H5

neuron
Figure 3

A  sucrose 0.5 M
    unadulterated
    following gurmarin 10 μg/ml

B  sucrose 0.5 M
    unadulterated
    following gurmarin 10 μg/ml

NaCl 0.1 M
    unadulterated
    following gurmarin 10 μg/ml

1 s
Figure 4

A. Sucrose 0.5 M

B. Sucrose 0.5 M following gurmarin 10 μg/ml

C. Difference

Legend:
- < 35%
- > 35%
- > 50%
- > 90%
Figure 5

Class S

Class N

Class H

Net spikes / s

Stimulus

Unadulterated

Following gurmarin 10 µg/ml
Figure 6

A

Neuron N13

B

Neuron N10

Neuron N15

sucreose 0.5 M presentation trial

net spikes / s
Figure 7

The figure shows the relationship between sucrose concentration and net spikes per second. The data points are plotted with error bars, indicating the variability in the measurements. Two lines are depicted:

- Solid line: unadulterated
- Dashed line: following gumarin 10 µg/ml
Figure 8

Neuron N14

Neuron N11

Neuron N15

Neuron N7

Neuron S3

Neuron S5

Neuron H5

net spikes / s

stimulus

unadulterated
following gurmarin 10 µg/ml