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Taste coding of complex naturalistic taste stimuli and traditional taste stimuli in the parabrachial pons of the awake, freely licking rat

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Sammons JD, Weiss MS, Victor JD, Di Lorenzo PM. Taste coding of complex naturalistic taste stimuli and traditional taste stimuli in the parabrachial pons of the awake, freely licking rat. *J Neurophysiol* 116: 171–182, 2016. First published April 27, 2016; doi:10.1152/jn.01119.2015.—Several studies have shown that taste-responsive cells in the brainstem taste nuclei of rodents respond to sensory qualities other than gustation. Such data suggest that cells in the classical gustatory brainstem may be better tuned to respond to stimuli that engage multiple sensory modalities than to stimuli that are purely gustatory. Here, we test this idea by recording the electrophysiological responses to complex, naturalistic stimuli in single neurons in the parabrachial pons (PbN, the second neural relay in the central gustatory pathway) in awake, freely licking rats. Following electrode implantation and recovery, we presented both prototypical and naturalistic taste stimuli and recorded the responses in the PbN. Prototypical taste stimuli (NaCl, sucrose, citric acid, and caffeine) and naturalistic stimuli (clam juice, grape juice, lemon juice, and coffee) were matched for taste quality and intensity (concentration). Umami (monosodium glutamate + inosine monophosphate) and fat (diluted heavy cream) were also tested. PbN neurons responded to naturalistic stimuli as much or more than to prototypical taste stimuli. Furthermore, they convey more information about naturalistic stimuli than about prototypical ones. Moreover, multidimensional scaling analyses showed that across unit responses to naturalistic stimuli were more widely separated than responses to prototypical taste stimuli. Interestingly, cream evoked a robust and widespread response in PbN cells. Collectively, these data suggest that natural foods are more potent stimulators of PbN cells than purely gustatory stimuli. Probing PbN cells with pure taste stimuli may underestimate the response repertoire of these cells.

taste; gustatory; neural coding; temporal coding; neurophysiology

NEW & NOTEWORTHY

Cells in the parabrachial nucleus of the pons (PbN), the second neural relay for gustation in the rodent brainstem, respond to exemplars of the five basic taste qualities, sweet, salty sour, bitter, and umami. Here we show that natural foods are more potent stimulators of PbN cells than purely gustatory stimuli. Probing PbN cells with pure taste stimuli may underestimate the response repertoire of these cells.

AN IMPORTANT GOAL OF THE FIELD of gustation is to understand how taste-related structures intersect and interact with the feeding pathway. Taste information from the tongue is acquired via three cranial nerves, the facial, glossopharyngeal, and vagus, all of which project to the nucleus of the solitary tract (NTS) in the brainstem. From there, the main target of NTS projections in the rodent and other nonprimate mammals is the parabrachial nucleus of the pons (PbN). Recent behavioral, pharmacological, and genetic studies have pointed to the PbN as an important node in the feeding circuit (Wu et al. 2012), thus providing an opportunity for information about the taste of food to influence ingestion. Evidence that neurons in the NTS (Escanilla et al. 2015; Van Buskirk and Erickson 1977) and PbN (Di Lorenzo and Garcia 1985) respond to odors as well as taste suggests that these structures may provide a richer assessment of the sensory aspects of food than simply its taste. That is, the sense of taste alone does not fully account for the ways in which cells in the classical taste pathway respond to natural foods.

When food enters the mouth, there are several sensory modalities, including taste, olfaction, and somatosensation, that are engaged. While stimulation of these systems may seem to evoke disparate sensations, their combination underlies the unified perception of flavor. Studies of cells in the NTS and PbN that respond to the olfactory (Di Lorenzo and Garcia 1985; Escanilla et al. 2015; Van Buskirk and Erickson, 1977), thermal (Ogawa et al. 1988; Schwartzbaum 1983; Wilson and Lemon 2013), and tactile (Ogawa et al. 1984; Travers and Norgren 1995) components of food as well as its taste suggest that these nuclei may in fact be multimodal in their responses to natural foods. However, the anatomic projections of these cells have not been described, so the nature of these multimodal cells is as yet unclear. Conversely, natural foods are more complex than the taste stimuli that are traditionally tested in the laboratory. That is, they are complex mixtures that may contain multiple tastants, odorants, and particulates and may vary in texture. The rich complexity of such stimuli may support better identification of a stimulus than its taste qualities could in isolation. If so, then naturalistic stimuli might be more effective at stimulating cells in the classical gustatory brainstem than their reductions to pure taste stimuli. To test this idea, we recorded from PbN cells in awake, freely licking rats while they licked aqueous solutions of chemicals that were prototypical of each of the five "basic" or "primary" taste qualities, sucrose for sweet, NaCl for salty, citric acid for sour, caffeine for bitter, and monosodium glutamate (MSG) plus inosine monophosphate for umami. In addition, we tested liquid foods that were matched for the intensity (concentration) of the predominant, if not the only, taste quality that these chemicals evoked. These included grape juice for sweet, clam juice for salty, lemon juice for sour, and coffee for bitter. We also tested dilute heavy cream as an exemplar of fat taste.

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Results of these experiments show that naturalistic stimuli evoke electrophysiological responses that are more readily distinguishable than responses to their intensity-matched prototypical taste stimulus counterparts, even though these responses are generally of similar magnitude. In all, the data presented here provide a strong argument that neurons in the parabrachial pons may be more appropriately characterized as "food-responsive" rather than purely taste-responsive.

MATERIALS AND METHODS

Subjects

For the "electrophysiological taste response" experiments, nine male Sprague-Dawley rats (300–425 g) were used. For the "behavioral taste acceptance" experiments, six other male Sprague-Dawley rats (450–550 g) served as subjects. Rats were pair-housed and kept on a 12-h light-dark cycle with lights off at 0900. Animals were provided with standard LabDiet 5001 rodent diet (Land O'Lakes, St. Louis, MO) ad libitum and water for 1 h daily, not including fluid received during the testing period. All electrophysiological and behavioral experiments were performed during the rats' dark cycle under dim light conditions. All procedures were approved by the Binghamton University Institutional Animal Care and Use Committee.

Electrophysiological Taste Response Experiments

Methods for electrode fabrication, implantation, and recording, as well as behavioral procedures for taste presentation, data analysis methods, and histology are established laboratory practices (Roussin et al. 2012; Weiss et al. 2014; Escanilla et al. 2015) and are summarized here.

Electrode Implantation Surgery

Following pretreatment with 0.01 mg/kg buprenorphine (sc) and 0.05 mg/kg atropine (sc), animals were anesthetized with isoflurane (3% induction, 1.5–2.5% maintenance) or a ketamine-xylazine mixture (100 mg/kg ketamine; 14 mg/kg xylazine ip). After anesthesia induction, the crown of the head was shaved. Artificial tear gel was applied to the eyes to prevent drying. A thermistor attached to a heating pad maintained body temperature at 37°C. Animals were placed in a stereotaxic apparatus (Kopf Model 1900, Tujunga, CA) using blunt ear bars. The head was swabbed several times with betadine followed by 70% ethanol. A longitudinal incision was then made in the scalp and the underlying fascia was resected with blunt dissection. The head was angled so that bregma was 4 mm below lambda, resulting in an ~25° head tilt. Five screws were implanted in the skull. A hole, 2 mm in diameter, was drilled above the PbN at ~12.0–12.5 mm posterior and 1.4–1.8 mm medial to the bregma. The dura was punctured and moved aside for insertion of microwire electrode assembly, which was slowly lowered to a depth of ~6 mm. After the electrode bundle was 4 mm below the cerebellar surface, the tongue was periodically bathed in a 0.1 M NaCl solution followed by artificial saliva (AS) or water to electrophysiologically monitor for taste responses. Once a taste response was observed, the electrode was cemented in place with dental acrylic. A stainless steel wire from the microwire assembly was wrapped around a skull screw to provide an electrical ground.

Upon completion of the surgery, animals were given ~10 ml of sterile isotonic saline (sc) to prevent dehydration as well as replenish fluids lost during surgery. Animals were then moved onto a warming bed. Once spontaneously mobile, animals received 0.02 mg/kg buprenorphine (sc) and 6 mg/kg gentamicin (sc). These injections continued for 2 days postsurgery. Additionally, topical antibiotic with analgesic (Neomycin Sulfate-Polymyxin B Sulfate-Pramoxine HCl cream) was applied around the headcap for 5 days. DietGel 76A (Clear H2O, Portland, ME) or Ensure (Abbott Laboratories) was placed in the animals' home cage to encourage eating and recovery. Animals were monitored daily 5 days postoperatively for general well-being (weight loss, grooming, activity, gait, etc.). Testing began once animals regained 95% of preoperative weight.

Microwire Electrode Assembly

Electrode assembly has been described previously (Roussin et al. 2012; Weiss et al. 2014). Briefly, eight tungsten microwires (25-μm diameter; 1–3 MΩ impedance) insulated with Formvar (California Fine Wire, Grover Beach, CA) were soldered onto pins 1–8 of a 10-pin Omnetics connector (Omnetics, Minneapolis, MN). The ninth pin was soldered to a stainless steel wire to wrap around a bone screw and serve as an electrical ground. The 10th pin was soldered to a 10-mm tungsten strut (127-μm diameter). The microwires were then gathered around the tungsten strut, passed through polyimide tubing (0.008-in. diameter; FHC, Bowdoin, ME), and trimmed so that they were staggered across 1 mm and extended 1–2 mm past the strut. The whole assembly was then coated with liquid plastic insulation (Insulating Coating; GC Electronics) to secure the wires to the connector. The microwire ends were dipped into a warmed liquefied sucrose-gelatin mixture and left to dry overnight. Finished electrode bundles were stored in the refrigerator until they were used for implantation.

Taste Stimuli and Delivery

Two groups of taste stimuli were tested. The first group included prototypical taste stimuli consisting of sucrose (0.24 M), NaCl (0.03 M), citric acid (0.017 M), caffeine (0.002 M), and MSG (0.1 M) + inosine monophosphate (0.01 M). All prototypical taste stimuli were made from reagent grade chemicals. The second group included complex tastants representing naturalistic counterparts to the prototypical taste stimuli matched for concentration. These included 50% grape juice (0.24 M sugar, an approximately equal mixture of fructose and glucose; Santa Cruz Organic, Chico, CA) as a sweet stimulus, 50% clam juice (0.03 M NaCl; Snow's Bumblebee, San Diego, CA) as a salty stimulus, 10% lemon juice (0.017 M citric acid; Santa Cruz Organic) as a sour stimulus, and coffee (2 g of instant Nescafe Tasters Choice coffee dissolved in 175 ml AS; 0.002 M caffeine) as a bitter stimulus. Additionally, 25% heavy cream (2.5 g fat/30 ml; Wegmans, Rochester, NY) was used as the putative taste of fat. All taste stimuli were dissolved AS, which was also used as a rinse and as a "taste" stimulus control. AS consisted of a mixture of 0.015 M NaCl, 0.022 M KCl, 0.003 M $CaCl_2$, and 0.0006 M $MgCl_2$ at a pH of 5.8 $\pm$ 0.2 (Hirata et al. 2005). (Heavy cream, an emulsified solution, stays in solution when diluted with AS, much as adding cream to coffee does not dissociate the fat from the solvent.)

To test for taste responses, rats were placed in a Plexiglas experimental box housed in a soundproof chamber with an observation window (Med Associates, St. Albans, VT). The taste stimulus delivery system consisted of 12 20-gauge stainless steel tubes housed in an 8-mm-diameter stainless steel sipper tube. A 16-into-1 mini-manifold AutoMate perfusion system (AutoMate Scientific, Berkeley, CA) housed the taste stimulus reservoirs that were maintained under 10 psi pressure. Computer-activated solenoids (Parker Hannifin, Elyria, OH) delivered 12 $\pm$ 2 μl of fluid within 10 ms after the rat broke an infrared beam close to the sipper tube in the licking recess. The stimulus delivery system was calibrated daily before the rats were tested.

Tastant trials consisted of five consecutive licks of a given taste stimulus. Taste stimulus trials were separated by five AS rinse licks delivered on a variable ratio 5 (VR5) schedule. That is, each AS lick was preceded and followed by four to six "dry" licks where no liquid was delivered. On average, that meant that the interstimulus interval

was ~4 s, given an average lick rate of 7/s. However, the rat was free to lick, or not, at its own pace so there was variability in this interval. Taste stimulus trials were presented in pseudorandom order without repetition such that all 10 taste stimuli (prototypical and naturalistic) plus AS presented as a taste stimulus control were delivered before any stimulus was repeated.

Electrophysiological Recording

Before testing, animals were water deprived for 20–22 h. Once the headstage was attached to the animal's head cap, a house light signaled the start of a recording session, which lasted for 30 min to 1 h. We recorded a minimum of seven trials for each taste stimulus.

Neural activity was monitored using Plexon's (Dallas, TX) SortClient software package. Timestamps of both waveforms and stimulus events were recorded with a 25-μs resolution. Waveforms were then imported into OfflineSorter (Plexon, Dallas, TX) for further analysis and isolation. The criterion for isolation of a waveform was a >3:1 signal-to-noise ratio and a refractory period of >2 ms (Stapleton et al. 2006).

After the recording session, rats were returned to their home cage. They were given 1 h free access to water no less than 1 h after being returned to their home cage. Because it was impossible to predict when a recording on a given day would contain a well-isolated taste-responsive cell, each rat underwent multiple daily sessions until the quality of the electrophysiological activity on all channels appeared degraded. This took approximately 3 wk of every-weekday test sessions.

Data Analyses for Electrophysiological Experiments

Electrophysiological responses: analysis of basic response characteristics. Data analyses were performed with MATLAB (Mathworks, Natick, MA) and Microsoft Excel (Redmond, WA). Spontaneous firing rates of single neurons were calculated from 10-s samples when the animals were not licking. For each taste stimulus, a peristimulus-time histogram (PSTH) was constructed across all trials (100-ms time bins) aligned with the first stimulus lick of each trial as the zero time point. Baseline firing rates for each stimulus were calculated in spikes per second as the mean ± SD of activity in the 500 ms before the first stimulus lick across trials. Taste responses were measured by a sliding window (100 ms, 20-ms increments) after the first stimulus lick until a significant difference from baseline was detected. Significant responses were characterized as activity ≥2.58 SD (99% confidence interval) above (excitatory) or below (inhibitory) the mean baseline firing rate, present for at least three consecutive 100-ms bins. Response magnitude was calculated as the difference between the average stimulus-evoked firing rate and the average baseline firing rate. The first time bin (in 20-ms increments) that was statistically above or below the baseline firing rate was defined as the response latency. Numerical values are expressed as mean ± SE unless otherwise stated.

Electrophysiological responses: temporal coding analysis. We characterized the contribution of the temporal aspects of taste coding by the metric space method of Victor and Purpura (1996, 1997). These analytical methods provide a rigorous way to determine whether the precise times of individual spikes have the potential to carry sensory information. We briefly review the approach here. The analysis centers on a family of metrics that measure "distance" (i.e., dissimilarity) between spike trains. The distance is given by the total "cost" of transforming one spike train into another by changing specific aspects of the spike trains that are being compared. These aspects include the number of spikes and the timing of individual spikes. For the simplest of this family of metrics, D^{count}, insertion or deletion of a spike incurs a cost of 1, and moving a spike in time has no cost; therefore, D^{count} is simply the arithmetic difference between the number of spikes in each response. To take into account spike timing, the metric $D^{\text{spike}}[q]$ keeps the cost of adding or deleting a spike equal to 1 but, in addition, sets the cost of moving a spike by an amount of time t at qt, where q is in units of 1/s. That is, for two spike trains to be similar in the sense of $D^{\text{spike}}[q]$, they have to have the same number of spikes, and the spike times must match to within $1/q$ s.

To estimate the amount of information (H in bits) conveyed by rate or temporal coding, we determined the degree to which pairs of responses to the same stimulus tended to be more similar to each other than pairs of responses to different stimuli, according to the metrics D^{count} and $D^{\text{spike}}[q]$. This was accomplished by decoding each spike train in the following manner: a spike train was determined to signal a particular stimulus S if the average metric distance from that spike train to each of the spike trains elicited by S was shorter than the average distance to the group of responses elicited by any another stimulus S'. Information, H, was then calculated from the confusion matrix between the actual stimulus that elicited each response and the stimulus into which it was decoded by the above procedure. For each metric, D^{count} or $D^{\text{spike}}[q]$, the information conveyed at various levels of precision (values of q) was calculated, and the value of q at which information is maximized was obtained. Therefore, information at $q = 0$ was called H_{count}, and information at q_{count} was called H_{count}.

Several additional analyses served as controls and refinements. To exclude spurious results due to small sample size, results were compared with analyses of surrogate data sets in which the labels assigned to stimuli were randomly permuted. Information that resulted from this analysis was called H_{shuffle}. To distinguish between coding via a time-varying firing rate (e.g., an inhomogeneous Poisson process), vs. coding in which the timing of individual spikes is critical, we used "exchange resampling," which compares the results to surrogate data sets in which spike times are randomly shuffled within responses to the same stimulus (see Di Lorenzo and Victor 2003 for further details). Information that resulted from this analysis was called H_{exchange}. Calculations included the Treves-Panzeri-Miller-Carlton bias correction for the limited number of samples (for review see Panzeri et al. 2007). Values of H_{count} and H_{count} that did not significantly exceed the shuffled amount (i.e., did not exceed H_{shuffle} + 2SD) were considered nonsignificant, and the final value of these quantities was taken to be 0.

If either H_{count} or H_{count} exceeded H_{shuffle} + 2SD, the response conveyed at least some information about the stimulus. We then classified the way that this information was carried as follows. If $H_{\text{count}} = H_{\text{count}}$, i.e., if measuring response similarity without regard to spike timing provided the most faithful decoding, we said that information was conveyed by rate coding. If $H_{\text{count}} > H_{\text{count}}$ but H_{count} did not exceed H_{exchange}, then the rate envelope conveyed more information than spike count alone, but the timing of individual spikes within each response did not matter, so we said that information was conveyed by rate envelope. Finally, if $H_{\text{count}} > H_{\text{count}}$ and also $H_{\text{count}} > H_{\text{exchange}}$, then the arrangement of individual spikes in each response made a measurable contribution to the information, and we said that a temporal code was present.

All analyses were performed for several response durations: the first 200 ms, 500 ms, 1 s, 1.5 s, and 2 s following the initial reinforced stimulus lick.

Histology for electrophysiological experiments. Rats were euthanized with a lethal dose of Sleepaway (1 ml/kg; Fort Dodge Animal Health, Fort Dodge, IA). Once deeply anesthetized, a DC current (1 mA for 10 s) was passed through the microwire on which the last single neuron was recoded. Rats were first perfused transcardially with isotonic saline followed by a 10% formalin in isotonic saline solution. Following perfusion, brains were extracted and placed in 10% formalin for a minimum 24 h. The day before sectioning, brains were washed three times with phosphate buffered saline (PBS) and placed in 20% sucrose in PBS for cryoprotection. Coronal sections (40-μm thick) were obtained from a cryostat and mounted onto superfrost plus slides (Fisher Scientific) and stained with cresyl violet for lesion site reconstruction.

Table 1. *Behavioral acceptance experimental protocol*

	Animal					
	1	*2*	*3*	*4*	*5*	*6*
10-s Exposure*						
Day 1	P	N	P	N	P	N
Day 2	N	P	N	P	N	P
Day 3	P	N	P	N	P	N
Day 4	N	P	N	P	N	P
Day 5	P	N	P	N	P	N
Day 6	N	P	N	P	N	P
30-s Exposure*						
Day 1	P	N	P	N	P	N
Day 2	N	P	N	P	N	P

P, prototypical; N, naturalistic. *3 trials of each stimulus in random order.

Behavioral Taste Acceptance Experiments

Behavioral acceptance for all taste stimuli was assessed in six naïve rats that were water deprived for 22 h before testing. Rats received either prototypical or naturalistic tastants for six daily sessions, with prototypical and naturalistic taste stimuli presented on alternate days. Three trials of each tastant were available within a session for 10 s at a time, separated by five AS licks presented on a variable ratio 5 (VR5) schedule. On each day, tastant trials were presented in pseudorandomized order. For each tastant, the trial with the greatest number of licks was recorded. These three single-day values were averaged across days to obtain a behavioral acceptance score for each tastant, in licks per second. Following these sessions, the same animals were run for 2 additional days in the same paradigm but with 30-s tastant presentations. The presentation protocol and analysis for these longer presentations were the same as for the 10-s presentations. See Table 1 for detail of experimental paradigm.

Data Analyses for Behavioral Taste Acceptance

The number of licks for the 10- and 30-s tastant presentation sessions were compared for each matched pair of stimuli. That is, we compared the number of licks for sucrose vs. grape juice, NaCl vs. clam juice, citric acid vs. lemon juice, and caffeine vs. coffee. Although umami was included in the group of prototypical tastants and cream was included in the group of naturalistic stimuli, we did not compare these acceptance scores with each other because they were not matched for taste quality, as the other pairs of stimuli were. All stimuli were presented at the same concentration as that used in the electrophysiological recordings. Significant differences in the number of licks for each pair of taste stimuli in the 10- and 30-s sessions were determined by Student's *t*-tests. Alpha was set at 0.01 to correct for multiple comparisons by the Bonferroni method.

RESULTS

Taste Responses in PbN

We recorded electrophysiological responses from taste responsive PbN neurons (32 cells) from nine rats during presentation of prototypical and naturalistic taste stimuli. In addition, there were 47 cells that were not taste-responsive but that showed lick-related activity. The average spontaneous firing rate of taste-responsive cells, taken from 10-s samples when there was no lick activity, was 27.2 ± 6.0 SE spikes/s (median: 11.1 spikes/s; range: 0–129 spikes/s). PbN cells were generally broadly tuned across the basic taste qualities. Figure 1 shows the proportion of cells that responded to multiple taste stimuli in each category of taste stimuli, prototypical and naturalistic. Of 32 PbN cells, 30 (94%) showed excitatory responses to at least one prototypical tastant while all 32 (100%) were excited by at least 1 naturalistic tastant. Across prototypical and naturalistic tastant categories, the proportions of cells that spanned the spectrum from broadly to narrowly tuned were comparable; a Wilcoxon rank-sum test showed no significant differences ($P = 0.52$).

Figure 2 shows the taste responses recorded from two PbN cells to both prototypical and naturalistic taste stimuli. Figure 2, *A* and *B*, *top*, shows a raster display where each dot represents the occurrence of a spike and each line of dots represents a trial. Colored triangles show the occurrence of taste stimulus licks; light blue triangles indicate AS rinse licks. Figure 2, *A* and *B*, *bottom*, shows a PSTH of responses to each tastant. In both cells, some prototypical tastants evoked responses while their naturalistic counterparts did not and vice versa. There were also some tastant pairs, e.g., sucrose and grape juice in *cell #2*, that evoked similar response magnitudes but with clearly different time courses of response.

The mean firing rates and latencies of excitatory and inhibitory responses for all taste stimuli are shown in Table 2. There were no statistically significant differences among responses magnitudes or latencies of response (paired *t*-tests, *P*s > 0.1). These observations are underscored by scatterplots shown in Fig. 3, *left*, where mean firing rates of response to prototypical tastants in each cell are plotted against those evoked by the corresponding naturalistic stimuli. Although there were some cells that responded to one type of stimulus and not the other (shown as gray symbols), for the majority of cells, both types of stimuli evoked

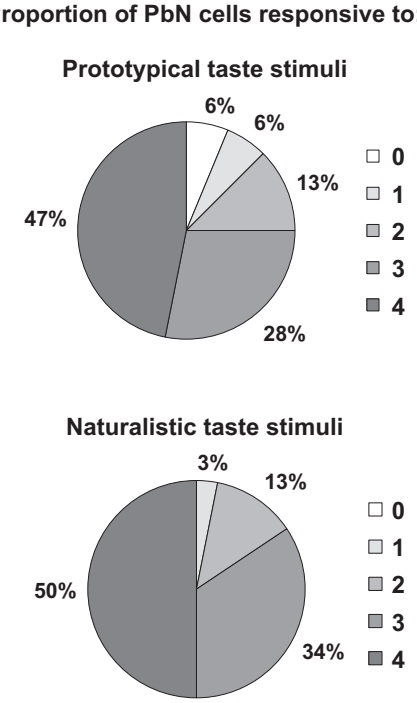

Fig. 1. Proportion of parabrachial pons (PbN) cells (total $n = 32$) with responses to 0, 1, 2, 3, and 4 prototypical (NaCl, sucrose, citric acid, and caffeine) or naturalistic (clam juice, grape juice, lemon juice, and coffee) tastants.

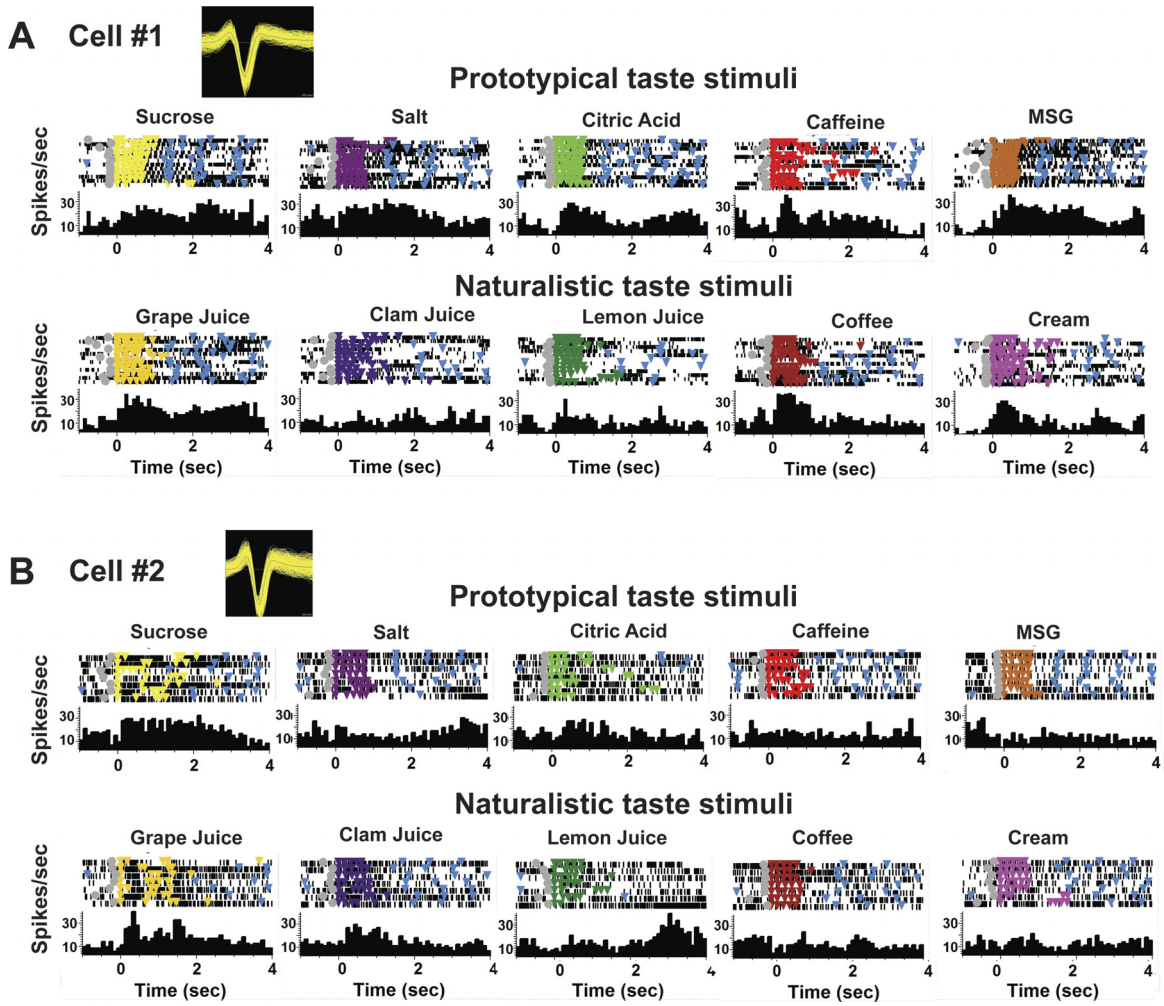

Fig. 2. *A* and *B*: responses to prototypical and naturalistic taste stimuli in 2 PbN cells. *A* and *B*, *top*: shows a trial-by-trial raster where black markers indicate spikes, blue triangles indicate artificial saliva (AS) licks, and other colored triangles indicate taste stimulus licks. The occurrence of dry licks during the AS presentation are not shown. *A* and *B*, *bottom*: peristimulus-time histogram for each taste stimulus; time bin = 100 ms.

similar firing rates. For latencies of response, a similar set of scatterplots (Fig. 3, *right*) showed evidence of differences between responses to prototypical vs. naturalistic tastants. That is, naturalistic stimuli evoked shorter latency responses than prototypical tastants for sweet, sour, and bitter taste qualities in the majority of cells. For salty stimuli, the opposite was true. Responses to grape juice ($n = 13$), lemon juice ($n = 12$) and caffeine ($n = 13$) with shorter latencies than their prototypical counterparts outnumbered those with longer latencies (sweet, $n = 5$; sour, $n = 4$; bitter, $n = 6$). Conversely, NaCl responses occurred at shorter latencies than those to clam juice in 10 cells and longer latencies in

Table 2. *Mean taste response magnitude for each tastant*

	Salty	Sweet	Bitter	Sour	MSG	Cream
Response magnitudes						
Excitatory						
Prototypical	18.5 ± 2.8	18.3 ± 3.1	15.7 ± 2.5	19.2 ± 3.2	19.2 ± 3.3	
Naturalistic	16.1 ± 2.2	20.8 ± 2.9	15.0 ± 1.8	19.8 ± 2.6		16.7 ± 1.6
Inhibitory*						
Prototypical	−11.1 ± 2.9	−13.2 ± 2.6	−9.2 ± 1.3	−18.7 ± 4.9	−14.4 ± 2.0	
Naturalistic	−15.8 ± 3.6	−21.8 ± 5.7	−17.2 ± 5.7	−17.7 ± 4.6		−13.0 ± 3.4
Response latencies						
Excitatory						
Prototypical	0.70 ± 0.12	0.84 ± 0.12	0.68 ± 0.11	0.88 ± 0.10	0.75 ± 0.12	
Naturalistic	0.78 ± 0.12	0.71 ± 0.11	0.56 ± 0.09	0.63 ± 0.12		0.81 ± 0.10
Inhibitory						
Prototypical	0.65 ± 0.24	0.84 ± 0.12	0.46 ± 0.13	0.66 ± 0.20	0.32 ± 0.13	
Naturalistic	0.48 ± 0.07	1.39 ± 0.31	1.11 ± 0.29	0.71 ± 0.21		1.33 ± 0.24

Values are means ± SE in spikes/s. MSG, monosodium glutamate. *Inhibitory responses are expressed as the mean deviation below baseline ± SE.

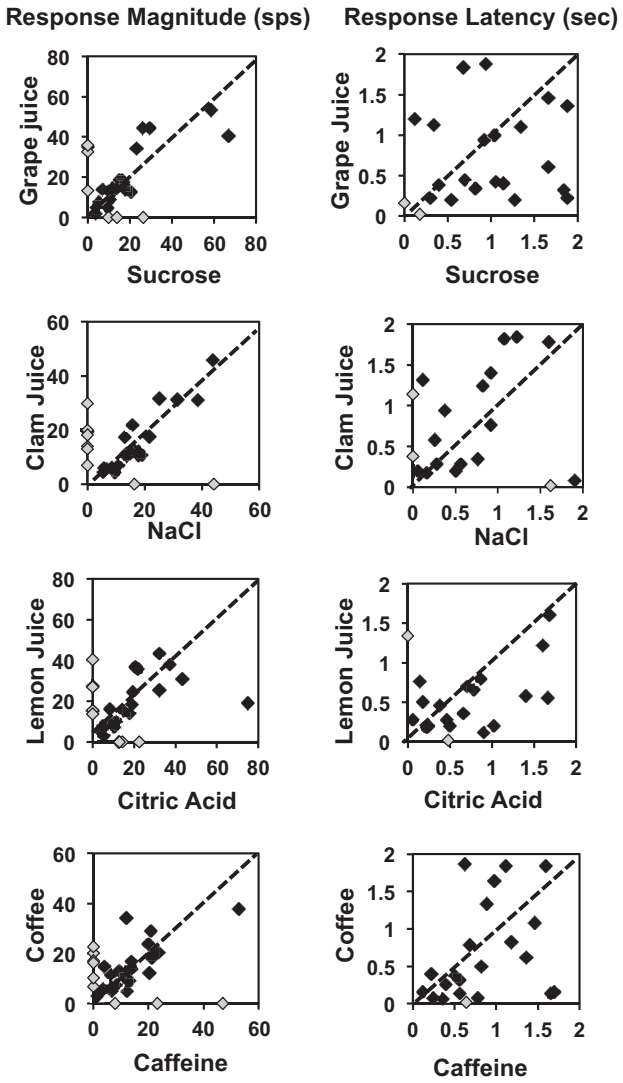

Fig. 3. Scatterplots of response magnitudes (*left*) and latencies (*right*) for prototypical vs. naturalistic tastants. Black diamonds indicate neurons that responded to both stimuli; grey diamonds indicate neurons that responded to either the prototypical or the naturalistic taste stimulus, but not both. Dashed diagonal lines indicate equal response magnitudes or latencies for both tastants.

six cells. However, the diversity of latencies within the population was quite large, and (as mentioned above) there was no overall difference between latencies in response to prototypical vs. naturalistic tastants.

Across Neuron Patterns of Response

Because of the diversity of responses of individual neurons, systematic differences in response properties across the population might not be evident from the responses of individual neurons to individual stimuli. Therefore, to identify possible differences in population responses, we applied multidimensional scaling (MDS). We considered the response magnitudes for prototypical and naturalistic tastants and used Pearson product-moment correlations as a measure of similarity so that neurons with high firing rates would not contribute disproportionately. Results are shown in Fig. 4. Both prototypical and naturalistic groups of stimuli are spaced widely apart. However, surprisingly, the naturalistic tastants were not placed close to their prototypical counterparts in the taste space constructed from the MDS analyses, as one might expect. This is especially noticeable, for example, for coffee vs. caffeine.

This map of "taste space" has two striking features. First, the group of naturalistic tastants is clearly segregated from prototypical taste stimuli. Notably, this separation is along *dimension 1*: the dimension that accounts for the greatest amount of the variance. Thus there is an overall difference in the way that the PbN responds to naturalistic tastants, compared with isolated tastes, even though this difference is not evident in the pairwise comparisons of firing rates, shown in Fig. 4. A second feature of the data is that the naturalistic stimuli are more widely dispersed in the taste space. Specifically, the mean distance between naturalistic taste stimuli was significantly greater than the mean distance between prototypical taste stimuli (Student's t-test, $P < 0.01$). Collectively, these data suggest that naturalistic stimuli evoke across neuron patterns that are different than their prototypical counterparts and are also more easily distinguishable from one another. We note that that these differences are not driven by the inclusion of the unpaired stimuli (cream and MSG), as very similar results were obtained in an MDS analysis that excluded them.

Temporal Coding Analyses

To directly test whether the naturalistic taste stimuli were more discriminable at the level of single neurons, we applied the metric space analysis. As detailed below, this supported the idea that responses to naturalistic taste stimuli convey more information than responses to prototypical taste stimuli.

Figure 5 shows the results of metric space analyses of the first 2 s of response in one PbN cell. (For all metric space analyses we excluded responses to MSG and cream so that each prototypical tastant had a matched naturalistic counterpart.) These graphs plot the information (in bits) conveyed by original neural data, the shuffled control ± 2SD, and the exchange control ± 2SD, across various levels of temporal

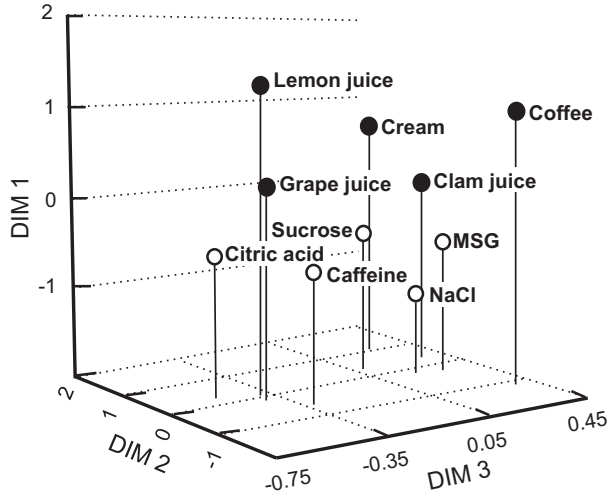

Fig. 4. Multidimensional scaling of responses to prototypical and naturalistic tastants. Pearson product-moment correlations were used as measures of similarity. The solution for 3 dimensions, which accounted for 98.4% of the variance, is shown. Pairs of quality-matched stimuli are depicted with the same colored symbols; open symbols are prototypical tastants, filled symbols are naturalistic stimuli. Cream and monosodium glutamate (MSG; black symbols) are not considered quality-matched stimuli. Guttman stress levels for each of 5 dimensions were as follows: *1*, 0.288; *2*, 0.111; *3*, 0.062; *4*, 0.035; *5*, 0.022.

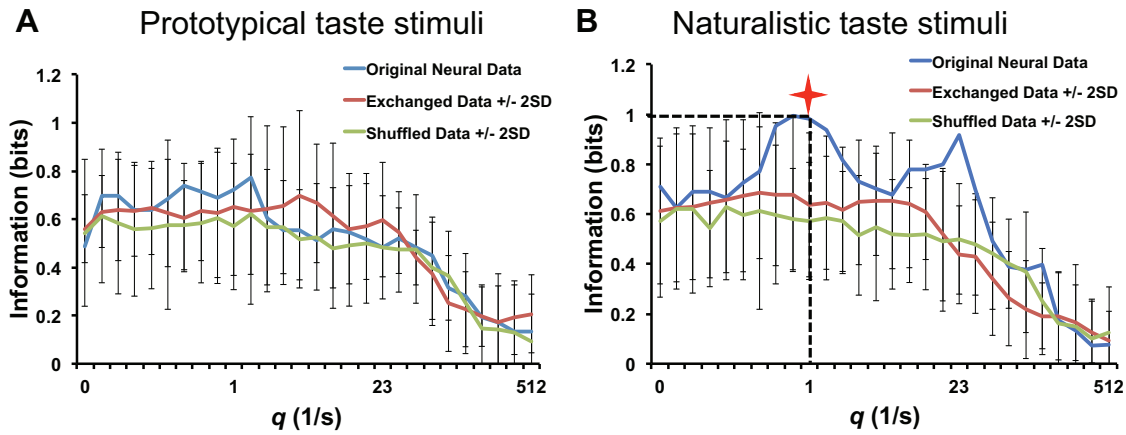

Fig. 5. Metric space analyses for prototypical (*A*) and naturalistic (*B*) taste stimuli in one neuron. *A* and *B* show the amount of information, *H*, in bits, at a variety of levels of temporal precision, *q*. Results shown in *A* indicate that there was no significant information conveyed about prototypical tastants since the information from the neural response did not exceed the shuffled control + 2SD. The red star in *B* indicates that the information conveyed by the spike timing in the neural response to naturalistic tastants is significantly larger than either the shuffled control + 2SD or the exchange control + 2SD. Four taste quality-matched tastants were used for each analysis, omitting umami and cream.

precision (*q*). Figure 5, *left*, shows the results for the prototypical tastants. In this plot, the information conveyed by the neural data did not differ from either control, indicating that there was no significant information conveyed by these responses. Figure 5, *right*, shows that for naturalistic taste stimuli (in the same cell), information is significantly above chance. The peak is at $q = 1$, indicating that there is a significant contribution of spike timing to information conveyed by this cell about differences among naturalistic tastants. Overall, there were 22 cells (of 32, 69%) that showed $H_{count} > H_{shuffle}$ for naturalistic taste stimuli, but only 17 cells (of 32, 53%) for prototypical tastants. Seven cells for naturalistic and six cells for prototypical taste stimuli also showed $H_{count} > H_{exchange}$, indicating that spike timing conveyed a significant amount of information discriminating among taste stimuli.

Figure 6 compares information conveyed about prototypical vs. naturalistic taste stimuli in those cells where H_{count} exceeded the shuffled control + 2SD value for both categories of taste stimuli in the first 2 s of the responses. There were 12 PbN cells that fit these criteria (black symbols). Also shown are the information values of those cells that conveyed a significant amount of information about naturalistic stimuli but not for prototypical tastants ($n = 10$) and vice versa ($n = 5$) (gray symbols). Figure 6, *left* and *middle*, shows that responses to naturalistic tastants convey as much or more information about taste quality as prototypical tastants in most PbN cells. For both H_{count} and H_{count}, there were only one or two cells respectively that showed less information for prototypical vs. naturalistic taste stimuli. Temporal precision at which information was maximized (q_{max}) was similar for responses to both stimulus categories (paired Student's *t*-test, $P = 0.72$).

Figure 7 shows the amount of information conveyed about prototypical and naturalistic tastants over cumulative response intervals from 200 ms to 2 s. For each response interval, the total information across all cells where $H_{count} > H_{shuffle}$ + 2SD was divided by the number of cells in the sample, i.e., 32. With very short responses intervals, the information conveyed by either stimulus group was similar. However, as the response unfolded over time, the information conveyed in responses to naturalistic taste stimuli became larger than the information in responses to their prototypical counterparts. This held for the information conveyed by spike count, as well as the total information conveyed by spike count (H_{count}, dotted lines) and timing (H_{count}, solid lines). We also performed parallel analyses of lick patterns associated with each stimulus to ensure that differences in information conveyed by a cell was not a

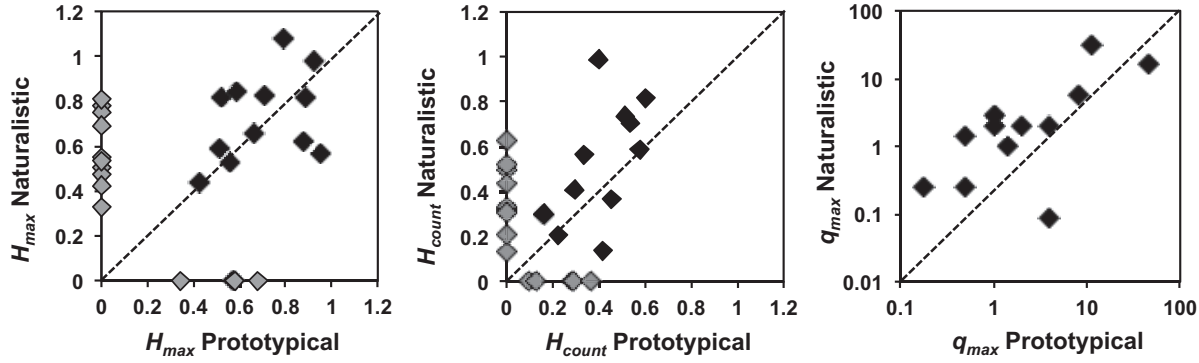

Fig. 6. Comparison of results of metric space analyses of responses to prototypical vs. naturalistic taste stimuli. Data from neurons that conveyed a significant amount of information about taste quality ($H_{max} > H_{shuffle}$) for both prototypical and naturalistic stimuli are shown by black symbols ($n = 12$). Cells that showed a significant amount of information about taste quality for either prototypical or naturalistic stimuli but not both are shown by grey symbols. *Left*: maximum amount of information conveyed about taste quality (H_{max}) by either spike count, the rate envelope or spike timing. *Middle*: amount of information about taste quality conveyed by spike count alone (H_{count}). *Right*: temporal precision (the value of *q* at H_{max}).

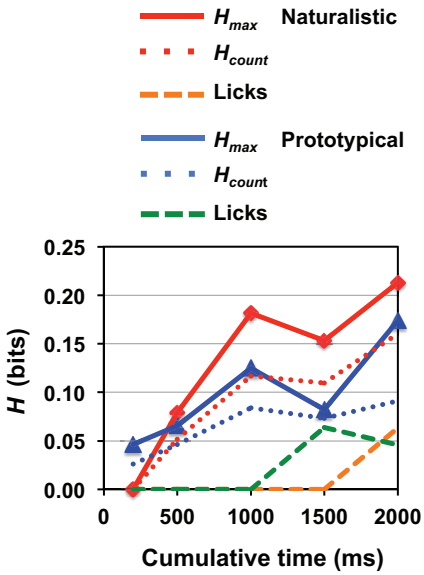

Fig. 7. Information in neural responses (average per cell) conveyed by spike timing (solid lines), by spike count alone (dotted lines) or by lick microstructure (dashed lines). Each point is the average of H_{count} across the entire population, with contributions from cells for which H_{count} did not reach significance set to 0. For the longer response intervals, the amount of information carried by temporal coding is larger for naturalistic taste stimuli than for prototypical taste stimuli.

byproduct of differences in the lick microstructure. As in other similar analyses that we have conducted, Fig. 7 shows that lick pattern was not as informative as the spike response (e.g., Escanilla et al. 2015; Roussin et al. 2012; Weiss et al. 2014).

Responses to Cream

In addition to testing prototypical tastants and their naturalistic counterparts, we also tested responses to a 25% solution of heavy cream diluted in AS. This taste stimulus solution was used as a stimulus representing fat taste: the diluted heavy cream solution had a fat content of 8.33 g of fat per 100 ml, 5.83 g of which was saturated fats. It should be noted that heavy cream contains lactose, a potentially effective taste stimulus; however, the concentration in 25% heavy cream is 2.6 mM, which is below the stimulus detection threshold for lactose (Joesten et al. 2007).

Of the 32 PbN cells recorded, 27 produced excitatory responses to cream. Figure 8 shows the evoked responses magnitudes for cream in all cells. The average excitatory response

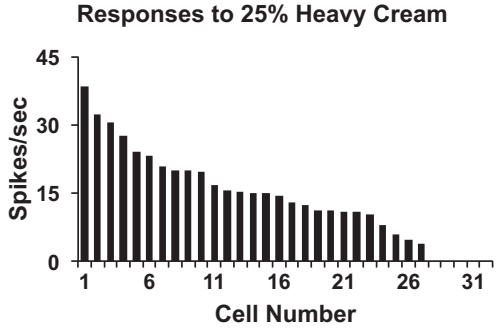

Fig. 8. Response magnitudes (spikes/s) to a 25% dilution of heavy cream in all PbN cells recorded. Responses are arranged in descending order of magnitude. Only 2 cells (of 32, 6%) did not respond to this stimulus.

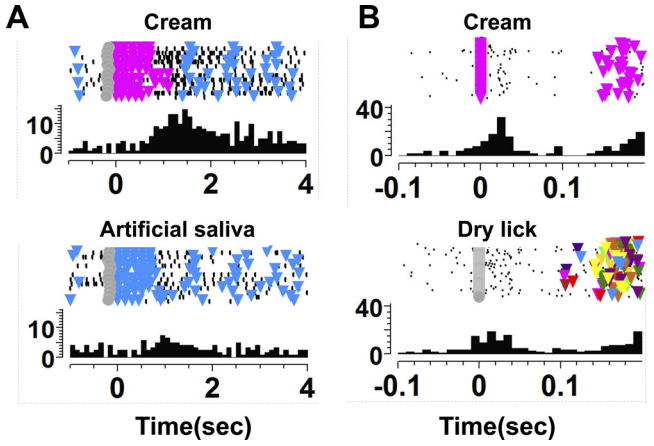

Fig. 9. Examples of responses to 25% heavy cream in 2 PbN cells. *A*: vigorous response to cream (*top*) compared with a nonsignificant response to artificial saliva. *B*: response to cream following each lick (*top*) compared with the response to dry licks (*bottom*). Details of *A* and *B* are as in Fig. 2.

magnitude was 16.7 ± 1.6 spikes/s above baseline firing rate, and the average latency was 0.81 ± 0.10 s. Figure 9 shows two examples of robust responses to cream in two different cells. The responses on the left showed a long latency and occurred over ~2 s. The response to AS in the same cell is shown below for comparison. On the right, the response to cream is very brief and peaks at ~20 ms after the lick. Responses to dry licks in the same cell are shown below for comparison.

Histology for Electrophysiological Experiments

Figure 10 shows the results of histological analyses of recording sites. As can be seen, all neurons were recorded from the medial PbN. Six lesion sites were located in the central and dorsal medial nuclei and three lesion sites were in the ventromedial nucleus. There were no apparent differences in taste or lick-related responsivity across these locations.

Behavioral Aceptance

To evaluate the possibility that differences between prototypical vs. naturalistic stimuli were due to a difference in their hedonic qualities, we carried out behavioral acceptance testing. Figure 11 shows the results. With the exception of salty tastes, each stimulus pair evoked similar numbers of licks. For salty tastants, there was a reliable difference between NaCl and clam juice at 10 s (NaCl preferred, $P < 0.001$, with Bonferroni correction). This difference was not significant when both taste stimuli were presented for 30 s. There were no other significant differences between any other pair of tastants for either the 10- or 30-s tastant presentation sessions.

DISCUSSION

Rather than "simply" relaying basic taste information from NTS upstream, the present data suggest that PbN cells are capable of encoding a much richer repertoire of sensory information than has been traditionally conceptualized. Results also suggest that a full functional characterization of the response properties of neurons in taste-related structures, such as the PbN, requires examination of their responses to exemplars of complex ingesta that are found in the natural environment. Excluding such stimuli risks misrepresenting the most effective

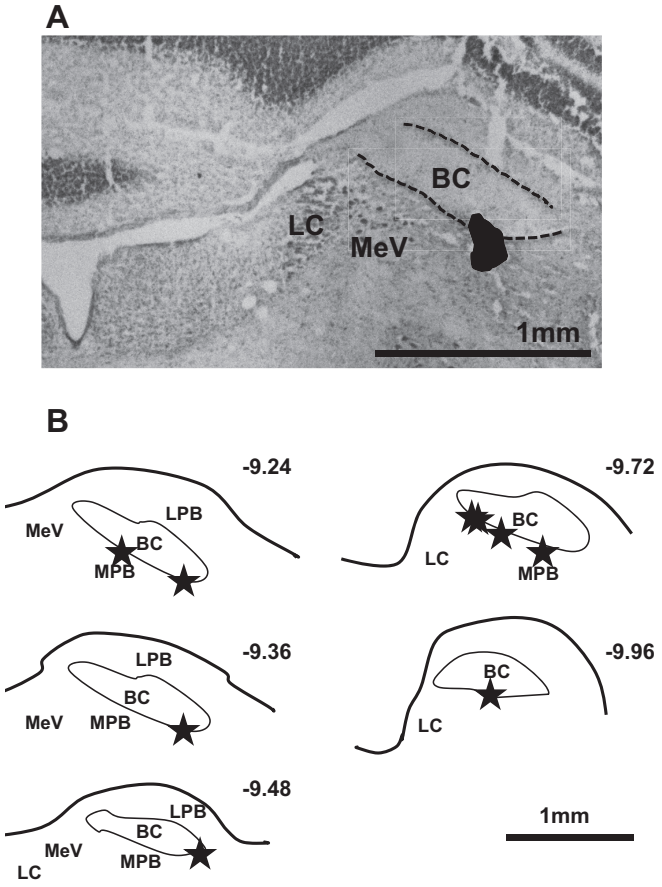

Fig. 10. Histological analyses of recording sites in the PbN of 9 rats. *A*: photomicrograph of a coronal section through the PbN showing the site of the lesion created to mark the recording site (filled black area). *B*: line drawings of the area surrounding the PbN showing the sites of recordings for all animals. Numbers in the upper right of each panel indicate distance posterior to bregma (mm). BC, brachium conjunctivum; LC, locus coeruleus; LPB, lateral parabrachial nucleus; MeV, mesencephalic nucleus of the trigeminal nerve; MPB, medial parabrachial nucleus.

stimuli that drive these cells. Moreover, although it may be difficult to replicate some of these stimuli with mixtures of their various components, they nevertheless represent more ethologically relevant stimuli than pure chemicals.

Converging evidence from several analyses support the idea that PbN cells convey more information about naturalistic tastes (food) than about concentration-matched prototypical taste stimuli. The across-neuron response patterns were more dissimilar (i.e., more widely separated) for naturalistic tastes than for prototypical taste stimuli. Within individual neurons, the metric space analyses also showed that most PbN cells conveyed as much or more information about naturalistic taste stimuli than about prototypical tastants. Specifically, on average, the amount of information conveyed by both temporal coding and firing rate over the first 2 s of the response was greater for naturalistic tastants than prototypical tastants. Results of the behavioral tests show that prototypical taste stimuli and their naturalistic counterparts evoke similar lick rates, suggesting that behavioral reactivity cannot account for differences in electrophysiological responses. Collectively, these data underscore the idea that the more effective and informative stimuli for PbN cells are natural foods and not the singular sapid stimuli that have traditionally served to characterize taste response profiles of PbN cells.

The naturalistic tastants that were used in the present study were chosen because each could be characterized as having a major component that matched a prototypical taste stimulus, but also had complexity in odor and texture. Concentrations of each major component were matched in prototypical-naturalistic stimulus pairs to enable direct comparisons. For example, clam juice is essentially salty; we chose a brand of clam juice that contained no MSG so as to avoid confounding the saltiness of the clam juice with the flavor-enhancing aspect MSG. Similarly, grape juice is predominantly sweet; lemon juice, which is almost exclusively citric acid, is sour; and coffee is bitter. However, despite our efforts to choose naturalistic stimuli dominated by a single "basic" taste, it is possible, perhaps likely, that some of our naturalistic tastes are actually mixtures of more than one basic taste. For example, grape juice is naturally slightly acidic (pH of 4 compared with pH of 4 for citric acid and lemon juice; pH of 6 for sucrose). Coffee may also contain bitter stimuli in addition to caffeine and may therefore stimulate several different types of "bitter cells" (Geran and Travers 2009). Nevertheless, we would expect that PbN responses to these naturalistic tastants would be about the same as the responses to the most effective component of the mixture (Travers and Smith 1984; Vogt and Smith 1993a,b; 1994), especially because the secondary components, if any, are below threshold. Not surprisingly, then, the average response magnitudes and average lick rates (as shown by behavioral acceptance tests) to each of our naturalistic tastants were comparable to the responses to their quality- and concentra-

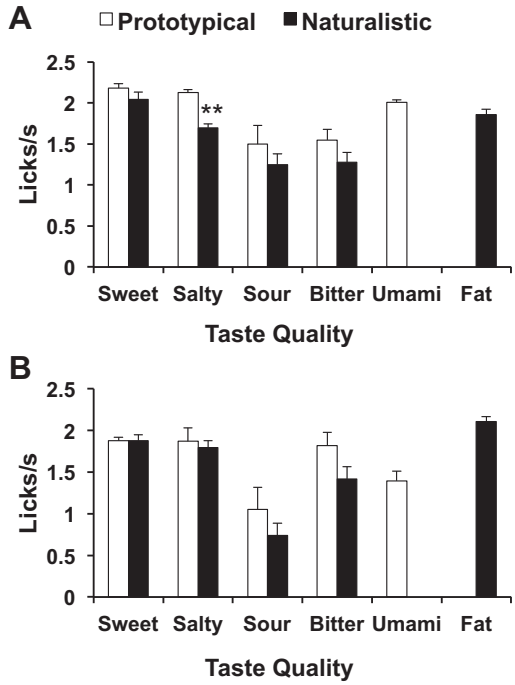

Fig. 11. Mean number of licks ± SE for each pair of quality-matched tastants plus umami and fat. Sweet, sucrose vs. grape juice; salty, NaCl vs. clam juice; sour, citric acid vs. lemon juice; bitter, caffeine vs. coffee; umami, MSG + inosine monophosphate (IMP); fat, heavy cream. Concentrations were the same as those used in electrophysiological experiments (see text). *A*: mean licks/s ± SE for 10-s presentations for each taste stimulus. *B*: mean licks/s ± SE for 30-s presentations for each taste stimulus. $^{**}P < 0.01$.

tion-matched prototypical stimuli. This might contribute to the spreading of the representation seen in MDS (Fig. 4). In addition, the presence of more than one taste quality along with odorant and particulate components might also contribute to the greater amount of information conveyed by naturalistic testate stimuli (Fig. 7).

Results of the behavioral acceptance tests showed that the prototypical and their naturalistic counterparts evoked similar lick rates with both 10- and 30-s exposures. Although NaCl evoked a significantly higher lick rate than clam juice over 10 s, this difference was gone with 30-s exposure. Also, with 30-s exposure, unpalatable tastants, such as caffeine and coffee, produced lick rates that were comparable to highly palatable tastes such as sucrose and grape juice. This may reflect the fact that animals were tested in a water-deprived state such that their motivation to drink overcame their aversion to relatively weak concentrations of bitter tastants. Rats were tested in a water-deprived state, identical to the water-deprived state during which taste responses were recorded; however, if rats had been tested without water deprivation, differences between prototypical and naturalistic taste stimuli might have emerged. We chose to test behavioral acceptance in these brief access tests, rather than using longer term exposure tests such as conditioned taste aversion generalization or 48-h two-bottle preference tests, because our tests preclude the influence of postingestional factors. Similar lick rates by prototypical and matched naturalistic taste stimuli imply that potential differences in behavioral reactivity cannot account for differences in information conveyed by the electrophysiological responses.

Recent studies from Palmiter's group (reviewed in Wu et al. 2012) have provided a map of the feeding circuit wherein the PbN is an essential component. Specifically, they have shown that when inhibitory input to the PbN from the hypothalamus is impaired, mice starve themselves voluntarily. This inhibitory input arises from cells in the arcuate nucleus that coexpress agouti-related protein (AgRP), neuropeptide Y, and GABA and modulates a powerful excitatory drive that originates in the NTS. Blocking glutaminergic output from the PbN following AgRP neuron ablation prevents the starvation that normally accompanies this manipulation. These authors concluded that the PbN is central to the neural circuit that regulates feeding and body weight. As such, it is not surprising that the cells in this area are more sensitive to the constellation of sensations produced by food rather than the arguably more limited sensations produced by prototypical taste stimuli.

It might be argued that the keen sensitivity to naturalistic stimuli reflects an evolutionary mandate of PbN cells to detect food and regulate ingestion. The observation that responses to naturalistic tastes occur at shorter latencies than prototypical tastants in most cells and that responses to naturalistic tastants convey more information than prototypical taste stimuli supports this idea. Along this line, it has been shown that both PbN (Di Lorenzo and Garcia 1985) and NTS (Escanilla et al. 2015; Van Buskirk and Erickson 1977) cells respond to both taste and olfactory stimuli. In addition, we have shown that binary mixtures of the prototypical taste stimuli can sometimes evoke responses in NTS cells that are not predicted by the responses to the components of the mixture (Chen and Di Lorenzo 2008). The same may be true of PbN cells. Since naturalistic tastes evoke more olfactory stimulation and are themselves mixtures of several tastes, responses in these cells to prototypical tastes may be only an approximation of their true repertoire of sensitivity.

It is noteworthy that there was some variability across PbN cells in the relative responses to naturalistic vs. prototypical taste stimuli. That is, there were some cells that responded more poorly to naturalistic tastes than to prototypical taste stimuli and others that conveyed less information, rather than more, about naturalistic tastants compared with prototypical taste stimuli. There are several possible (nonexclusive) explanations for this finding, beyond the obvious conclusion that the PbN is heterogeneous in its composition. First, it raises the possibility that there are some cells that respond to taste alone, i.e., those favoring prototypical taste stimuli, while others respond preferentially to naturalistic stimuli. Such "taste-only" cells could convey the role of identifying (parsing) the gustatory component of a complex stimulus such as a food and sending that information along the main central gustatory pathway. This conceptualization would be compatible with a "labeled line" organization of the taste system, although it would be applicable to only a limited number of cells in the PbN. Second, independent of a specific role for taste-only cells, a diversity of tuning properties may be generally advantageous for sensory coding, as it reduces the redundancy among neurons. Third, it is possible that cells that appeared to be taste-only are in fact multisensory but did not manifest this behavior in response to the limited library of stimuli used here.

We emphasize that the findings here likely represent an underestimate of the response repertoire of PbN cells: it is likely that there are foods that would evoke even larger responses than the ones that were tested here or demonstrate multimodality in neurons that appeared taste-only in these recordings. Naturalistic tastes are essentially multisensory. Thus it can be argued that these naturalistic stimuli are not normally dissected cognitively into their components but instead are processed as food objects, that is, complex mixtures with their own unique identities, by cells that respond preferentially to naturalistic stimuli.

Responses to Cream

It has been argued that fat is a basic taste quality, deserving equal status with sweet, sour, salty, bitter, and umami (Running et al. 2015). To test this hypothesis, many investigators have studied responses to free fatty acids (FFAs) as representatives of "fat taste" since fats are rapidly broken down into FFAs in the mouth by lingual lipase (Kawai and Fushiki 2003). However, FFAs alone do not evoke electrophysiological responses in the chorda tympani nerve (which innervates taste buds on the rostral 2/3 of the tongue) but instead modify responses to other tastes (Stratford and Contreras 2009; Stratford et al. 2008). In addition, Rolls et al. (1999) and Verhagen et al. (2003, 2004) recorded responses from a few "fat-responsive" cells from the orbitofrontal and opercular cortices in primates and concluded that these cells responded to the oral feel of fats (such as cream and silicone oil) and not the chemosensory properties. Evidence that neurons in other parts of the central nervous system respond to fat or fatty acids has been lacking. Here, we showed robust and widespread sensitivity to dilute heavy cream in the sample of PbN cells. Heavy cream at 25% dilution is 9.3% fat and 89.4% solvent (artificial saliva and water), leaving little else that might stimulate a response. That

is, the concentrations of all other ingredients, including Na^+, are less than 0.001 M after dilution. These minute concentrations are near or below threshold and could not account for the vigorous responses that we recorded. It is possible that the lubricity of the cream might have contributed to the responses to cream, but even if that were the case, it is difficult to imagine that lubricity alone could generate such vigorous responses in such a large proportion of PbN cells. In other studies, it has been argued that FFAs are perceived through their ability to stimulate somatosensory (texture) or olfactory sensations (Ramirez 1993; Takeda et al. 2001; Verhagen et al. 2004; Oberland et al. 2015). However, it has been argued that these assertions are not as compelling when tested with more tightly controlled experiments (reviewed in Running et al. 2015). Instead, it may be the combination of sensory modalities that embodies the sensation of "fat" whether or not fat alone has a taste. Interestingly, we showed that cream was placed relatively far from all other stimuli in the MDS space, indicating that the across neuron responses were distinguishable from across-neuron responses to other taste stimuli, both prototypical and naturalistic. These data suggest that the responses to fat in the PbN are separate from responses to other tastants. As with other naturalistic taste stimuli, the extra-gustatory components add significantly to the overall sensory experience.

Summary and Conclusions

Rats are omnivores and as such incorporate a wide variety of foodstuffs in their diet, if given the chance. It is therefore unsurprising that the sensory systems that alert these animals to the taste of food might also incorporate the hedonic and nutritional value of food into their responses. Previous work has shown that PbN (Ogawa et al. 1982) and NTS (Halsell et al. 1993; Travers and Norgren 1995) neurons respond to tactile and olfactory (Di Lorenzo and Garcia 1985) stimuli in addition to taste. The present study shows that this multimodality is relevant to real food: the majority of taste-responsive neurons in the PbN respond best to and convey the most information about complex, naturalistic stimuli. Thus the response properties of PbN neurons are appropriate not only for an obligatory relay for gustatory information but for a critical structure in the neural circuit underlying ingestion.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.D.S. and M.S.W. performed experiments; J.D.S., M.S.W., and P.M.D.L. analyzed data; J.D.S., M.S.W., J.D.V., and P.M.D.L. interpreted results of experiments; J.D.S. and M.S.W. drafted manuscript; J.D.S., M.S.W., J.D.V., and P.M.D.L. edited and revised manuscript; J.D.S., M.S.W., J.D.V., and P.M.D.L. approved final version of manuscript; P.M.D.L. conception and design of research; P.M.D.L. prepared figures.

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