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Temperature systematically modifies neural activity for sweet taste

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Temperature and sweet taste processing

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Temperature can modify neural and behavioral responses to taste stimuli that elicit "sweetness", a perception linked to intake of calorie-laden foods. However, the role of temperature in the neural representation of sweet taste is poorly understood. Here we made electrophysiological recordings from gustatory neurons in the medulla of inbred mice to study how adjustments in taste solution temperature (°C) to cool (18°), ambient (22°), and warm (30° and 37°) values changed the magnitude and latency of gustatory activity to sucrose (0, 0.05, 0.1, 0.17, 0.31, and 0.56 M). Analysis of 22 sucrose-best neurons revealed temperature markedly influenced responses to sucrose, which, across concentrations, were largest when solutions were warmed to 30°. Yet reducing solution temperature from warm, to ambient, to cool progressively steepened the slope of the sucrose concentration-response function computed across cells ($P < 0.05$), indicating mean activity to sucrose increased more rapidly with concentration steps under cooling than with warming. Thus, the slope of the sucrose concentration-response function shows an inverse relation with temperature. Temperature also influenced latency to the first spike of the sucrose response. Across neurons, latencies were shorter when sucrose solutions were warmed, and longer, by hundreds of milliseconds, when solutions were cooled ($P < 0.05$), indicating temperature is also a temporal parameter of sucrose activity. Our findings reveal temperature systematically modifies the timing of gustatory activity to sucrose in the mammalian brain, and how this activity changes with concentration. Results further highlight how oral somatosensory cues function as physiological modulators of gustatory processing.

Keywords: taste, sucrose, temperature, latency, slope
Introduction

The study of neural coding involves, in part, relating features of sensory stimuli to neural activity. For taste, this pursuit has largely focused on two stimulus features: the perceptual quality of the taste chemical and its concentration while dissolved in solution. Taste quality is a descriptive characteristic of taste stimuli transmitted, in part, through substitution (cf. Stevens 1961) of responses by different neurons. For instance, exchanging a taste stimulus of one quality (e.g., “sweet”) for another (e.g., “salty”) can cause a substitutive change in the neurons that respond maximally to the stimulus, leading to an associated change in the evoked response pattern. Concentration, on the other hand, is a physical property of taste stimuli that guides the magnitude of gustatory responses.

Temperature is an additional physical property of taste stimuli that can modulate gustatory processing. Temperature strongly influences neural and behavioral responses to sweet stimuli. Gustatory activity to sucrose in peripheral nerves carrying sensory input from the tongue to brain is greater when taste solutions are warmed than cooled (Breza et al. 2006; Lu et al. 2012; Talavera et al. 2005; Yamashita and Sato 1965). This effect arises, in part, from the actions of temperature on the transient receptor potential (TRP) ion channel TRPM5: a heat-activated cation channel that plays a key role in the molecular receptor transduction cascade for sweet stimuli (Perez et al. 2002; Talavera et al. 2007; Talavera et al. 2005; Zhang et al. 2003). In humans, the detection and perceived intensity of sweet substances can vary with change in solution, and also tongue, temperature (Bartoshuk et al. 1982; Calvino 1986; Green and Frankmann 1988; Green and Frankmann 1987; Green and Nachtigal 2012; McBurney et al. 1973), indicating temperature importantly guides the generation of sweet taste perceptions.

Studies on the influence of temperature on gustatory responses have largely focused on peripheral and psychophysical effects, leaving only a paucity of data on the influence of temperature on neural representations of sweet stimuli in the brain. A recent electrophysiology
study on gustatory neurons in the mouse nucleus of the solitary tract (NTS) showed that warming taste solutions increased responses to 0.1 M sucrose in a supralinear manner, and that cooling taste solutions largely inhibited sucrose activity (Wilson and Lemon 2013). These results suggested temperature modified the gain of the neural response to sucrose. However, a broad focus of this work on multiple taste qualities afforded only limited exploration of this hypothesis.

The goal of the present study was to quantitatively define how stimulus temperature operates on the neural representation of sucrose taste in the brain. We recorded electrophysiological responses from sucrose-best NTS neurons in inbred mice during oral delivery of 6 concentrations of sucrose tested at 4 temperatures. Concentrations included perithreshold to strong intensities, and temperatures ranged from cool to physiological warm. Analysis of neural data focused on the effect of temperature on the slope of the sucrose concentration-response function, which indexes rate of growth in response with concentration, and on latency to first spike to sucrose. The latency of a gustatory response could conceivably influence the evolution of taste perceptions during the time course of flavor (Green and Frankmann 1988) and contribute information about gustatory stimuli (Breza et al. 2010; Graham et al. 2014; Hallock and Di Lorenzo 2006). Results showed change in temperature induced systematic change in both the slope and latency parameters of neural responses to the sucrose concentration series. Our discussion centers on how temperature operates as a parameter of the magnitude and timing of gustatory activity to sucrose. Moreover, the present data further build on the postulate that oral somatosensory cues importantly guide and modulate the operation of neurobiological substrates for taste (e.g., Green 2002; Green and Nachtigal 2012; Wilson and Lemon 2013).
Materials & Methods

Mouse line and preparation

Twenty-five adult mice (8 females, mean weight = 24.8 g ± 2.1 standard deviations [SD]; 17 males, mean weight = 28.3 g ± 1.9 SD) from the C57BL/6J (B6) inbred strain (The Jackson Laboratory, Mar Harbor, ME) were used. B6 mice were selected as this line harbors the “taster” allele of the genetic locus Sac, the saccharin-preference locus, and, accordingly, shows robust sensitivity to and preference for stimuli that taste “sweet” to humans (Fuller 1974; Lush 1989). Sac is associated with the T1r3 taste receptor protein involved with sweet taste (Bachmanov et al. 2001; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Sainz et al. 2001).

Mice were housed in a vivarium that maintained a 12/12-hour light/dark cycle and an air temperature of approximately 23°C. All mice were naïve to experimentation. Room temperature water and standard rodent chow were available in the colony ad libitum.

All procedures were performed on mice under anesthesia in accordance with National Institutes of Health guidelines and protocols reviewed and approved by the Saint Louis University Institutional Animal Care and Use Committee. Mice were acutely anesthetized using a mixture of ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) to facilitate insertion of a tracheostomy tube. Atropine (0.024 mg/kg, i.p.) was administered to reduce bronchial secretions. Each mouse was positioned in a non-traumatic head holder, and a custom “nose cone” for gas anesthesia was placed over the open end of the tracheostomy tube, which was angled away from the mouth. The nose cone was intentionally designed not to seal onto the tracheostomy tube, but to allow for continuous weak pressure delivery and vacuum removal of anesthetic gas to and from the airspace surrounding the distal opening of this tube. Mice then freely respired this gas through their tracheostomy tube, without the aid of a mechanical ventilator. Anesthesia was maintained throughout recording sessions using 1 to 1.5% isoflurane in oxygen, where a high-precision vaporizer (Dräger Medical, Lübeck, Germany) regulated isoflurane concentration.
Other surgical and electrophysiological recording procedures from mouse NTS were as described (Lemon and Margolskee 2009; Wilson et al. 2012).

Stimuli

The presentation of taste solutions was carried out using a custom apparatus, as described (Lemon and Margolskee 2009; Wilson and Lemon 2013). Briefly, stimuli were loaded one at a time into a funnel and tubing system suspended above the preparation and drawn into the mouse oral cavity by gravity. A three-way, computer-controlled fluid valve precisely regulated the timing of taste stimulus flow, and switching between taste and rinse solutions during a trial, as below. This valve was positioned such that a small plastic elbow (approximately 1.5 mm inner diameter) extended the output passage of the valve into the mouse oral cavity. A small, sealable hole was punctured in the ventral end of the elbow for insertion of a fine, fast-response thermocouple probe, which continuously monitored the temperature (to 0.1°C accuracy, sampled at 1 kHz) of solution flow at the moment of oral delivery. This system facilitated broad-field application of taste and rinse solutions to multiple oral epithelia, including rostral, caudal, and lateral regions of the tongue, and also the palate.

Taste solutions included 0.3 M D-fructose (monosaccharide), 0.03 M NaCl (sodium salt), 0.003 M citric acid (organic acid), 0.01 M quinine-HCl (alkaloid; bitter), and six concentrations of sucrose (disaccharide), including 0 (purified water), 0.05, 0.1, 0.17, 0.31, and 0.56 M. Sucrose concentrations were approximate quarter-log steps selected to cover a dynamic behavioral response range in mice, from perithreshold (0.05 M) to strongly appetitive (0.31, 0.56 M; e.g., Treesukosol and Spector 2012). Concentrations of other stimuli followed from our previous neural recording studies in mice. All taste chemicals (Sigma, St. Louis, MO) were high purity and dissolved in purified water. Taste solutions were stored in airtight glass bottles; dark glass was used for quinine, a light-sensitive compound.
Bottles of taste solutions were placed into circulated refrigerated and warming water baths to regulate their temperature. A separate bath and group of stimulus bottles was used for each temperature condition. Solutions of one concentration of sucrose tested at multiple temperatures were generally drawn from one stock to ensure concentration was invariant across thermal conditions. Sucrose solutions were cooled and warmed to four target temperatures (in °C): 18° (cool), 22° (ambient), 30° (warm), and 37° (warm; physiological). Temperature values were selected to provide non-noxious cooling and warming (cf. Wilson and Lemon 2013). The actual mean temperatures measured within condition, as sampled during the last 3 sec of stimulus delivery across all sucrose trials, were (in °C/msec ± SD) 17.8° ± 1.3°, 21.8° ± 1.2°, 30.1° ± 1.4°, and 37.1° ±1.2°. Temperature was expressed as the rate °C/msec to index the stability of the actual stimulus temperature relative to the target and facilitate use of change in the thermal signal for detection of stimulus onset time, described below. Other references to stimulus temperature in this manuscript refer to the target values above. Fructose, NaCl, citric acid, and quinine were used only for neuronal classification and were tested at 22°. Oral delivery of a rinse of purified water at 22° preceded and followed presentation of each taste stimulus, and also continued in between trials during data collection. This rinse aimed to maintain a stable oral adapting temperature, and to adapt mechanosensory activity associated with taste stimulation. A stainless steel, brazed plate, fluid-to-fluid heat exchanger (AIC Inc., model LA14-10X) controlled the temperature of the rinse solution, which flowed through the exchanger under light pressure. A closed-loop water supply set to 22° fed the temperature regulation circuit of the heat exchanger. The actual mean temperature of the adapting rinse at the oral cavity, as acquired during the last 3 sec of the pre-stimulus period across all sucrose trials, was 21.8°C/msec ± 1.2° SD.

Data acquisition
Electrophysiological activity was recorded using a high-impedance (2 to 5 MΩ) tungsten microelectrode. The NTS was located in mice as described (Lemon and Margolskee 2009; Wilson et al. 2012; Wilson and Lemon 2013). When searching for single units, the electrode was lowered into the nucleus and neural activity to oral delivery of 0.5 M sucrose was monitored. The use of an appetitive concentration of sucrose as the primary search stimulus intended to purposefully target units with high sensitivity to sucrose, cells at the focus of the present work. Nonetheless, data were recorded from isolated taste-sensitive neurons, with the sucrose-orientation of cells identified post-hoc, as below. Unit activity in mouse NTS was digitized at 25 kHz (1401 interface and Spike2 software, CED, Cambridge, UK) and spikes from single units identified based on waveform consistency (e.g., Figures 1, 4). The thermocouple circuit that monitored the temperature of the rinse and stimulus flow was also linked to the data acquisition system. Thus, temperature data were recorded simultaneously alongside neural activity.

Each isolated neuron was first tested with 22° solutions of fructose, NaCl, citric acid, and quinine, in random order. Next, neurons were tested with each of the six concentrations of sucrose adjusted to 18°, 22°, 30°, and 37°. To randomize presentation order, the four temperatures used for one sucrose concentration were grouped into a single block, and the sequence of the six concentration blocks was randomized for each cell. The ordering of temperature trials within each block was also randomized.

Each data acquisition trial lasted 15 sec. The 22° adapting water rinse was presented during the first 5 sec of a trial. Flow then immediately switched to stimulus delivery for 5 sec. Flow retuned to 22° water after the taste presentation epoch. The inter-trial interval was approximately 2 to 3 min. Mice did not ingest solutions, which fell into a drain positioned beneath the mandible.

Data analysis proceeded in two phases. Phase 1 assessed how stimulus temperature interacted with concentration to influence neuronal response magnitude to sucrose. Phase 2 assessed how stimulus temperature influenced the latency and timing of neural responses to
intensity-varied solutions of sucrose. The types of data and analytic approach were unique to each phase, as follows.

**Phase 1: analyses of response magnitude**

Analyses in phase 1 were performed on the magnitude of neural activity to taste solutions. For each trial, the number of action potentials that arose during the 5 sec stimulus presentation period was defined as response magnitude. A “long” 5 sec response window was used to capture general effects. The pre-stimulus firing rate for each trial was calculated as the average number of spikes that emerged in one sec during the 5 sec period that preceded taste stimulus presentation. Although indexed, pre-stimulus activity was not subtracted from spike counts collected during taste presentation to avoid negative responses in regression analysis of log-transformed data, as below. Nonetheless, inspection of average trends in activity to sucrose both corrected and uncorrected for pre-stimulus firing revealed similar effects of temperature between these conditions.

Hierarchical cluster analysis was used to sort neurons into groups with unique response profiles across stimuli, and to identify neurons most responsive to sucrose. Cluster analysis was performed on a matrix of pairwise correlation distances among neurons computed from their responses to 22° solutions of fructose, NaCl, citric acid, quinine, and all six concentrations of sucrose. Group average amalgamation was used. Scree plots determined groupings in the cluster solution. Because cluster analysis was used only to detect neurons with strong sucrose sensitivity, the clustering solution (e.g., dendrogram) was not a main finding and, thus, is not shown here.

Double-log regression was applied to sucrose concentration-response data at each temperature, operating on the logarithm (base 10) of response magnitude and the logarithm of concentration, to study how temperature influenced the rate by which activity to sucrose grew with concentration. The slope of the regression line fitted to sucrose responses in doubly
logarithmic coordinates estimated the percentage change in the response expected when concentration increased by 1% (cf. Wooldridge 2009), assuming all other variables were constant. For this analysis, unit responses to sucrose were transformed as $\log(resp + 1)$, where $resp$ was the response magnitude, and adding 1 to $resp$ allowed the logarithm of magnitude to be taken when $resp = 0$. A $t$-test evaluated the deviation of slopes from 0 or 1. The 95% confidence interval of the mean (95% CI) was computed for responses to sucrose and regression slopes measured under different stimulus conditions. If the 95% CIs of two estimates did not overlap at all, they were interpreted as different at the $\alpha = 0.05$ level (cf. Whitlock and Schluter 2009). Where reported, 95% CIs were described as [lower bound, upper bound].

Phase 2: analyses of timing of neural activity to sucrose

Analyses in phase 2 focused on the influence of solution temperature on the latency to the first spike of the sucrose response. To measure this effect, we estimated on each trial the time of sucrose entry into the mouth, defined as stimulus onset, and the time post-onset when a statistically significant increase in neuronal discharge to sucrose occurred, defined as latency.

Time of stimulus onset during sucrose trials

The continuous monitoring of solution temperature at the moment of oral delivery afforded use of change in temperature from baseline as a marker of stimulus onset during presentations of cooled and warmed sucrose. For these trials, the mean temperature (in °C/msec) of the adapting pre-stimulus rinse, $\bar{T}_{\text{adapt}}$, was calculated, and the solution temperature sampled during each consecutive msec of the taste stimulation period was compared to this value using $\Delta T = 0.5^\circ$ as a threshold for temperature change. For trials that tested cooled (18°) sucrose solutions, stimulus onset was defined as the time (1 msec
resolution) during taste stimulation when oral solution temperature fell below $T_{\text{adapt}} - 0.5^\circ$ (cf. Figure 1A). For trials that tested warmed (30° and 37°) solutions, onset was defined as the time at which stimulus temperature exceeded $T_{\text{adapt}} + 0.5^\circ$. In contrast to cooling and warming trials, sucrose solutions tested at 22° were isothermal with the adapting baseline temperature. Thus, stimulus onset for each particular concentration of sucrose at 22° was estimated independently for each neuron by averaging the onset times for that concentration measured at 18° and 30°.

Analysis of stimulus onset times revealed solution delivery rate varied nominally with cooling and warming of taste solutions. The mean stimulus onset time for all trials tested at 18° (5.45 sec ± 0.02 SD; min = 5.42; max = 5.47) was slightly delayed (Bonferroni-adjusted pairwise comparisons, $P < 0.001$; repeated measures ANOVA, $F_{2,42} = 519.7, P < 0.001$), by approximately 100 msec, relative to the mean onset times for trials at 30° (5.35 sec ± 0.02 SD; min = 5.32; max = 5.37) and 37° (5.34 sec ± 0.01 SD; min = 5.32; max = 5.37). Using these data and the known flow rate of the stimulus delivery system at room temperature (approximately 1.4 ml/sec), flow rates during cooling and warming trials were estimated at, respectively, 1.33 ml/sec (velocity = 72.75 cm/sec) and 1.47 ml/sec (80.75 cm/sec), with an approximate difference of 150 μl/sec (8 cm/sec).

The subtle variance in stimulus delivery rates across cool and warm temperature conditions likely produced only negligible, if any, effect on measurement of latency to neuronal activation. For instance, time to stimulus onset also subtly varied within each temperature-concentration condition for sucrose (average SD = 30 msec), albeit, within each condition, there was no significant correlation ($P > 0.06$; mean $r = -0.13$) between stimulus onset time and latency to neural response. Moreover, changes in temperature were found here to induce shifts in neuronal response latency to sucrose on the order of many hundreds of milliseconds (e.g., Figure 1, 4, 5A). Such shifts in time were substantially larger than the observed variation in stimulus onset.
The time of stimulus onset on each trial was set as the zero point along the time scale of the neuronal spiking response. All spike times on a trial were expressed relative to this zero point, as was the latency to the first spike of the sucrose response.

Latency to neural response to sucrose

Latency to first spike during sucrose presentation was defined as the time of the first significant elevation in firing rate from the pre-stimulus rate. Only significant increases in responding were assessed, as some cells had low or zero pre-stimulus activity, precluding detection of inhibition. A binless algorithm (cf. Bair and Koch 1996; Chase and Young 2007; Schumacher et al. 2011) quantified latency on individual sucrose trials. For description, let us assume $s$ represents a counter for spikes that arose in series following stimulus onset. For each of these spikes considered in sequence, a Poisson-based statistical model estimated the probability that a firing rate equal to or greater than $s$ was due to lingering pre-stimulus activity at time $t_s$, which was the time lag (precision = 0.1 msec) from stimulus onset to spike $s$. This probability was given by:

$$Pr[\geq s] = 1 - \sum_{i=0}^{s-1} \frac{(t_s \lambda)^i e^{-t_s \lambda}}{i!}$$

where $\lambda$ was the mean pre-stimulus firing rate (spikes/sec) for the neuron under consideration, as averaged across all 24 temperature-concentration combination trials for sucrose. When iteratively computing $Pr[\geq s]$, $\lambda$ was multiplied by the period, in sec, of the current spike count window, $t_s$. This scaled $\lambda$ according to the size of the potential response window (cf. Chase and Young 2007). If $Pr[\geq s]$ became less than $10^{-6}$, a firing rate of $s$ spikes at time $t_s$ was unusually high relative to pre-stimulus firing (Bair and Koch 1996; Chase and Young 2007; Schumacher et
al. 2011), and the time of spike \( s \) (i.e., \( t_s \)) was taken as latency to first spike. If this criterion was not met for spikes falling within 4 sec of stimulus onset, response latency was left undefined. This statistical criterion for latency worked reasonably well for NTS gustatory units, as based on visual inspection of all latencies plotted against spike rastergrams (e.g., Figure 4).

Due, in part, to imbalance in the number of latencies obtained between certain temperature-concentration conditions, a bootstrap-\( t \) resampling procedure (cf. Wilcox 2003) was used to approximate the sampling distribution of median response latency for each condition, and to estimate the confidence interval for the median. To do this, \( n \) replicates of the actual \( n \) latencies measured for one thermo-concentration condition were randomly resampled, with replacement, 1000 times. Following each resample, a Studentized bootstrap-\( t \) (\( T^* \)) was computed as given by: 

\[
T^* = (m^* - m) / s^* ,
\]

where \( m^* \) was the median of the current bootstrap resample, \( m \) was the median of the actual latency data, and \( s^* \) was the standard deviation of the medians of 100 nested bootstrapped resamples of the current bootstrap resample. The resulting distribution of \( T^* \) values was used to calculate a 95% bootstrapped confidence interval (95% CI*). The 95% CI* for sample median \( m \) was given by:

\[
[T^*_0.025 \times s_{M^*} + m, T^*_0.975 \times s_{M^*} + m],
\]

where \( T^*_0.025 \) and \( T^*_0.975 \) were the respective 2.5 and 97.5 percentile \( T^* \) values, and \( s_{M^*} \) was the standard deviation of the distribution of medians for the 1000 bootstrap-generated resamples of latency. Confidence intervals were compared between different temperature-concentration conditions to assess trends in the effect of temperature on latency to first spike to sucrose.

ANOVAs were performed using SPSS (version 20, IBM, Somers, NY). All other mathematical, statistical, and plotting procedures were carried out using custom code and proprietary functions in MATLAB (version 8.1, The MathWorks, Inc., Natick, MA). Regression and bootstrap resampling procedures were performed using methods included with the MATLAB statistics toolbox (version 8.2).
Results

Trains of action potentials were recorded from 35 NTS neurons in B6 mice. Cluster analysis of responses to all stimuli tested at 22° revealed twenty-two of these cells responded “best” to 0.56 M sucrose; these cells were called S-type neurons. Other identified neuron types included cells that responded best to quinine \((n = 3)\), those that responded best to NaCl \((n = 9)\), and one acid best unit. When collapsed across all concentrations excluding 0 M, sucrose tested at 22° induced significantly larger responses (non-overlapping 95% CIs) in S-type neurons (mean response magnitude = 66.5 spikes, 95% CI [56.4, 76.5]) compared to Na⁺-best (mean response magnitude = 16.4 spikes, 95% CI [12.7, 20.2]) or quinine-best (mean response magnitude = 32.6 spikes, 95% CI [12.5, 52.7]) cells. Because this study focused on the neural signaling of sucrose taste, all analyses herein involved response data from only S-type units, which composed 63% of recorded cells. In NTS, S-type cells arise at a reduced frequency in mouse lines with relatively lower behavioral sensitivity to sucrose (McCaughey 2007) and do not emerge in mice genetically deficient for the T1r3 taste receptor protein (Lemon and Margolskee 2009), which is a component of the neural circuit that guides normal behavioral preference for sucrose (Damak et al. 2003; Zhao et al. 2003). Thus, S-type neurons likely play a key role in the central processing of sucrose taste in mice. Digital oscilloscope sweeps depicting responses by one S-type neuron to 0.56 M sucrose tested at cool, ambient, and warm temperatures are shown in Figure 1. Each of the 22 S-type neurons was tested with all six concentrations of sucrose at all four temperatures, which made 528 trials for sucrose available for analysis. S-type cells displayed low activity during the pre-stimulus period of all sucrose trials (mean spiking rate = 0.62 Hz ± 1 SD).

Temperature influences the slope of the concentration-response function to sucrose in S-type neurons.
We first studied how variation in stimulus temperature would impact the magnitude of the neuronal response to sucrose. Figure 2A depicts the mean concentration-response functions for sucrose, in linear coordinates, measured across temperature conditions. Visual inspection of this plot revealed that at each temperature, activity to sucrose generally, and expectedly, increased monotonically with increasing concentration steps. However, the magnitude of the sucrose concentration-response function varied markedly across temperatures. This observation was statistically explored through a two-way repeated measures ANOVA on responses to 0.1, 0.17, 0.31, and 0.56 M sucrose tested at 18°, 22°, 30°, and 37°; responses to 0 and 0.05 M sucrose violated assumptions of ANOVA and were not included in this analysis. Following a significant concentration × temperature interaction ($F_{9,189} = 7, P < 0.001$), tests of simple effects of temperature revealed neuronal responses to sucrose, at all concentrations considered, systematically increased with warming from 18°, to 22°, to 30°, then decreased with further warming to 37° (Bonferroni-adjusted pairwise comparisons, $P < 0.04$). Thus, under the present conditions augmentation of neural activity to sucrose by warming must asymptote near a temperature of 30°, as further increases in temperature began to reduce responses (cf. Wilson and Lemon 2013). In fact, the response to 0.56 M sucrose, albeit increased by warming from 22° to 30°, did not differ between 22° and 37° (Bonferroni-adjusted pairwise comparison, $P = 1$).

In addition to its influence on magnitude, temperature appeared to markedly influence the shape of the sucrose concentration-response function (Figure 2A), which reflects the degree of change in spike discharge induced by stepping concentration. This observation was quantitatively explored through log transformation of the data. Plotting neural activity to sucrose against concentration in doubly logarithmic coordinates, using the logarithm of the response and the logarithm of concentration, yielded points for averaged activity to 0.1, 0.17, 0.31, and 0.56 M sucrose that tightly adhered to a least squares line ($0.91 < r^2 < 1$) with a slope that appeared to differ between cool, ambient, and warm temperatures (Figure 2B). As an aside, the response to 0.05 M sucrose at each temperature clearly fell below each line (Figure 2B). This suggests the
relationship between response growth, sucrose concentration, and temperature may markedly shift when transitioning from perithreshold concentrations to intermediate or greater intensities (cf. Calvino 1986).

It is noteworthy that the slope of a least squares line computed in doubly logarithmic coordinates estimates the percent growth in the dependent variable expected while the predictor is increased by 1%. Following this, we performed least squares regression on individual responses to 0.1, 0.17, 0.31, and 0.56 M sucrose at each temperature, operating on the logarithms of firing rate and concentration (Figure 3), to determine how temperature influenced the rate of growth of the sucrose concentration-response function. Regression slopes were significantly greater than 0 at each temperature (one sample $t$-tests, $t_{20} > 5.5$, $P < 0.001$), and significantly steepened with reductions in sucrose temperature from warm (30° or 37°), to ambient (22°), to cool (18°) values (non-overlapping 95% CIs between conditions; Table 1). At 18°, the slope of the sucrose concentration-response function was significantly greater than 1 (one sample $t$-test, $t_{20} = 3.5$, $P = 0.002$), predicting that, under cooling, a 1% increase in concentration would cause, on average, a greater than 1% increase in neuronal firing. On the other hand, the slope fell to significantly less than 1 at 30° (one sample $t$-test, $t_{20} = -8.5$, $P < 0.001$) and 37° (one sample $t$-test, $t_{20} = -5.6$, $P < 0.001$), predicting that, under warming, a 1% increase in sucrose concentration would increase neuronal firing by less than 1%, on average. The slope of the regression line fit to responses to sucrose at ambient temperature did not differ from 1 (one sample $t$-test, $t_{20} = -0.6$, $P = 0.6$), reflecting relatively intermediate growth in activity with concentration.

The results above show that, for 0.1 M and higher intensities, temperature systematically modified the slope of the concentration-response function to sucrose observed across S-type neurons. This slope was flattest with warming and steepest with cooling, and holding sucrose at
ambient temperature produced a relatively intermediate slope. Thus, steps in concentration
induced the largest relative change in mean activity to sucrose under cooling.

Temperature influences latency to respond to sucrose in S-type neurons

We were able to observe during recordings a strong influence of temperature on latency
to first spike to sucrose. This effect was readily seen in S-type neurons with zero pre-stimulus
activity, as illustrated in Figure 1. For this cell, increasing the temperature of the sucrose
solution, while holding concentration constant, systematically decreased the latency to first
spike. Latencies across the four temperatures were: 18°, 917 msec; 22°, 658 msec; 30°, 451
msec; 37°, 278 msec. The shortest and longest lags across these trials differed by 639 msec,
indicating temperature could markedly impact the time at which this cell generated its response
to sucrose.

Unlike the example unit in Figure 1, most neurons produced some spikes during the pre-
stimulation period of sucrose trials, presumably reflecting, in part, "spontaneous" activity
commonly reported in taste-sensitive units. The presence of pre-stimulus activity during a trial
questions if a spike observed just after the onset of stimulus presentation reflects lingering
spontaneous drive or the beginning of a taste response. Given this uncertainty, latency to
response on each sucrose trial was detected using a statistical algorithm (methods). Figure 4
demonstrates application of this algorithm to estimate, for two neurons, latency to first spike to
temperature- and also concentration-varied solutions of sucrose. As shown in Figure 4, sucrose
temperature strongly influenced latency to first spike, which showed a general trend across
sucrose concentrations to decrease with warming. Increasing sucrose concentration could also
reduce latency in these cells, particularly for the unit in Figure 4A, albeit this effect was obvious
only at cool (18°) and ambient (22°) temperatures. For both neurons, warming solutions to 30°
and also 37° markedly reduced the dependence of latency on concentration. Warming also
yielded the shortest latencies measured across all thermo-concentration conditions. Multiple
concentrations of sucrose at 30° and 37° induced latencies in these units that were visibly shorter than lags measured to the highest concentration of sucrose tested at ambient temperature (Figure 4), the standard stimulus temperature used in many taste neurophysiology studies.

Statistical assessment of latency data from all S-type neurons recapitulated the effects observed in the example units above. Across units, temperature ubiquitously modified the dependence of latency on sucrose concentration. For instance, at 18°, latencies for 0.56 M (median latency \( \mu = 1.12 \) sec) were quicker than latencies measured for 0.17 M (\( \mu = 1.75 \) sec), as evidenced by non-overlapping 95% CI’s between concentrations (Figure 5A). At 22°, latencies for 0.31 M (\( \mu = 0.99 \) sec) and 0.56 M (\( \mu = 0.85 \) sec) were shorter than latencies for 0.1 M (\( \mu = 1.42 \) sec) or 0.05 M (\( \mu = 1.69 \) sec), as suggested by lack of overlap between 95% CI’s (Figure 5A). On the other hand, warming solutions to 30°, or 37°, reduced all latencies such that median latency for each concentration generally overlapped with the 95% CI’s of the median for every other concentration (Figure 5A). Thus, concentration can modulate first spike latency to sucrose at cool and ambient temperatures, but has little to no influence on latency when sucrose is warmed.

The reduced latencies with warming were the shortest observed among all thermo-concentration conditions. In example, latencies to 0.31 M (\( \mu = 0.50 \) sec), 0.17 M (\( \mu = 0.56 \) sec), and 0.1 M (\( \mu = 0.58 \) sec) sucrose warmed to 37° were shorter than latencies for even the highest concentration of sucrose, 0.56 M, tested at ambient temperature (\( \mu = 0.85 \) sec), as suggested by non-overlapping 95% CI’s (Figure 5A). What is more, latencies for particular concentrations of sucrose systematically decreased with warming. For instance, latencies for 0.56 M sucrose progressively fell as temperature was raised from 18° (\( \mu = 1.12 \) sec), to 22° (\( \mu = 0.85 \) sec), to 37° (\( \mu = 0.48 \) sec), as revealed by non-overlapping 95% CI’s between temperatures (Figure 5A). Latencies for 0.56 M at 30° (\( \mu = 0.67 \) sec) and 37° were also
significantly reduced relative to 18° (Figure 5A). These data indicate the “speed” of neural activation to sucrose is importantly controlled by stimulus temperature. Warming systematically speeds up neural activation to sucrose, and cooling slows it down.

Finally, warming significantly increased the percentage of units activated (i.e., units with defined latencies) by perithreshold (0.05 M, cf. Treesukosol and Spector 2012) and low (0.1 M) concentrations of sucrose ($\chi^2 > 8, df = 3, P < 0.04$, Figure 5B). It is noteworthy that only 1 S-type neuron responded to 0.05 M sucrose at 18°, albeit all S-class cells responded to this concentration tested at 30°. Thus, the ability of S-type neurons to register the presence of weakly sweet solutions of sucrose is augmented by warmth.

Our previous work indicated many NTS units can respond to warmed water applied to oral epithelia, in the absence of taste input (Wilson and Lemon 2013). Thus, we explored whether the early phase of reduced-latency firing to warmed sucrose represented a bona fide chemosensory response, or the appearance of an initial “warming” response induced solely by temperature. Using data from S-type neurons, spike trains for all concentrations of sucrose tested at 30° and 37° were divided into 50 msec bins, where each bin held a spike count. Median spike counts for synchronized bins were compared, within temperature, between trials for warmed water (0 M sucrose) and each sucrose concentration to address if firing to warmed sucrose surpassed that to warmth alone in early response windows.

Analysis of binned data revealed firing to sucrose became greater than activity to warmed water by 600 to 800 msec following stimulus onset. Specifically, at 30°, binned activity to 0.1, 0.17, 0.31, and 0.56 M sucrose significantly exceeded, under a conservative $\alpha$, activity to water at, respectively, 800, 700, 750, and 750 msec post-stimulus (Bonferroni-adjusted sign tests, $P < 0.003$, Figure 6A). At 37°, firing to 0.1, 0.17, 0.31, and 0.56 M sucrose became significantly greater than activity to water at, respectively, 700, 600, 650, and 600 msec post-stimulus (Bonferroni-adjusted sign tests, $P < 0.003$, Figure 6B). For each temperature-concentration condition noted, the time bin where activity to sucrose rose above activity to
warmed water was within or immediately followed the 95% CI* for latency to first spike to 
sucrose (Figure 5A). Thus, reductions in latency with warming appeared to result from a 
reduction in lag to a chemosensory response to sucrose, rather than the emergence of an early, 
purely thermal signal.
Here we report two novel effects of temperature on neural responses to sucrose. Recordings from S-type neurons in mouse NTS revealed cooling and warming solutions of sucrose induced marked change in (1) the slope of the concentration-response function to this stimulus, and (2) the time of onset of neural activation. Temperature systematically modified these parameters of sucrose activity, with both slope and latency showing an inverse relation with temperature. These results reveal temperature is an operational parameter of the central code for sucrose taste. Yet few studies have considered temperature in the analysis of central responses to sweet, or other, taste stimuli (cf. Wilson and Lemon 2013).

Cooling and concentration effects

Change in solution temperature changed the slope of the response function for 0.1 to 0.56 M sucrose, as assessed by log-log regression. Decreasing temperature from warm (30° or 37°), to ambient (22°), to cool (18°) systematically steepened the regression slope (Figures 2, 3). Because this slope is proportional to the percent change in response expected, on average, for a given concentration step, neural responses to sucrose increased more rapidly with concentration under cooling compared to warming. This effect associates with mathematical trends reported in human psychophysical studies on the temperature dependence of sucrose taste perception. For instance, the slope of the psychometric function for sucrose is steeper when the tongue is cooled to 20°C than when held at 36°C (Green and Frankmann 1987), and this slope progressively steepens when solution temperature is stepped from 44° down to 12°C (Bartoshuk et al. 1982). Thus, the perceived sweetness of sucrose also appears to increase more rapidly with concentration when solutions are cooled rather than warmed (see also Calvino 1986). This trend and the present data converge on the postulate that rate of growth in the brain's taste response to increasing concentrations of sucrose is augmented by cooling.
The observed increase in slope while cooling, with similar intercept across temperatures (Table 1), suggests raising the concentration of sucrose progressively weakened the effect of temperature on neural activity (cf. Green and Frankmann 1988). Indeed, temperature induced larger effects on responses to low concentrations of sucrose. For instance, increasing temperature from 18° to 30° caused a 1049% increase in the mean response to 0.1 M sucrose, but only an 152% increase in mean activity to 0.56 M sucrose (Figure 2A). What is more, systematic increases in slope due to cooling predict responses to sucrose should show independence from temperature at high stimulus concentrations (cf. Green and Frankmann 1988). Here this was observed, in part, for neuronal concentration-response functions to sucrose at 23° and 37°, where responses at these temperatures increased in similarity as concentration was raised, and became indistinguishable between 0.31 and 0.56 M (Figure 2). An analogous effect was reported in human psychophysical studies, where the perception of sucrose intensity begins to operate independently of temperature at concentrations greater than around 0.4 M (Bartoshuk et al. 1982; Calvino 1986; Green and Frankmann 1987). Although comparing data across species and levels of analysis can be performed only with caution, similarities between prior psychometric and the present neurometric data suggest the ability of temperature to modulate responses to sucrose in the mammalian brain depends on concentration, and that greater modulation arises at low to weak concentrations than high. Moreover, the similar quantitative effects of temperature noted in humans and mice suggest neurophysiological mechanisms for sucrose taste in the mouse partly model these mechanisms in humans.

Only a few peripheral electrophysiological studies have measured responses to sucrose across several concentrations and temperatures. These studies have focused on the chorda tympani (CT) nerve, which innervates taste receptors on only the anterior tongue. Neurons in rostral regions of the NTS can receive convergent input from afferents innervating gustatory epithelia located on different regions of the tongue and mouth (Corson and Erisir 2013;
Grabauskas and Bradley 1996; Travers and Norgren 1995; Travers et al. 1986), which were broadly bathed with solutions by our taste stimulation method (Wilson et al. 2012). Along this line, there exist similarities and differences between prior peripheral and the present central data on thermo-concentration effects on gustatory activity to sucrose. For instance, recordings from the CT nerve in B6 mice showed responses to 0.1 M sucrose were elevated with warming, as opposed to cooling, and appeared similar when solutions were tested at warm temperatures ranging from 29° to 38°C (Lu et al. 2012). As found presently, warming also augmented responses to 0.1 M sucrose by central S-type neurons in B6 mice, albeit responses to this concentration, and others, were significantly larger when sucrose solutions were warmed to 30° compared to 37°C (Figure 2A). The range of warm temperatures that maximizes activity to sucrose may be narrower in the CNS (cf. Wilson and Lemon 2013). What is more, the present finding that the influence of temperature on gustatory responses to sucrose weakens with increasing concentration (Figure 2) was not uniformly found across studies of CT nerve activity to temperature- and concentration-varied solutions of sucrose (Lu et al. 2012; Talavera et al. 2005). Although methodological discrepancies between studies may account for these differences, it is certainly possible that input from multiple nerves and central processing may contribute to the effects of temperature on gustatory activity to sucrose in the NTS.

Our understanding of how temperature influences orosensory behavioral responses to sucrose in rodents is nascent. Torregrossa et al. (2012) described complex effects of temperature on sucrose intake in rats, with dependence on experimental procedure. Inspection of their data (Fig. 4D, p. 288) suggests water-replete rats performing in brief-access intake tests can, under certain conditions, show greater licking responses to warmed (e.g., 30°) sucrose than cooled (e.g., 10°), yet cooling appears to cause greater percentage increase in the rate of licking when sucrose concentration is raised. In addition to its effects on sucrose-guided behavior, temperature can serve as salient cue in oral sensory learning (Smith et al. 2010) and also regulate preference towards water (Gold et al. 1973; Kapatos and Gold 1972) in rats. As
with humans, temperature appears to importantly guide oral sensation in rodents, albeit more work is needed in this area.

Temperature and timing

Prior studies on peripheral neurons by Marowitz and Halpern (1977) and Breza et al. (2010) showed that latency to respond to sodium salts inversely followed concentration. The present analysis revealed an inverse relationship between concentration and response latency to sucrose in central neurons, and showed this relationship was temperature dependent. Change in sucrose concentration could modulate latency for solutions held at cool and ambient temperatures, but largely had no effect on latency when solutions were warmed (Figure 5A). Moreover, increasing solution temperature from cool or ambient to warm, while holding concentration constant, generally decreased lag-to-response to sucrose (Figures 1, 4, 5A). The longest latencies (e.g., > 1200 msec) were found with cooling, and the shortest (e.g., < 500 msec) were obtained under warming. The shortened latencies for warmed sucrose were unprecedented by latencies measured for sucrose at ambient temperature, the common stimulus temperature used in taste neurophysiological studies.

The ability of temperature to modify latency has implications for gustatory coding. For one, latency was proposed to convey neural information used to represent taste stimuli (Breza et al. 2010; Hallock and Di Lorenzo 2006). However, the present data introduce complications for this hypothesis for sucrose. Median latencies for select concentrations of sucrose varied by hundreds of milliseconds with cooling and warming (e.g., for 0.1 M, median lag was 1418 msec at 22°, but 580 msec at 37°, Figure 5A), indicating latency was a poor marker of concentration. Although latencies generally decreased with warming, lags for low and high concentrations of sucrose were sometimes indifferent at different temperatures (e.g., 0.56 M at 22° and 0.05 M at 30° showed overlapping medians/95% CI’s; Figure 5A), suggesting latency does not encode temperature. Although latency is evidenced in other systems to signal features of sensory
stimuli (e.g., Chase and Young 2007; Gollisch and Meister 2008), it is unclear if latency could serve as a marker for information about sucrose. Differences in neuronal response latency were reported for stimuli of different gustatory qualia (Breza et al. 2010; Pritchard and Scott 1982; Yamamoto et al. 1984), albeit this awaits evaluation using temperature-varied stimuli.

Alternatively, the present data suggest that temperature will impact the timing and evolution of responses to sucrose across central neurons, and may also influence the synchrony of sucrose activity with that for other qualia bound with sucrose in a mixture.

Temperature-imbued change in the speed of the signal for sucrose relative to input about another tastant, delivered alongside, could change which message reaches the central network first. Differences in the timing and order of delivery of sucrose and other taste stimuli can modulate firing to sucrose in NTS units (Di Lorenzo and Lemon 2000; Di Lorenzo et al. 2003), and this modulation may partly originate through a central mechanism (Lemon and Di Lorenzo 2002). Although the influence of temperature on responses to taste mixtures that include sucrose awaits investigation, temperature does uniquely modify unit responses in NTS to sucrose compared to sodium salt, acidic, and bitter tastants (Wilson and Lemon 2013).

Moreover, taste receptors for sucrose (Talavera et al. 2005) and other stimuli, such as sodium salts (Askwith et al. 2001), can show inverse sensitivity to a thermal gradient. This predicts temperature change, in one direction, would simultaneously enhance and attenuate features of mixture responses to sucrose and select stimuli.

Considerations and conclusion

There are several conditions to the present data to consider for interpretation. For one, we used a statistical threshold to define extracellular spiking latency to sucrose for single units, which may only approximate the time lag to an actual physiological change in the recorded neurons. Considering the delayed latencies noted under cool and ambient temperatures, it is possible there exist earlier stimulus-induced perturbations in, for example, the membrane
potential of the recorded cells that precede significant spiking activity but are undetected by our recording or statistical methods. The present definition of latency might reflect, in part, the influence of oral temperature, and stimulus concentration, on the efficiency or speed by which taste transduction processes can convert the chemical energy of sucrose to a set of electrical impulses, rather than a time point of absolute change in physiology. However, although our neuronal data likely do incorporate the upstream influence of temperature on taste receptors for sucrose (e.g., Lu et al. 2012; Talavera et al. 2007; Talavera et al. 2005), the exact neurobiological mechanism contributing to the present effects, and the potential contribution of central processing, remains unknown. It is noteworthy that long latencies to respond to sucrose under cool and ambient temperatures, as shown presently (Figures 1, 4, and 5), are not atypical for medullary neurons. Other studies of NTS units in mice and rats have shown latencies to respond, and to maximal activation, to room temperature sucrose of near 1 sec from stimulus onset (Chen and Di Lorenzo 2008; McCaughey 2007). Considering the present effects of cooling on latency, these lags would be expected to further increase with reduction in solution temperature.

It is also important to consider that our measurements of response latency were made relative to when stimuli entered the mouth space, and are an overestimate of latency relative to time of receptor activation. Moreover, latencies, and spiking responses, were “static” in that our measurements could not account for any dynamic modulations in activity imposed by tongue and mouth movements in awake animals. On the other hand, anesthesia promoted recording of sensory responses to sucrose across multiple thermo-concentration conditions in the absence of non-specific influences, such as behavioral differences across animals (Chapuis and Wilson 2011). Finally, the rinse temperature (22°, ambient) used, albeit common in taste neurophysiology studies, is probably lower than the normal oral temperature of an awake mouse (cf. Wilson and Lemon 2013), and other adapting temperatures need to be tested in future studies.
Caveats notwithstanding, the present results agree with prior data suggesting the taste of sucrose is strongly sensitive to temperature, and delineate how temperature operates as a systematic parameter of the gustatory neural representation of sucrose. Our results are part of a growing body of data that argue temperature is equally important to stimulus concentration and quality as a parameter of gustatory coding, and should be included as a standard factor in neurophysiological studies on sweet and other tastes (Breza et al. 2006; Lu et al. 2012; Lundy and Contreras 1999; Talavera et al. 2005; Wilson and Lemon 2013; Yamashita and Sato 1965). Temperature is an omnipresent property of taste stimuli that can modify taste experience. Thus, elucidating thermodynamic principles of gustatory processing may greatly facilitate our understanding of neural substrates for taste (Talavera et al. 2007).
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Disclosures

The authors declare no competing financial interests.
References


Treesukosol Y, and Spector AC. Orosensory detection of sucrose, maltose, and glucose is severely impaired in mice lacking T1R2 or T1R3, but Polycose sensitivity remains relatively normal. *Am J Physiol Regul Integr Comp Physiol* 303: R218-235, 2012.


Figure captions

Figure 1. Example recordings from one S-type neuron. A, Family of traces depicting real-time measurement of rinse and stimulus temperature, at the moment of oral delivery, on four separate trials where a fixed concentration of sucrose (0.56 M) was tested at 18°, 22°, 30°, and 37°. B, Electrophysiological sweeps depicting trains of action potentials recorded in synchrony with temperature during each trial in panel A. Sweeps are aligned by time of stimulus onset (see methods). Upward and downward arrows indicate stimulus onset and offset, respectively.

Figure 2. The effect of temperature on averaged concentration-response functions to sucrose. A, Mean (± 1 standard error) responses across 22 S-type neurons to 0, 0.05, 0.1, 0.17, 0.31, and 0.56 M sucrose tested at 18°, 22°, 30°, and 37°C. Data are plotted in linear coordinates. Solid lines represent quadratic fit of mean responses at each temperature. B, Mean response values in panel A, except for 0 M, plotted in doubly logarithmic (base 10) coordinates. Solid lines represent least squares fits applied to mean response values for 0.1, 0.17, 0.31, and 0.56 M sucrose at each temperature, operating on the logarithm of the mean response and the logarithm of concentration. Dotted lines extend fits for each temperature to allow visual comparison against activity to 0.05 M sucrose.

Figure 3. Responses by each of 22 S-type neurons to 0.1, 0.17, 0.31, and 0.56 M sucrose tested at 18°, 22°, 30°, and 37°C. Data are plotted in doubly logarithmic (base 10) coordinates. Solid lines represent least squares fits applied to all individual responses to these concentrations at each temperature, operating on the logarithm of neural activity and the logarithm of concentration. See methods for details, and Table 1 for regression results.

Figure 4. Rastergrams for two separately recorded S-type neurons (A and B) showing detection of latency to first spike across 24 unique temperature-concentration combination trials for
sucrose. The electrophysiological sweep recorded for 0 M sucrose at 18°C is shown for each cell to demonstrate conversion of neurophysiological data to raster spikes. A blackened raster spike on a trial represents the time during stimulus delivery when the firing rate of the neuron became unusually high compared to the average pre-stimulus firing rate of the cell (see methods). The absence of a blackened spike indicates no significant elevation in firing was detected for that trial.

**Figure 5.** Effect of temperature on latency to first spike to sucrose across 22 S-type neurons. **A**, Median latency (filled circles) and 95% confidence limits for the median (whiskers) for each of the 24 sucrose temperature-concentration conditions. Confidence limits were approximated using a bootstrap resampling procedure (see methods). n/a, interval not applicable: no or too few cells activated. **B**, Percent of neurons within each temperature-concentration condition for sucrose where latency to first spike could be detected (i.e., neurons that showed significant activation to sucrose). ø, no cells activated (0 M sucrose at 18° and 22°).

**Figure 6.** Time course of neuronal responses to 0, 0.05, 0.1, 0.17, 0.31, and 0.56 M sucrose tested at 30° (panel **A**) and 37°C (panel **B**). In each panel, a family of traces (gray lines) depicts time-evolved activity by 22 S-type neurons to multiple sucrose concentrations, where traces for different concentrations are denoted by line thickness (legend). To construct each plot, individual spike trains were binned (50 msec) and spike counts in time-aligned bins were summed across cells. Each trace connects points for sequential response bins for one concentration. Bins are referenced along the abscissa by their time of closure (e.g., data for the 0 to 0.05 sec bin are plotted at 0.05 sec on the abscissa). Filled circles denote bins where median activity to sucrose was significantly greater than median activity to isothermal water (i.e., 0 M sucrose). Response traces represent smoothed data (lowess method), albeit statistical comparisons were made using raw spike counts.
Table 1. Results of least squares regression applied to responses by 22 S-type neurons to 0.1, 0.17, 0.31, and 0.56 M sucrose at each temperature (Figure 3). Calculations operated on the logarithm of the spiking response and the logarithm of concentration. All regression slopes were significantly greater than zero ($P < 0.001$). The group column indicates whether there was separation, denoted by different characters, between the 95% CIs for two slopes.

<table>
<thead>
<tr>
<th>°C</th>
<th>slope [95% CI lower, 95% CI upper]</th>
<th>intercept</th>
<th>$r^2$</th>
<th>group</th>
</tr>
</thead>
<tbody>
<tr>
<td>18°</td>
<td>1.44 [1.18, 1.70]</td>
<td>2.25</td>
<td>0.61</td>
<td>a</td>
</tr>
<tr>
<td>22°</td>
<td>0.94 [0.74, 1.15]</td>
<td>2.42</td>
<td>0.52</td>
<td>b</td>
</tr>
<tr>
<td>30°</td>
<td>0.39 [0.25, 0.54]</td>
<td>2.38</td>
<td>0.26</td>
<td>c</td>
</tr>
<tr>
<td>37°</td>
<td>0.50 [0.31, 0.68]</td>
<td>2.29</td>
<td>0.26</td>
<td>c</td>
</tr>
</tbody>
</table>
A 30°C

- [sucrose] (M)
  - 0.56
  - 0.31
  - 0.17
  - 0.1
  - 0.05
  - 0

- P < 0.003 (vs. 0 M)

B 37°C

- spikes
- lag (sec)