Dynamic taste responses of parabrachial pontine neurons in awake rats

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1Biology Department, Brandeis University, Waltham, Massachusetts; 2Psychology Department, Brandeis University, Waltham, Massachusetts; 3Volen National Center for Complex Systems, Brandeis University, Waltham, Massachusetts; 4Department of Neurobiology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; 5Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel; and 6Department of Neurobiology and Anatomy, Wake Forest School of Medicine, Winston-Salem, North Carolina

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Baez-Santiago MA, Reid EE, Moran A, Maier JX, Marrero-Garcia Y, Katz DB. Dynamic taste responses of parabrachial pontine neurons in awake rats. J Neurophysiol 115: 1314–1323, 2016. First published January 20, 2016; doi:10.1152/jn.00311.2015.—The parabrachial nuclei of the pons (PbN) receive almost direct input from taste buds on the tongue and control basic taste-driven behaviors. Thus it is reasonable to hypothesize that PbN neurons might respond to tastes in a manner similar to that of peripheral receptors, i.e., that these responses might be narrow and relatively “dynamics free.” On the other hand, the majority of the input to PbN descends from forebrain regions such as gustatory cortex (GC), which processes tastes with “temporal codes” in which firing reflects first the presence, then the identity, and finally the desirability of the stimulus. Therefore a reasonable alternative hypothesis is that PbN responses might be dominated by dynamics similar to those observed in GC. Here we examined simultaneously recorded single-neuron PbN (and GC) responses in awake rats receiving exposure to basic taste stimuli. We found that pontine taste responses were almost entirely confined to canonically identified taste-PbN (t-PbN). Taste-specificity was found, furthermore, to be time varying in a larger percentage of these t-PbN responses than in responses recorded from the tissue around PbN (including non-taste-PbN). Finally, these time-varying properties were a good match for those observed in simultaneously recorded GC neurons—taste-specificity appeared after an initial nonspecific burst of action potentials, and palatability emerged several hundred milliseconds later. These results suggest that the pontine taste relay is closely allied with the dynamic taste processing performed in forebrain.

parabrachial nucleus; taste processing; temporal coding; gustatory cortex

THE TASTE SYSTEM EXISTS to facilitate identification of nutrients and toxins in an animal’s potential diet (Boudreau et al. 1985; Scott et al. 1999). Much of this process is performed within the central nervous system (CNS), a fact evident in the ease and speed with which taste preferences are changed by experience (see Galef 1986; Monk et al. 2014; Spector et al. 1988). However, much remains to be learned about how such identifications arise in the CNS—specifically, what neural mechanisms assign meaningful labels (e.g., chemical identity, hedonic attributes) to tastes and where these mechanisms reside.

It is clear that the nature of taste coding changes between the periphery, where processing units appear to be simply labeled for specific stimulus types (Scott 2004; Zhao et al. 2003), and cortex, where neurons respond to tastes with complex, perceptually relevant dynamics—responding first to the presence of a taste on the tongue, then to its physical properties, and finally to its palatability, all across the first second of firing (Bahar et al. 2004; Grossman et al. 2008; Katz et al. 2001; Maier and Katz 2013; Piette et al. 2012; Sadacca et al. 2012; Samuelsen et al. 2012). It is less clear whether the regions between the tongue and forebrain are more “periphery-like” or “cortex-like.”

Of particular interest in this regard are responses of pontine parabrachial nucleus (PbN) neurons in the brainstem. In the rodent, PbN neurons receive taste information almost directly from the tongue, via a single intervening way station in the rostral nucleus of the solitary tract (rNTS; Granata and Kitai 1989; Halsell et al. 1996; Suwabe and Bradley 2009). PbN plays a central role in gustation (Sewards 2004) and is necessary for palatability processing (Spector 1995), taste-reward processing (Mungamdee et al. 2008), and taste learning (Gregson et al. 1997; Reilly et al. 1993; Sakai and Yamamoto 1998; Yamamoto et al. 1995). This involvement of PbN in the most basic of taste processes likely reflects its placement as a bottleneck in the ascending signal, and is thus certainly consistent with the suggestion that taste responses in PbN might best reflect simple representations of taste input ascending from the periphery.

In line with this prediction, most examinations of PbN taste responses have reported little in the way of cortexlike dynamics. These taste responses, which have been observed exclusively in the brachium conjunctivum, medial PbN, and external lateral and ventral lateral subregions of PbN, are typically summarized in terms of spikes per second averaged across 5–10 s, with the primary realm of controversy being whether they are best described as narrowly (Geran and Travers 2009; Nishijo and Norgren 1990, 1991, 1997) or broadly (Di Lorenzo 1988) tuned.

On the other hand, the majority of input to PbN is not ascending signals from rNTS but rather feedback from forebrain structures such as gustatory cortex (GC) (Zhang et al. 2011). Such feedback has been shown to have a significant impact on PbN taste responses (Li et al. 2005; Lundy and Norgren 2004; Smith et al. 1998) and on taste-induced behavior (Grill and Norgren 1978; Tokita et al. 2004, 2007; Touzani et al. 1997). It is therefore reasonable to hypothesize that PbN taste responses may be dominated by temporal structures similar to those observed in cortex, produced via this pathway. Recent work has in fact suggested that responses in PbN (Di...
Lorenzo and Victor 2003; Roussin et al. 2012), and PbN itself (Geran and Travers 2013; Weiss et al. 2014), do vary through time in an information-rich manner (dynamics that are masked in analyses of multisecond averages of firing).

Here we further probe the possible temporal structure of taste responsiveness of PbN neurons in light of the above considerations, testing the specific hypothesis that PbN taste response dynamics will be similar to those of simultaneously recorded GC neurons. GC neurons were simultaneously assayed with those in taste-PbN (t-PbN) to ensure that comparisons of the two structures were made using data obtained under identical conditions. Our data support this hypothesis: as in GC, PbN taste responses progress from fluid responsive to taste specific, and from there to palatability related, all on a timescale similar to simultaneously recorded cortical responses.

MATERIALS AND METHODS

Subjects

Female Long-Evans rats (n = 19, 275–340 g at time of surgery) from Charles River Laboratories served as subjects. Females were chosen for docility and because our previous work has suggested little to no difference between males and females with regard to response dynamics, at least in cortex. While shifting estradiol levels undoubtedly impact some aspects of taste activity (Curtis et al. 2004; Curtis and Contreras 2006; Di Lorenzo and Monroe 1989; Stratford et al. 2006), these effects would, if anything, reduce the reliability of our results; thus any observed results are those that are robust enough to be insensitive to estrus state. Rats were maintained on a 12:12-h light-dark schedule and given ad libitum access to chow and water, unless otherwise specified. All sessions took place within the light period, and all surgical and experimental protocols were reviewed and approved by the Brandeis University Institutional Animal Care and Use Committee (IACUC).

Surgery

Rats were anesthetized with intraperitoneal injections of a ketamine-xylazine cocktail (100 and 10 mg/kg rat weight, respectively). Stable levels of anesthesia were maintained with 0.1 ml/h injections of the same cocktail. Rats were placed in a Kopf stereotaxic apparatus, and four sterile stainless steel ground screws were inserted into the skull. Craniotomies were then made, through which bundles of 16 formvar-coated 25-μm nichrome wires attached to a movable mini-microdrive (Katz et al. 2001) were implanted unilaterally 1 mm above PbN (−9.1 6 mm AP and ±2 mm ML from bregma; 6–7 mm DV from dura) and GC (1.4 A and ± 5.0 ML from bregma; −5.5 DV). To avoid puncturing the transverse sinus, the PbN microelectrode bundle was oriented 20° off the vertical with the tip pointing caudal. To avoid any risk of damaging the target region before recording (and to avoid neuron preselection), we did not use a search electrode before electrode implantation. Although this method increased the placement variability, that variability proved useful, as recordings obtained from areas outside the “waist” and lateral taste regions of PbN were used as control data.

After insertion of electrode bundles, bilateral intraoral cannulas (IOCs) were implanted and secured near the headstage (Phillips and Norgren 1970) to allow for tastant delivery, and the entire headstage was cemented to the skull with dental acrylic. After surgery, rats were given postoperative analgesics (Rimadyl, 4.4 mg/kg) and antibiotics (Pro-Pen-G, 150,000 U/kg) once a day for at least 2 days. Rats recovered for 7 days after surgery before undergoing habituation and experimental sessions (see below). During the recovery period, rats were provided with ad libitum access to chow and water.

Experimental Design

After recovery from surgery, rats were placed on water restriction (15 ml/day, given during the late morning) and habituated to the recording chamber and to fluid delivery through the IOC. During subsequent recording sessions, the responses of small ensembles of PbN and GC single neurons to passive administration of reagent-grade chemicals dissolved in double-distilled water [room temperature 40-μl aliquots of (in mM) 100 sucrose, 100 NaCl, 10 citric acid, and 1 quinine HCl] were recorded (see below); concentrations were selected to approximate those used in previous studies of this sort (Chen et al. 2011b; Frank et al. 2003; Weiss et al. 2014).

Taste solutions were maintained under slight nitrogen pressure and delivered directly into the mouth by means of a manifold of five miniature polymide tubes slid to the bottom of the IOC (to ensure there was no taste mixing). The combination of manifold and pressure ensured that relatively broad tongue coverage was achieved within only a few milliseconds of the computer-controlled solenoid opening—a similar delay for each taste (variability on the order of a few milliseconds) that was easily accounted for in our analyses. Tastes were selected randomly without replacement and interleaved with water rinse. There was a 15-s gap between each delivery to ensure resumption of basal firing. Recording sessions lasted 25–35 min, such that rats received between 12 and 15 trials of each tastant overall (total fluid volume ~2.5 ml).

Each rat underwent two or three recording sessions (1/day). Electrode tips were lowered by 75 μm between each recording day, enabling recordings within t-PbN, outside of t-PbN (non-t-PbN), and outside of PbN (non-PbN) in the same animal. The electrodes were moved up to a total of 0.5 mm in depth by the end of the recording.

Electrophysiology

During recording sessions, neural signals were collected from electrode bundles in PbN (16 electrodes) and GC (16 electrodes). Differential recordings were fed into a parallel processor, which digitized, amplified, filtered, and saved the signals (Plexon). Action potentials of signal-to-noise ratio > 3:1 were isolated online and set to cross a designated amplitude threshold. Single-unit action potentials were discriminated and isolated with a template algorithm and cluster cutting techniques (Offline Sorter, Plexon). All other analyses of electrophysiological data were performed with MATLAB (http://www.mathworks.com/).

Data Analysis

All analyses were performed on the evoked nonnormalized firing rates in the 2,000 ms after stimulation—a period encapsulating the time that it takes an animal to identify the taste, decide on its current hedonic value, and act upon that decision (Halpern and Tapper 1971; Perez et al. 2013). Peristimulus time histograms (PSTHs) were produced from the firing of isolated single neurons, and these PSTHs served as the basic input to statistical analysis. In a small number of cases, electrical noise caused by solenoid opening contaminated the first 30 ms of the PSTHs; this contamination was removed by an algorithm that ignored above-baseline numbers of “action potentials” within the −10 to +30 ms period of the response, a period that did not overlap any portion of the taste responses themselves. Fluid responsiveness. Classification of fluid-responsive (and taste-responsive, see specific, below) units followed definitions used in previous publications (Maier et al. 2012; Piette et al. 2012; Sadacca et al. 2012): A neuron was deemed “fluid responsive” if any of the tantants significantly altered that neuron’s baseline firing rate. For this analysis, evoked nonnormalized responses to tastant administration were averaged for each tastant (across 2,000 ms) and compared to baseline firing rate (1,000 ms before tastant delivery) with paired t-tests. If stimulus-induced firing rates were significantly higher or lower than
the baseline firing rate (with \( P < 0.001 \)), they were considered fluid responsive. This analysis was then repeated for small time windows (200 ms, slid through the 2-s response window in 50-ms increments).

Note that this measure does not differentiate or specify differences in magnitude to the evoked response of each tastant or temporal differences between the evoked responses—that is, it identifies neurons that were modulated by fluid delivery, without determining whether those neurons were modulated distinctly by different tastants.

**Taste-specificity.** To identify which fluid-responsive neurons were “taste specific” (i.e., for which the normalized response to at least one tastant differed significantly from that to at least one other, in terms of either magnitude or time course), we applied a two-way ANOVA with taste and time factors to 10 consecutive, nonoverlapping time windows of that neuron’s evoked responses (window size: 250 ms; time span: 0–2,000 ms relative to taste delivery). A significant main effect of taste in such an ANOVA implies taste-specificity in the overall 2-s average of firing (i.e., the overall firing rate response to at least one tastant was significantly different from the overall average firing rate response to at least one other tastant). A significant taste × time interaction, meanwhile, can be interpreted as indicating “temporal coding” (i.e., different time courses of responses for different tastes; Li et al. 2013).

Note again that it is possible to have fluid-responsive neurons for which responses are not taste specific; furthermore, by the above definitions, neurons can also be taste specific without being fluid responsive. This is due to the fact that fluid responsiveness is evaluated with a paired t-test that compares a large bin of poststimulus time to baseline and that this long time average may possibly obscure brief modulations (even a short brief response to a single taste may be nonsignificant when all tastant trials are averaged). A biphasic response, for example, initially excitatory then inhibitory, can be canceled when averaged across time. Alternatively, a neuron might produce a mild inhibitory response to one stimulus and a mild excitatory response to another, such that neither manages to deviate significantly from baseline even though they differ significantly from each other. In each of these cases, the neuron’s responses would be classified as “taste specific” despite not being deemed “fluid responsive” overall.

Ancillary analyses shed light on the magnitude of taste-specificity uncovered in the two-way ANOVAs. We used the sum of squares differences (\( \eta^2 \), a step in the quantification of variability leading up to the ANOVA F-test) as estimates of effect size. Effect size estimates for all neurons from these analyses, and from similar sets of one-way ANOVAs examining the impact of taste delivery on firing rate of individual neurons in a sliding window over time (window size: 500 ms; step size: 100 ms), were themselves evaluated by ANOVA and/or paired t-test.

**Palatability relatedness.** To determine whether neurons produced “palatability-related” responses, we calculated the absolute correlation coefficient (\( r \)) between each neuron’s firing rate responses for each tastant and a simple established linear palatability function (which were estimated, based on the above references, to be sucrose > NaCl > citric acid > quinine). The magnitude of the correlation provided a measure of how well between-stimulus differences in response were accounted for by palatability. A neuron’s response was considered “palatability related” if this correlation achieved statistical significance (Fontanini et al. 2009; Li et al. 2013; Piette et al. 2012). It is worth noting that the temporal resolution of the above analysis is dependent on bin and sample sizes. We therefore performed one ancillary analysis of the above-described palatability relatedness plot—a normalized CUSUM chart. This procedure, a part of “change point” analysis (Kass-Hout et al. 2012; Page 1954; Weinberger et al. 2006), is commonly used to assess shifts in the mean of any kind of time series. In this case, we examined the time series of correlations between firing and palatability, using CUSUM to better visualize the time point at which correlations between the neuronal firing and palatability along the neuronal responses between neurons from different regions increase.

To that end, we first calculated the firing correlation to palatability in a short time window (200 ms), slid the window forward by 50 ms, and recalculated the correlation. Once we had a time series of correlations (starting 1,000 ms before stimulus delivery and ending 2,000 ms after stimulus delivery), we transformed the time series by subtracting the mean correlation from each of these instantaneous correlations. The sum of these differences of successive comparisons was then accumulated (hence “CUSUM”), and the PnB and GC charts were normalized to one another (with their minimum at zero) to facilitate interregional comparison.

If the mean correlations in such plots remain stable across the entire period, this time series will therefore wander randomly around a single value. If, however, the correlation increases within a particular time window (as we propose), the mean value will be intermediate between the two extremes, and therefore the differences between the individual points and the mean will be negative before the time of the increase and positive afterward; in this case, the CUSUM will become more and more negative until the time point of the correlation change, and will then head positive.

Each of these analyses offers information regarding the time course of palatability-related firing. Of course, no across-trial measure of this firing will provide precise evaluation; any useable bin size will necessarily smooth the data and smear the information.

**Histology**

Analysis of the electrophysiological data was done blind to histological confirmation of recording sites. After the last experimental session, rats were deeply anesthetized with an overdose of the same ketamine-xylazine cocktail used in surgeries, and small electrolytic lesions were made to mark the final location of electrode tips from which neurons of interest were recorded (via 7-μA DC current for 7 s). Rats were then perfused with 10% formalin, and brains were harvested and sectioned at a thickness of 60 μm and stained with cresyl violet (Sigma-Aldrich) to visualize Nissl substance in the neurons. Electrode trajectories were reconstructed based on information regarding their implantation, movement, and visible tracks, in reference to sections containing the electrode tracks and lesions, in relation to the standard rat brain atlas (Paxinos and Watson 2007); we extrapolated from our records of the electrode movements across recording days to identify the anatomical locations of each recording.

**RESULTS**

The responses of histologically verified (Fig. 1) brain stem neurons (\( n = 46 \)) to solutions of basic tastes (sucrose, NaCl, citric acid, and quinine) were recorded from 19 awake rats (\( 2.0 \pm 0.2 \) brain stem neurons/ensemble). In a subset of these rats (\( n = 5 \)), cortical neurons were simultaneously recorded with brain stem neurons. A total of 8 PbN-GC ensembles were isolated, with an average of \( 2.0 \pm 0.4 \) brain stem neurons/ensemble and \( 3.0 \pm 0.8 \) GC neurons/ensemble. A minimum of 13 responses to each taste were assayed in each recording session, along with spontaneous activity between each stimulus delivery.

The mean spontaneous firing rate (average activity in the 1.0 s before tastant delivery) of the PbN sample was \( 13.9 \pm 3.7 \) (SE) spikes/s, a number that is in good agreement with those reported in other studies using chronically implanted PbN electrodes in rats (Di Lorenzo 1988; Nishijo and Norgren 1990, 1991, 1997; Weiss et al. 2014). The mean spontaneous firing rate for the simultaneously recorded GC neurons was lower—\( 4.3 \pm 1.0 \) (SE) spikes/s, a number also consistent with previous

Example raster plots and PSTHs generated from t-PbN (Fig. 2A), non-t-PbN (Fig. 2B), and non-PbN (Fig. 2C) neurons reveal the expected “fluid responsiveness”—that is, their post-stimulus firing rates differed significantly from baseline on average—emerging almost immediately after taste delivery and peaking at ~200–250 ms after stimulus onset. This fluid responsiveness proved representative of the whole data set (as we demonstrate in greater detail below), in which ~70% (32/46) of the recorded brain stem sample were fluid responsive—46% (6/13) of t-PbN neurons, 67% (12/18) of non-t-PbN neurons, and 93% (14/15) of non-PbN neurons were modulated by oral delivery of fluid stimuli, according to paired t-tests (P value = 0.001).

The high rate of nonspecific responsiveness, the striking of the tongue by fluid, may reflect the prevalence of somatosensory innervation in the regions in and surrounding t-PbN (Baird et al. 2001; Halsell and Travers 1997; Karinnamazi and Travers 1998), as it did in previously recorded GC neurons (Katz et al. 2001). While we did not deliver water as a “taste stimulus,” examination of responses to water rinse trials (immediately following delivery of another tastant) suggested that indeed the same is true in the pons: 11 of 13 neurons showing either excitatory or inhibitory early responses (numbers of these were about equal) to tastes also showed the same response to the water rinse; these responses in general did not appear to be taste related (but see DISCUSSION).

However, while there were high rates of simple responsivity in all areas examined, “taste-specificity” was focused more in t-PbN responses than in non-t-PbN and non-PbN responses. Ninety-two percent (12/13) of t-PbN neurons responded distinctly to different tastes (that is, they were not only fluid responsive but also taste specific), whereas fewer non-t-PbN and non-PbN units—61% (11/18) and 73% (11/15), respectively—reacted in a taste-specific manner.

Furthermore, even those non-t-PbN and non-PbN neurons that did produce taste-specific responses failed to do so robustly. This can be seen in the example neurons in Fig. 2: Although all three example neurons produced significant
plots showing firing (each colored mark in a 2-way ANOVA; see text for details). Above each set of PSTHs is raster (x-axis is time, whereas those of the non-t-PbN neuron shown in Fig. 2B and the non-PbN neuron shown in Fig. 2C do not; the “best stimulus” for the t-PbN neuron shifted at ~750 ms after taste delivery (at the time that the evoked response to citric acid decreased), whereas the stimulus causing the strongest response was stable across a full 2 s for both of the other examples.

Again, the example results proved representative of the whole data set, according to which the differential taste-specificity of t-PbN responses (described above) to a large extent reflects the greater prevalence of temporal coding. Figure 3A presents the proportion of neurons that showed taste-specificity (i.e., a significant main effect of taste) and temporal coding (i.e., a significant taste × time interaction). This presentation reveals that the most noticeable difference between the entire sample of t-PbN neurons and that of other brain stem neurons had to do with the proportion of neurons for which taste-specificity changed through time. Whereas approximately half of the neurons in t-PbN produced time-varying patterns of taste-specificity, only a small subset of the neurons outside of t-PbN showed the same effect; specifically, 54% of taste-specific t-PbN neurons showed temporal complexity, while only 6% of non-t-PbN and 27% of non-PbN neurons did so, that is, 10 times as many t-PbN neurons as non-t-PbN neurons produced time-varying responses.

Furthermore, analysis performed on all of the taste-specific neurons in the data set (i.e., restricted to only those neurons that produced significant taste and taste × time effects, thus removing the confound of nonresponsive neurons) revealed that it was the time-varying component of the two-way ANOVAs (i.e., the values for the taste × time interaction) that differentiated neurons in the three regions [F(2,34) = 9.02, P = 0.0008]; the main effect for taste did not differentiate the groups [F(2,34) = 1.15, P = 0.3287]. Roughly half of the taste-specific responses recorded from t-PbN (a number that far exceeds chance and that is significantly higher than that observed outside of t-PbN) were time varying.

The above two results—that taste-specificity in brain stem responses outside of t-PbN is of relatively low magnitude and lacking in temporal features—imply that what little taste-specificity is apparent outside t-PbN should only be visible in multiple-second-long time averages of activity. We tested this additional prediction by performing a moving-window analysis in which we explicitly examined taste responses in 500-ms time bins (with a 100-ms step size). Figure 3B presents the results of this reanalysis, showing the percentage of brain stem neurons with significant taste-specific responses as a function of response time. This presentation confirms the suggestions that taste-specific responses (in that 2-way ANOVAs showed significant main effects of taste), visual inspection suggests that the variance of the responses as a function of taste was broader (that is, there were larger differences in responses to different tastes, i.e., more taste-specificity) for the neuron in t-PbN (Fig. 2A) than for those of the other brain stem neurons (Fig. 2, B and C). Statistical analysis of the total data set confirmed these appearances, showing that the average effect size for taste (η, essentially the raw SS taste value from the ANOVA) across the taste-specific subset of neurons (a data selection criterion that removed any confound that might emerge because of the factoring in of a higher percentage of nonsignificant responses in nontaste regions) was significantly higher for t-PbN neurons than for other neurons [t(33) = 2.56, P = 0.015].
that 1) t-PbN taste responses are more robust than those observed elsewhere, and in fact are for the most part the only pontine taste responses that are large enough in magnitude to be significant in small-bin analyses, and 2) these responses appear only after a 200- to 250-ms period of poststimulus time in which responses are nonspecific.

This latter point (which can also be observed in Fig. 2, in which the PSTHs essentially overlie one another for the first few hundred milliseconds) reveals a specific similarity between PbN taste response dynamics and those previously observed in forebrain structures such as basolateral amygdala (Fontanini et al. 2009), central amygdala (Sadacca et al. 2012), lateral hypothalamus (Li et al. 2013), and most noticeably primary gustatory cortex (Fontanini and Katz 2006; Katz et al. 2001; Maier and Katz 2013; Piette et al. 2012; Sadacca et al. 2012). Despite being relatively close to the peripheral taste cells, which react to taste input quickly (Chen et al. 2011b; Zhang et al. 2003), t-PbN neurons appear, like those in forebrain structures, to demonstrate an initial period of nonspecific response followed by the receipt of specific taste information only 200–250 ms after taste delivery.

Statistical confirmation of this relatively slow onset of taste-specificity is shown in Fig. 4, which presents the average effect sizes ($\eta^2$) from the set of neurons showing taste-specificity for data compiled into moving windows. It can be seen that the aforementioned significant taste $\times$ time interaction to some degree reflects the fact that t-PbN taste-specificity does not begin to exceed that of nontaste neurons until 200–250 ms after taste delivery, just as is consistently reported for forebrain neurons. Thus a basic feature of cortical (and amygdalar and hypothalamic) taste responses also appears in PbN responses.

Of course, the most distinctive feature characterizing cortical single-neuron responses to taste is not their relatively “slow” onset but rather the emergence of palatability-related firing between 0.5 and 1.0 s after stimulus delivery, that is, the relatively late rise in correlation between firing rates and taste palatability shown in experiments using basic tastes (Fontanini and Katz 2006; Katz et al. 2001; Piette et al. 2012), a range of NaCl concentrations (Sadacca et al. 2012), and binary taste mixtures (Maier and Katz 2013). To determine whether brain stem taste responses contain this feature, we performed a moving-window analysis of the correlation between taste-induced firing and taste palatability on the entire t-PbN sample and directly compared the results of this analysis to those of an identical analysis of simultaneously recorded GC neurons—simultaneous recording being an important control against the possibility of some minor differences in how past GC data were gathered.

Figure 5A shows the rasters and PSTHs for a pair of simultaneously recorded t-PbN and GC neurons, in both of which palatability-related firing appeared by $\sim$1.0 s after stimulus delivery; insets show the quality of single-neuron isolation. The PbN neuron (Fig. 5A, left) responded most to citric acid and quinine, the more aversive tastes, and the GC neuron (Fig. 5A, right) responded most to the more palatable tastes, sucrose and NaCl, but both patterns were found in both samples. Analysis of the full data sets, including even nonresponsive t-PbN and GC neurons (Fig. 5B), reveals that the correlations of PbN and GC taste responses with taste palatability emerge in similar manners (see also Katz et al. 2001; Piette et al. 2012; Sadacca et al. 2012), at least to the limits of our present analytic resolution. The brief “chop” in the GC correlation function (not observed in any of the above-mentioned studies) between 750 and 950 ms likely reflects random noise courtesy of the small size of the GC sample and did not obscure the obvious similarity in timing—a moving-window analysis of the correlation between firing rates and taste palatability failed to reveal differences between the time in which palatability-related responses emerged for t-PbN and simultaneously recorded GC neurons [2-way ANOVA, location $\times$ time: $F_{(1,11)} < 1$, $P = 0.83$].

Figure 5C presents the CUSUM charts for the data presented in Fig. 5B, again normalized to one another. As predicted, these charts are not flat—rather, the cumulative sum of the differences between the instantaneous correlations and the average
correlations decreases until ~500 ms after stimulus delivery and then increases, reflecting the fact that the correlations with palatability increased sharply at that time point for neurons in both regions.

In sum, the taste responses of PbN neurons, the locations of which correspond well with previous reports, strongly resemble cortical responses in their dynamics. Specifically, they progress through a sequence of three epochs—an initial nonspecific response is followed by taste-specificity that emerges around 200–250 ms and finally gives way to palatability relatedness > 500 ms after taste delivery. This suggests that PbN, while relatively close to the periphery, produces taste responses that are part and parcel of the larger CNS taste processing system.

**DISCUSSION**

The goal of these experiments was to determine whether PbN responses recorded from awake rats are dominated by temporal structure akin to that observed in GC or whether, alternatively, PbN taste responsiveness is best described as being a simpler matter of response magnitude and breadth. Our results support the former conclusion. We demonstrate that while fluid-responsive neurons are present to some degree outside of t-PbN (and non-PbN as well), taste-specificity (i.e., distinct neural responses to different tastes) was to a large extent concentrated in t-PbN and stronger in t-PbN neurons than in even the few other brain stem neurons in which it was significant. Furthermore, the taste-specific responses of t-PbN neurons were time varying approximately four times as often as those recorded from outside this region—complexity that resembled that observed in GC neurons: taste-specificity and palatability-related responses emerge at what are at least similar time points after stimulation in t-PbN and GC.

This conclusion makes sense in light of the fact that PbN is not only an early taste relay but also part of a highly distributed system. PhN neurons target several forebrain structures, including insular GC (Kosar et al. 1986a, 1986b; Saper 1982), central amygdala (Ottersen 1981), and hypothalamus (Norgren 1974). Each of these regions in turn feeds information back upon PbN—feedback that has been shown to have a significant impact on basic responses to ascending taste signals (Li et al. 2005; Lundy and Norgren 2004; Smith et al. 1998; Zhang et al. 2011). It is therefore reasonable to expect that perception-related taste dynamics observed in forebrain should be associated with similarly time-varying properties in PbN taste responses. And indeed, taste responses in PbN ensembles appear to progress, as they do in GC (Katz et al. 2001; Piette et al. 2012; Sadacca et al. 2012), through coherent firing rate “epochs”—nonspecific firing appears first, followed by chemosensory and then evaluative information.

It is possible that responses in the very first central taste relay, the NST in the medulla, might demonstrate “less” of a dynamic quality than those in the PbN, although NST is also a target of substantial amounts of forebrain feedback (Cho et al.
We were actually somewhat surprised at the similarity of the PbN and GC dynamics. We naively expected information regarding taste identity/quality to appear earlier in PbN than in GC, for the simple reason that PbN is earlier in the nominal taste processing hierarchy. It is possible that such a difference exists below the resolution of the present analyses (see MATERIALS AND METHODS), or even that the opposite pattern exists (as a function of feedback pathways), and that a larger brain stem neuron sample (which is prohibitively difficult to obtain from awake, freely moving rats) would allow us to make finer-grained distinctions. Despite the resolution limits of the present analyses, PbN responses appear to become reliably taste specific only after an initial 200- to 250-ms period of either no response or nondistinctive response. Analysis of water rinse responses suggests that these early t-PbN responses are, as they are in GC, somatosensory rather than taste related, although the fact that traces of the previous taste might remain on the tongue at the time of water rinse delivery (and the fact that 2/13 neurons showing early taste responses showed no water rinse response, a proportion that could, in larger samples, prove to be higher than chance) means that we cannot conclusively rule out the possibility that these represent very broad taste responses. Regardless, these responses become reorganized to reflect taste palatability in the second half of the first response second, again like those in GC.

Behavioral studies have shown that rats can make taste-specific decisions in ~200 ms after taste delivery (Halpern and Tapper 1971; Stapleton et al. 2006). The discrepancy between our electrophysiological data and these results could reflect differences in experimental setups. Our rats received tastants through passive intraoral infusion. Animals that are trained to receive a stimulus at a specific time (or lick) can anticipate a taste stimulus, thus shortening the latency of the taste-specific activity (Fontanini et al. 2009; Stapleton et al. 2006). Behavioral context can also change the latencies of taste-specific activities. Rats are capable of quickly identifying and rejecting a taste stimulus to which they have learned to have an aversive response (Halpern and Tapper 1971). While the latencies to taste-specific activity in PbN may differ depending on the behavioral context, we suggest that the dynamics between PbN and GC will remain the same.

These findings emerge in what is admittedly a small sample of neurons. In this regard, our study is similar to several previous investigations of brain stem taste responses, which often make use of similarly small samples (such data being very difficult to collect; see Lei et al. 2007; Weiss et al. 2014). While it is possible that future larger studies will call our conclusions into question, the very fact that, despite the sample size, these conclusions are supported by statistical significance is a testament to the results’ robustness.

Using a neurophysiological recording approach in awake rats and simultaneous recordings in both PbN and GC, we show that temporal codes in the PbN differ from the classic time-averaged codes for taste. These results offer a new glimpse into early stages of central gustatory coding, showing that even at the level of the brain stem taste processing is temporally dynamic, coding more than just taste quality.
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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