Bursting by taste-responsive cells in the rodent brain stem

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Bursting by taste-responsive cells in the rodent brain stem. J Neurophysiol 113: 2434–2446, 2015. First published January 21, 2015; doi:10.1152/jn.00862.2014.—Neurons that fire in bursts have been well-characterized in vision and other neural systems, but not in taste systems. We therefore examined whether brain stem gustatory neurons fire in bursts during spontaneous activity and, if so, whether such cells differ from nonbursting cells in other characteristics. We looked at neurons in the nucleus of the solitary tract (NST) of C57BL/6ByJ (B6) and 129P3/J (129) mice, and in the NST and parabrachial nucleus (PBN) of Sprague-Dawley rats. Many NST cells fired frequently with short intervals characteristic of bursting, and such neurons differed from others in their responsiveness to taste compounds. In B6 mice and rats, there was a significant positive correlation between the prevalence of short-interval firing and the net spikes evoked by application of NaCl. In contrast, in 129 mice the prevalence of short intervals was positively correlated with the size of sucrose responses. We also compared breadth-of-tuning measures based on counting either all spikes or only those following short intervals, and we found narrower tuning for the latter in the NST of B6 mice and rats. There was little evidence of spontaneous bursting in the rat PBN, and firing patterns in this nucleus were not related to the size of taste-evoked responses. We suggest that bursting may be a strategy employed by the NST to amplify the postsynaptic impact of particular taste stimuli, depending on an animal’s needs. Another function may be to sharpen breadth-of-tuning and thus enhance the contrast between stimuli of different taste qualities.

THERE ARE SEVERAL MEASURES to characterize neural firing, with the simplest being the number of action potentials during a fixed period. However, this approach ignores the temporal pattern of activity, which is often complex and can vary under different conditions (DiLorenzo and Victor 2003; Gire et al. 2013). One way in which action potentials can deviate from a random time course is by occurring in a bursting pattern of two or more spikes that follow each other in quick succession.

Brain areas that receive visual input have been well-characterized in terms of whether their cells burst and on the consequences of such firing patterns, which include enhancing synchronization between different cells (Lesica and Stanley 2004; Martinez-Conde et al. 2002; Usrey et al. 1998). Bursts have also been shown to be especially efficient at driving postsynaptic firing compared with the same number of presynaptic spikes fired at longer intervals (Lisman 1997). This is true even for bursts in which the number of spikes fired in a row is small (e.g., double-firing; Usrey et al. 1998). Thus the presence of bursting by a neuron has important consequences for how it will impact its postsynaptic targets. Accordingly, bursts in the lateral geniculate of cats have been proposed to amplify certain visual features (Lesica and Stanley 2004). Bursting may also serve to enhance perceptual contrast: the receptive field maps of the primary visual cortex of macaques and the lateral geniculate of cats are sharpened if only spikes that follow the previous one with a short interval are counted, relative to maps created using all spikes (Reich et al. 2000; Rivadulla et al. 2003).

There have been few attempts to characterize bursting by gustatory neurons. Boudreau et al. (1985) described geniculate ganglion neurons’ firing patterns, which were classified based on distributions of interspike intervals (ISIs) during spontaneous firing (i.e., in the absence of taste stimulation). They found some cells with a peak in their distribution at short (<10 ms) intervals, which is consistent with bursting. Furthermore, the tendency to burst during spontaneous activity was related to the response profile of the neuron, albeit in ways that varied across cats, dogs, goats, and rats. Nuding et al. (1991) observed bursting by a subset of gustatory neurons in the nucleus of the solitary tract (NST) of hamsters, but they found that these cells were not different from nonbursting cells in their response profiles to taste compounds, although they did have higher spontaneous firing rates. Fujiwara et al. (2007) described bursting neurons in the gustatory cortex of rats but did not look at the relationships between bursting during spontaneous activity and taste-evoked response profiles. In addition, several investigators have examined bursting in terms of its contribution to taste-evoked activity and found that bursts occurred primarily during responses to compounds with a particular taste quality (Geran and Travers 2013; Mistretta 1972; Ogawa et al. 1974; Travers and Norgren 1989).

There are still many unresolved issues related to bursting for the taste system, including the initial question of whether or not particular neurons fire in bursts. For example, there have been no attempts to address this issue in mice. Moreover, there is no reason to assume that results will be uniform across nuclei or in different species, given the substantial regional variation in the visual system (Lisman 1997) and the species differences observed by Boudreau et al. (1985) for gustatory neurons in the geniculate ganglion.

One incentive to identify bursting and nonbursting neurons is that it provides an additional way to classify neurons with potential benefits over other currently used methods. For example, brain stem gustatory neurons are often categorized based on their response profiles, which depend on the stimuli and concentrations used, and even the rate of stimulus application can influence the size of taste-evoked responses (DiLorenzo and Victor 2007; Smith and Bealer 1975). Moreover, the magnitude of taste responses can be affected by the passage of time or changes in physiological state, with some
neurons switching their response-profile category as a result (McCaughey and Scott 2000; Roussin et al. 2008). Classifying neurons based on their tendency to burst is potentially advantageous in that it can be done in the absence of taste stimulation, which helps to ensure that similarly named cell types identified in different experiments are truly equivalent to each other.

We sought to address some fundamental issues related to bursting in brain stem gustatory nuclei. First, we were interested in how many neurons fire in bursts; we focused on firing during spontaneous activity to address a cell’s general characteristics, rather than ones associated only with responses to a particular taste compound. Second, we examined whether bursting cells differ from nonbursting ones in other features, such as responses to taste stimuli. Third, we characterized how firing with short intervals is related to other variables, such as firing with longer intervals. Fourth, we tested whether bursting during spontaneous activity tends to be maintained when taste stimuli are applied; we also calculated response sizes when only spikes following a short interval are included, as has been done in some studies of visual processing (Reich et al. 2000; Rivadulla et al. 2003), to examine the impact on breadth-of-tuning. Our primary interest here was in characterizing neurons in terms of their bursting behavior, rather than in identifying bursts during evoked responding and relating them to the coding of gustatory quality, which we consider a subject for future research. Our data were based on cells recorded from the NST of two mouse strains (C57BL/6ByJ, or B6; and 129P3/J, or 129), and both the NST and PBN of rats.

METHODS

Animals

Four groups were used: 1) mice from the C57BL/6ByJ (B6) strain, in which the activity of 38 NST neurons was measured from 20 animals; 2) 129P3/J (129) mice, in which the activity of 40 NST neurons was measured from 18 animals; 3) Sprague-Dawley (SD) rats, in which the activity of 39 NST neurons was measured from 11 animals; and 4) SD rats, in which the activity of 39 PBN neurons was measured from 19 animals. The mice weighed 20–36 g and were 8–30 wk old on the days of neural recording. The rats for the NST and PBN experiments weighed 325–580 g and 275–430 g, respectively, on the recording days. For the first three groups, we drew on data from published work (McCaughey et al. 2007; McCaughey et al. 2007), in which we had characterized taste-evoked responses without examining measures related to bursting; the use of existing data for the current experiment served to reduce the number of animals needed for the research. We included only neurons for which we obtained at least 100 ISIs, given recommendations that 99–100 is the minimum number of ISIs for defining firing patterns of gustatory cells (Boudreau et al. 1982; Nuding et al. 1991).

In all cases, recordings were made from adult male animals who were maintained on nutritionally complete rodent chow and water ad libitum. Their environments were temperature-controlled and they were kept on a 12:12-h light-dark cycle. None of the animals had prior experience with taste solutions prior to electrophysiological recording. The protocols of all studies were approved by the Institutional Animal Care and Use Committees of the Monell Chemical Senses Center or Amherst College.

Electrophysiology

All animals were anesthetized, given a trachea tube to prevent suffocation and an esophageal fistula to prevent ingestion of stimuli, and fixed in a headholder. For the surgeries conducted on mice, the anesthesia used was a mixture of ketamine, xylazine, and acepromazine (90, 20, and 3 mg/kg ip, respectively, with additional doses as necessary). For the surgeries conducted on rats, urethane was used as anesthesia (1.5 g/kg ip, with further doses as necessary) Animals remained deeply anesthetized throughout surgeries to minimize breathing artifacts and other possible contributors to instability. For the NST surgeries, a portion of skull was removed and the cerebellum aspirated to expose the surface of the brain stem over the nucleus, and the activity of individual neurons was isolated using glass micropipettes. For PBN recordings, the electrode was inclined 20° posterior to prevent transverse sinus rupture and advanced automatically using a microdrive (Burleigh InChworm 8200). From the brain stem surface, neural responses were evaluated in 25-μm increments. A mixture of the four basic tastants, followed by dH2O rinsing, was used as a search stimulus. Jaw stretch was tested by depressing the lower incisors. Oral tactile responses were evaluated using a glass or wooden probe to stroke the anterior tongue, foliate region, and occasionally the soft palate. If a jaw stretch response was observed (indicating mesencephalic trigeminal nucleus) the electrode was withdrawn and a new track begun. Tracks were made at 200- to 300-μm points along a grid over the exposed brain surface, until a stable cell was isolated for recording. Additional details for NST recording can be found in the original publications (McCaughey 2007; McCaughey et al. 2007), and the procedures for single-unit PBN recordings in rats were similar to those that have been reported previously (Baird et al. 2001).

Taste-Evoked Responses

For all animals stimuli were delivered as a spray that contacted the entire oral cavity, and stimulus applications were followed by a thorough water rinse and a delay of at least 1 min before the next taste solution was applied; additional details of stimulus application have been published previously (Baird et al. 2001; McCaughey 2007; McCaughey et al. 2007). A broad array of stimuli was used in all groups of animals, and solutions were presented in a semirandom order in which compounds with similar taste qualities were not applied consecutively; because of this, it is unlikely that relationships between spontaneous firing patterns and the size of taste-evoked responses actually represented a lingering effect of the prior stimulus. We used the baseline periods prior to application of all taste stimuli within an array to obtain ISIs related to spontaneous firing patterns (see next section), but response sizes to only four stimuli representative of the basic taste qualities were of interest here. For the mouse NST groups, these stimuli consisted of (in mM) 10 HCl, 100 NaCl, 20 quinine HCl, and 500 sucrose. For the rat NST group, they were (in mM) 10 HCl, 100 NaCl, 10 quinine HCl, and 500 sucrose. For the rat PBN, they were (in mM) 10 citric acid, 100 NaCl, 10 quinine HCl, and 500 sucrose. The unexamined stimuli were either similar in taste quality to the ones just mentioned, or else they had been chosen to represent nontraditional taste qualities that are not widely considered in taste experiments (e.g., trisodium pyrophosphate), and thus we decided there was little benefit to including them in analyses here.

When possible, stimuli were presented multiple times for a given cell, and response sizes were based on averaging across all applications. Net response sizes were based on a baseline period of either 3 s (for NST cells) or 30 s (for PBN cells) and a 5-s evoked period, and they were expressed in terms of mean net spikes/s, except where indicated otherwise. Cluster analysis was applied to these response sizes to identify neural subtypes with different profiles of responding across the four prototypical stimuli, as described previously (McCaughey 2007).
Data Analysis

Analyses were performed using the Systat software package. The criterion for significance was considered to be 0.05, except where noted otherwise.

Creating ISI distributions. We obtained ISIs from neural activity that had been recorded previously during multiple “baseline” or “spontaneous” periods interposed between taste solutions that were applied over a period of 15–60 min. Intervals from all of these periods were combined for a particular neuron, given that they all occurred after any prior taste stimuli had been fully rinsed off, and that there were no experimental manipulations or other factors that provided a clear rationale for considering them separately. In all cases we considered a total of at least 27 s of spontaneous activity for a neuron.

For the NST recordings, baseline periods typically consisted of the 3 s prior to application of each stimulus. In some cells from B6 and 129 mice, these periods yielded less than 100 interspike intervals total, and so we extended the baseline periods to raise the total number of ISIs to greater than 100 (the exact length of the new baseline periods varied across individual stimulus applications, depending on how much neural activity had been recorded originally, but in all cases these periods included only spontaneous firing that occurred after the prior stimulus had been completely rinsed off). For the rat NST recordings, the regular 3-s baseline period was used in all cases, because this captured more than 100 intervals in all cells. For the rat PBN recordings, baseline periods consisted of the 30 s prior to application of each taste stimulus, and all cells yielded more than 100 intervals total.

Frequency distributions of ISIs were constructed using 0.5-ms bins up to 3 s, although longer intervals were also included in analyses where appropriate (e.g., when a cell’s total number of ISIs was calculated). Given the large range of durations for intervals, we plotted them on a log scale that was modified to include similar widths within an order, so as to avoid distortion. That is, we grouped intervals that were 0–10 ms long into 10 bins of 1 ms each, we grouped intervals that were 10–100 ms long into 9 bins of 10 ms each, we grouped intervals that were 100–1,000 ms long into 9 bins of 100 ms each, and we grouped intervals that were 1,000–3,000 ms long into 2 bins of 1,000 ms each. Histograms were made with the y-axis as “percent of total intervals” so that distributions could be compared and averaged across cells.

Categorizing cells and comparing neural classes. There are numerous methods that have been used to identify bursting neurons or individual burst events, with none of them used universally. We considered several of these approaches, but found them all to be inappropriate for our data. For example, we were interested in characterizing neurons, rather than individual bursts or response patterns during different conditions, which have often been the targets of prior work. Furthermore, the other methods have typically been used to identify neurons with large burst sizes (e.g., 10–50 spikes in a row; in Legéndy and Salcman 1985). However, it was clear from inspection of our data that most of our short intervals were followed by a longer one (i.e., the intervals represented instances of double-firing). We therefore developed our own method for identifying bursting cells in which we focused on the shape of the ISI distribution, which shows a distinct peak at short intervals for neurons that often fire in doublets, but we also took into account the fact that some short intervals might occur simply due to a high mean firing rate.

The small size of our neurons’ bursts also presented a dilemma for our choice of terminology. The term “bursting” is often associated with distinctive events in which a cell fires multiple spikes in a row, all of which follow the previous one with a short interval, and so we considered substituting the term “double-firing” for our data. However, the latter choice would not have been completely accurate, given that all of our double-firing neurons also sometimes triple-fired or showed even larger burst sizes. Thus we have favored the accurate terminology “bursting,” but mention the dominance of double-firing multiple times.

Our method for identifying those neurons that showed the greatest degree of bursting was as follows: for each neuron we calculated a “Burstiness” score, which was as defined as follows: Burstiness = (% of the total intervals that were less than 5 ms) — (% of the total intervals that would be expected to be less than 5 ms for a random firing pattern).

The latter value was based on an exponential distribution calculated with the rate of change (lambda) equal to the inverse of a cell’s mean firing rate (expressed in spikes/ms) and a location equal to 1.5 ms (to take into account a refractory period of that duration). Next, we plotted a frequency histogram showing the distribution of Burstiness scores across all cells within a group of animals, and we looked for a break point to set a threshold used to categorize cells for that group. Neurons with Burstiness scores that exceeded the threshold were classified as “bursting” (or B) cells, whereas the remaining neurons were labeled “nonbursting” (or non-B) cells. Within B cells, each instance of an ISI less than 5 ms was considered to be part of a burst, which was assigned a size based on the number of spikes in a row with intervals of less than 5 ms separating them (e.g., double-firing was assigned a burst size of 2), as has been done in some prior considerations of bursting cells (Oswald et al. 2007).

We then conducted additional analyses to define the characteristics of B cells and compare them with those of non-B cells within each of the three of the groups of animals in which NST activity was measured; these analyses were not performed for the rat PBN because few B cells were identified. The cell types were compared on response sizes across the four stimuli using an ANOVA with cell type and taste compound as factors, followed by post hoc t-tests if there were significant main effects or interactions, to pinpoint compounds on which they differed. We used t-tests to compare neuronal subtypes on spontaneous firing rates and breadth-of-tuning measures; the latter included: 1) an entropy measure (H) developed for gustatory neurons by Smith and Travers (1979) and defined as follows:

\[
H = -k \sum p_i \log p_i
\]

where \( k = \) a scaling constant, which is 1.661 for four stimuli, and \( p_i = \) the proportion of the neuron’s response devoted to any one stimulus; and 2) a noise-to-signal ratio, which equaled the second largest response size to a basic stimulus divided by the size of the largest response (Geran and Travers 2013). Both measures have a maximum of 1, which would occur for an extremely nonselective neuron, and lower values represent greater specificity in responding. In the rare instances in which a net response size was negative, we used the absolute value for the entropy measure.

Characteristics of short-interval firing. The analyses described above suggested that firing with intervals of less than 5 ms was a special occurrence that merited further scrutiny. Thus, for each neuron we calculated the percentage of the total intervals that were less than 5 ms, and we looked at the relationship between this measure of short-interval firing and other variables, such as response sizes to each taste stimulus; this allowed us to look at the relationship between firing patterns and taste responsiveness along a continuum and ignoring membership of neurons in the B and non-B classes. We did not correct this percentage for mean firing rate (i.e., we did not subtract the percentage of short intervals expected for a random firing pattern) for several reasons. First, we wished to favor simplicity. Second, there are precedents for focusing on all spikes that followed intervals of less than a certain duration (5 ms, in our case; Reich et al. 2000 used 3 ms; Harris et al. 2001 used 6 ms). Third, this approach was advantageous because the brevity of an interspike interval, and not its identity as part of a burst, is the variable most directly related to temporal summation of postsynaptic neurons. That is, any instance in which two spikes follow each other quickly should result in a high degree of temporal summation in the postsynaptic neuron and help to trigger an action...
Potential, regardless of whether the short interval is due to bursting or to a high mean firing rate.

Pearson product-moment correlations were calculated to determine the association between the percentage of the total intervals that were less than 5 ms and the response sizes to each of the basic stimuli. The specificity of any such relationship was evaluated by performing similar correlations, but using the percentage of the total intervals that were 5–10 ms or 5–15 ms. We set a threshold for significance of $P < 0.0125$ (i.e., 0.05/4) for these tests, to control for the multiple comparisons across the four basic stimuli. We also calculated correlations between the percentage of the total intervals found in different time bins, to test whether the bins were independent of each other and to justify considering the 0- to 5-ms interval separately. These correlations assessed the relationship between the percentage of intervals that were 0–5 ms vs. 5–10 ms, or between the percentage that were 5–10 ms vs. 10–15 ms.

We also recalculated correlations between short-interval firing and taste-evoked response sizes using the Burstiness scores that were used to define B cells, rather than simply the observed percentage of intervals less than 5 ms. This was done to provide a closer match with how B and non-B cells were defined and to examine short-interval firing while also taking into account the mean firing rate of a cell. This reanalysis yielded nearly identical correlations to those we had obtained using the observed percentage of intervals less than 5 ms (as described above), and so we do not present the results for the former.

Fig. 1. Representative histograms of interspike interval distributions during spontaneous activity for individual neurons in the mouse nucleus of the solitary tract (NST). Labels on the right refer to strain-cell number. The left graphs plot intervals from 0–25 ms on a linear scale, and the right graphs plot intervals from 0–3 s on a log scale that was modified to keep each bin the same width within an order, so as to avoid distortion. Frequency is represented in terms of the percentage of intervals out of the total number counted for the cell. The right panels include the cell’s mean spontaneous firing rate (“spon”), and the left panels include the percentage of intervals less than 5 ms that were actually observed [% < 5 (Obs)] and the percentage of intervals less than 5 ms that would be expected for an exponential distribution [% < 5 (Exp)]. A and D: neurons that showed clear evidence of bursting, based on a distinct peak in the distribution at less than 5 ms. B and E: neurons that showed small peaks at less than 5 ms. C and F: neurons that did not fire in bursts.
Intervals counted during taste-evoked firing. Given the presence of bursting during spontaneous activity in many NST neurons, we were interested in whether their firing patterns were maintained during evoked activity, and so we determined ISIs during the 5-s evoked periods; we did not perform these calculations for the PBN group, due to the low prevalence of spontaneous bursting by the cells. For each NST neuron, we created an “evoked” ISI distribution that involved collapsing data across the four taste stimuli; for each B cell we also determined the distributions of evoked ISIs during responses to each stimulus.

Evoked ISIs were also used to recalculate response sizes using only spikes that followed an ISI of less than 5 ms (“short-interval spikes”), rather than all action potentials. These new net responses sizes were expressed in short intervals per second and were based on a 5-s evoked period and the baseline period that had been used to obtain spontaneous ISIs. Response sizes expressed in short intervals per second were then used to recalculate entropy and noise-to-signal ratios using the same formulas as for responses expressed in spikes per second. Some cells had an undefined entropy measure because they included at least one response size that equaled exactly zero when expressed in short intervals per second, and so they were dropped from the analyses.

RESULTS

Mouse NST

Comparing bursting and nonbursting cell types. The shape of ISI distributions for mouse NST cells varied widely, with the most obvious distinction being whether there was a peak at less than 5 ms (Fig. 1). Some neurons fired with such short intervals very frequently, whereas other cells rarely displayed them, and there were also neurons that fell between these two extremes. We plotted the distributions of Burstiness scores, which represented the extent to which the percentage of intervals less than 5 ms exceeded what would be expected for a random firing pattern (Fig. 2), and then selected those neurons in each strain with the highest values (e.g., those shown in Fig. 1, A and D) to be B (or “bursting”) cells. Neurons that lacked peaks in their ISI distributions (e.g., Fig. 1, C and F) or that had small peaks (e.g., Fig. 1, B and E) were classified as non-B cells. The B6 group contained 10 B and 28 non-B cells, and the 129 group contained 16 and 24, respectively (Fig. 2).

B cells contained a significantly larger percentage of intervals less than 5 ms than did non-B cells in both strains \([t(36–38) = 8.9, P < 0.001 in both cases]\), as is to be expected based on how the cell types were defined. However, B and non-B cells did not differ on their percentage of intervals that were 5–10 ms (Fig. 2). In most cases, the intervals less than 5 ms represented instances of double-firing; the most common burst size (i.e., the mode) for all B cells in B6 and 129 mice was 2. However, all B cells occasionally fired with bursts of size 3 (i.e., triple-firing) or more, and one cell from each strain had a maximum burst size of 8. Spontaneous firing rates and our two breadth-of-tuning metrics (entropy and noise-to-signal ratios) of interspike intervals during spontaneous activity in B (blue) and non-B (red) NST cells in B6 (top) and 129 (bottom) mice. Intervals between 0 and 3 s are plotted on a modified log scale similar to that for Fig. 1.
Table 1. Comparison of mean (± SE) spontaneous firing rates and breadth-of-tuning metrics (entropy and noise-to-signal ratio) between B and non-B cells in B6 and 129 mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Spontaneous Rate, spikes/s</th>
<th>Entropy</th>
<th>Noise-to-Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B cells</td>
<td>Non-B cells</td>
<td>B cells</td>
</tr>
<tr>
<td>B6</td>
<td>6.2 ± 0.9</td>
<td>4.6 ± 0.5</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>129</td>
<td>8.6 ± 1.0</td>
<td>8.0 ± 1.0</td>
<td>0.76 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. There were no significant differences between bursting (B) and nonbursting (non-B) cells within each strain.

Table 2. The number of B and non-B cells in subtypes of cells defined based on response profile in B6 and 129 mice

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>N-cells</th>
<th>H-cells</th>
<th>S-cells</th>
<th>N-cells</th>
<th>H-cells</th>
<th>S-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Non-B cells</td>
<td>5</td>
<td>3</td>
<td>20</td>
<td>15</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

S-cells, N-cells, and H-cells are sugar-, salt-, and acid-oriented cells, respectively.

ratios) did not differ between B and non-B cells within each strain (Table 1).

In contrast, there were differences between B and non-B cells in taste-evoked responses to particular stimuli. Cells were classified based on their profile of responding across stimuli representative of the basic tastes, and the analysis yielded three cell types: sugar-, salt-, and acid-oriented (S-, N-, and H-cells, respectively; Table 2). S-cells were more common in B6 than 129 mice, as described earlier (McCaughey 2007), which likely relates to strain differences in the allele for the taste receptor protein T1R3. In B6 mice, B cells were more common in N- and H-cells than they were in S-cells. In contrast, in 129 mice, B cells were most prevalent in S- and N-cells.

We compared B and non-B cells on their taste-evoked responses in B6 mice, and there was a significant cell type × compound interaction [F(3,108) = 11.1, P < 0.001]. Post hoc tests indicated that the B cells evoked significantly larger responses to NaCl and HCl than did non-B cells (P = 0.002 in both cases; Fig. 3); in contrast, responses evoked by sucrose were significantly smaller in B than non-B cells of B6 mice (P = 0.01).

Evoked responses also differed between B and non-B cells in 129 mice [main effect of cell type, F(1,38) = 7.4, P = 0.01], with significantly larger responses to sucrose in the former (P = 0.0015; Fig. 3).

Characteristics of short-interval firing. We looked at the correlations between the percentage of intervals less than 5 ms for a cell and the size of its taste-evoked responses to each of the basic stimuli (Table 3), to examine these relationships along a continuum. In B6 mice, there were significant positive correlations between the percentage of intervals less than 5 ms and the percentage of spikes between 5 and 15 ms (data not shown). These results reinforced our decision to set a cut-off at 5 ms to consider intervals that were shorter to be distinct from those that were longer. To further examine this issue, we also calculated correlations between the percentage of intervals less than 5 ms and the percentage of intervals that were 5–10 ms. In B6 mice, the correlation was +0.17, and in 129 mice it was −0.20, indicating the absence of a significant relationship for both strains (P > 0.05 in both cases). In contrast, the percentage of 5- to 10-ms intervals was significantly correlated with the percentage of 10- to 15-ms intervals in both strains (+0.50 and +0.60 in B6 and 129 mice, respectively; P < 0.002 in both cases).

ISIs counted during evoked firing. For both mouse strains the firing patterns were generally similar for the spontaneous and evoked conditions (Fig. 4). Within B cells, for example, the percentage of intervals that were 0–5 ms did not differ significantly between the spontaneous and evoked conditions, and at a 5- to 15-ms bin, which we used to match the 0- to 5-ms bin in mean percentage of total intervals. However, there were no significant correlations between taste-evoked response sizes and the percentage of spikes between 5 and 15 ms (data not shown). These results reinforced our decision to set a cut-off at 5 ms and to consider intervals that were shorter to be distinct from those that were longer. To further examine this issue, we also calculated correlations between the percentage of intervals less than 5 ms and the percentage of intervals that were 5–10 ms. In B6 mice, the correlation was +0.17, and in 129 mice it was −0.20, indicating the absence of a significant relationship for both strains (P > 0.05 in both cases). In contrast, the percentage of 5- to 10-ms intervals was significantly correlated with the percentage of 10- to 15-ms intervals in both strains (+0.50 and +0.60 in B6 and 129 mice, respectively; P < 0.002 in both cases).
Table 3. Correlations between response sizes to the basic stimuli (in net spikes/s) and the percentage of total ISIs that fell within a certain range (either 0–5 ms or 5–10 ms) in B6 and 129 mice

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>0–5 ms</th>
<th>5–10 ms</th>
<th>0–5 ms</th>
<th>5–10 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>0.47*</td>
<td>0.27</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.50*</td>
<td>0.24</td>
<td>0.24</td>
<td>0.15</td>
</tr>
<tr>
<td>Q</td>
<td>0.24</td>
<td>0.20</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Suc</td>
<td>−0.31</td>
<td>0.06</td>
<td>0.62*</td>
<td>−0.13</td>
</tr>
</tbody>
</table>

Interspike intervals (ISIs) were counted during spontaneous activity, so significant correlations reflect relationships between patterns of firing in the absence of taste stimulation and the size of taste-evoked responses. *P < 0.003.

ISI distributions were similar during evoked responding to the different stimuli. In addition, the distributions of non-B cells did not develop sharp peaks at less than 5 ms as a result of taste stimulation.

We also recalculated net response sizes by counting only spikes that followed an interval of less than 5 ms. Unsurprisingly, these new response sizes were generally smaller than the previously calculated response sizes, for which all spikes were included. However, the new across-neuron profiles were highly correlated with the previous ones for the same taste stimulus within each strain; (the 8 Pearson product-moment correlations between spike counting methods ranged from +0.72 to +0.93). Table 4 compares mean breadth-of-tuning measures calculated with the two methods in each strain. In B6 mice, both measures (entropy and noise-to-signal ratio) were significantly smaller when counting only short-interval spikes, compared with including all spikes [(t30–36) = 3.9, P < 0.001 in both cases]. In 129 mice, however, there was no difference on either measure when comparing spike counting methods.

Table 4. Comparison between two spike-counting methods in values of breadth-of-tuning metrics in B6 and 129 mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>All spikes</th>
<th>Short-interval spikes</th>
<th>All spikes</th>
<th>Short-interval spikes</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>0.82 ± 0.02</td>
<td>0.69 ± 0.04*</td>
<td>0.54 ± 0.04</td>
<td>0.42 ± 0.05*</td>
</tr>
<tr>
<td>129</td>
<td>0.74 ± 0.03</td>
<td>0.76 ± 0.03</td>
<td>0.49 ± 0.04</td>
<td>0.51 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. One method involved counting all spikes, whereas for the other only spikes that followed an inter-spike interval of <5 ms were counted. *P < 0.001, all spikes vs. short-interval spikes.

Rat NST

Comparing bursting and nonbursting cell types. As in the mouse NST, the cells varied widely in the extent to which there was a distinct peak at less than 5 ms (Fig. 5) and in their Burstiness scores (Fig. 6A). Of the 39 cells that were examined, 15 were designated B cells. The most common interval within B cells was 1–2 ms (Fig. 6B), which was shorter than the most common intervals found in B6 and 129 mice (2–3 and 3–4 ms, respectively); however, the mean percentage of intervals that were less than 5 ms in B cells of rats (28%) was similar to those observed for B6 and 129 mice (29 and 30%, respectively). For all B cells the most common burst size was 2, although they all sometimes fired with bursts of size 3 of larger, and one cell had a maximum burst size of 10.

When subtypes of cells were defined based on response profile, there were 17 N-cells, 11 H-cells, and 11 S-cells. The percentage of neurons that were B cells differed across them (41%, 63%, and 9%, respectively) and was lowest for S-cells. There were also significant differences between B and non-B cells in the size of their taste-evoked responses [Fig. 6C; cell type × compound interaction, F(3,111) = 5.9, P = 0.001]. Post hoc tests indicated this was due to larger responses to NaCl in B compared with non-B cells (P = 0.001). In addition,
spontaneous firing rates were significantly higher in B than non-B cells [24 ± 5 and 9 ± 1 spikes/s, respectively; t(37) = 3.6, P = 0.001]. Mean (+SE) entropy values, however, did not differ between B and non-B cells (0.74 ± 0.04 and 0.79 ± 0.02, respectively), nor did noise-to-signal ratios (0.39 ± 0.09 and 0.45 ± 0.05).

**Characteristics of short-interval firing.** We calculated correlations between the percentage of ISIs less than 5 ms and the size of evoked responses. There was a significant relationship for NaCl (r = +0.52, P < 0.001), but not for the other three stimuli (P > 0.05 in all cases). There were significant correlations between the response size to HCl and the percentage of 5- to 10-ms intervals (r = +0.45, P = 0.004) or 5- to 15-ms intervals (r = +0.49, P = 0.002), but the other stimuli were not significantly correlated with these time bins. The percentage of intervals less than 5 ms was not significantly correlated with the percentage that were 5–10 ms (r = +0.23, P > 0.05), but the latter was significantly correlated with the percentage that were 10–15 ms (r = +0.73, P < 0.001).

**ISIs counted during evoked firing.** As in mice, firing patterns for individual cells were similar during evoked and spontaneous firing (data not shown). We also recalculated net response sizes using only spikes that followed an interval of less than 5 ms. This resulted in smaller values than when all spikes had been counted, but the across-neuron profiles remained similar; the correlations ranged from +0.82 to +0.92 when each of the four stimuli were compared on their response sizes between the two methods. Mean (+SE) entropy values were significantly smaller when counting only short-interval spikes (0.73 ± 0.03) compared with counting all spikes [0.78 ± 0.02; t(35) = 3.2, P = 0.003]. The same was also true for noise-to-signal ratios [0.41 ± 0.05 vs. 0.49 ± 0.04; t(38) = 3.0, P = 0.004].

**Rat PBN**

Rat PBN neurons tended to have a smaller percentage of short intervals during spontaneous activity than did cells from the NST of rats and mice. For example, the percentage of ISIs that were less than 20 ms averaged only 8% across all PBN cells, compared with 25–34% in our three NST data sets. This was not due simply to lower firing rates in the PBN, as they matched B6 mice in mean spontaneous rate (5 spikes/s in both cases). There were also fewer examples of neurons with peaks in their ISI distributions at short intervals (Fig. 7), with only two instances in which ISI distributions had a peak at less than 5 ms that clearly exceeded the percentage expected for an exponential distribution (Fig. 8A). The most common burst size in both of these B cells was 2, although they sometimes fired in bursts of 3 or 4 spikes in a row.

The two B cells were characterized not only by a large percentage of very short intervals, but also by many long ones as well (Fig. 8B). One of the B cells had the highest percentage of intervals greater than 5 s of all PBN cells, and the other B cell’s percentage was higher than those of all but two of the non-B cells. We noticed no other distinguishing features of the two B cells, however, and we did not perform statistical tests to compare the response properties of B and non-B cells, due to the scarcity of the former. We also considered the relationship between firing with short (<5 ms) intervals and response sizes along a continuum. However, none of the correlations between the percentage of intervals less than 5 ms and evoked response sizes were significant. There was a significant correlation, however, between a cell’s percentage of intervals less than 5 ms and that were 5–10 ms long (r = +0.39, P = 0.04). Compared with NST neurons, those in the PBN were different in several respects: firing with intervals of less than 5 ms was uncommon; the prevalence of such intervals was not independent of the prevalence of slightly longer ones; and the percentage of intervals less than 5 ms was not related to any response sizes. We were concerned, however, that our definition of a “short” interval for the NST (i.e., 5 ms) may not be appropriate for the PBN, and so we also determined correlations using 10 ms or 20 ms as our cut-off. However, the percentage of intervals that were 0–10 ms was not correlated significantly with the size of any of the taste-evoked responses, nor was the percentage of 0- to 20-ms intervals; both of these
time bins, however, were correlated significantly with the next bin of the same length (i.e., 0 –10 ms with 10 –20 ms, and 0 –20 ms with 20 – 40 ms).

DISCUSSION

Spontaneous Bursting is Common in the NST but Not the PBN

Many mouse and rat NST neurons fired in bursts during spontaneous activity, with 26–40% of neurons classified as B (bursting) cells in the three NST data sets, compared with only 5% for the PBN data set. All B cells sometimes fired in bursts of three or more spikes, but most of the short intervals that we counted represented doublets (i.e., double-firing). The small size of the bursts, compared with those found in other regions (e.g., gustatory cortex, where burst sizes of five or more are common; Fujiwara et al. 2007) helps to explain why most investigators have ignored this feature of NST cells previously.

We chose an ISI limit of 5 ms for bursting by neurons, and we confirmed that these very short intervals are independent of slightly longer ones in the NST: the percentage of ISIs less than 5 ms was not significantly correlated with the percentage of 5- to 10-ms intervals in all three data sets. Furthermore, a preponderance of short intervals was not due solely to a high mean firing rate or to the presence of a certain kind of taste stimulus (or, indeed, to application of any taste stimulus). Overall, our results suggest that short intervals occur in many NST neurons based on some property intrinsic to the cell itself, and not based on input from other neurons. Presumably, some cellular mechanism causes the firing of an action potential to trigger another one less than 5 ms later in that same neuron. One possibility is back-propagation of spikes, which has been proposed for bursting neurons in other regions (Wang 1999).

We felt it was important to include data from multiple nuclei and kinds of animals, given the regional variation in bursting-related variables for the visual system (Lisman 1997) and the species differences reported for gustatory cells in the geniculate ganglion (Boudreau et al. 1985). We observed differences between B6 mice, 129 mice, and SD rats in terms of which stimuli evoked larger responses in B vs. non-B cells in the NST. However, all three NST data sets were similar in terms of the distribution of Burstiness scores, the percentage of neurons that were identified as B cells, and the mean percentage of intervals less than 5 ms within B cells. These variables, however, differed dramatically between the NST and PBN. Overall, then, our data suggest that there are at least two factors that regulate bursting in brain stem taste nuclei: one that determines the overall degree of bursting in the nucleus, which is expressed differentially between the NST and PBN; and a second mechanism that determines which cells show the greatest degree of bursting, which varies across strain and species.

We considered the possibility that our anesthetic may have affected bursting, given reports of this occurrence in nongustatory neurons (Katayama et al. 1980). However, our results do not support such a conclusion, because we used the same kind of anesthetic for the rat NST and PBN experiments and obtained different results; at the same time, different anesthetics were used for the NST experiments in B6 mice and rats, and we observed similar results. Admittedly, we did not examine enough data sets to completely rule out roles for anesthetic or other unknown factors in affecting bursting, and more work will be needed to explore these possibilities. However, the overall picture provided by our data is that the most important variable related to bursting is region, not anesthetic, type.

Gustatory Neurons Can Be Classified Based on Their Tendency to Burst

Investigators have sometimes assigned neurons into bursting and nonbursting (or “bursty” and “non-bursty”) categories (Lenz et al. 1998; Bastian and Nguyenkim 2001). We did the same, based on the extent to which a neuron fired with intervals less than 5 ms, relative to what would be expected for a random firing pattern. For the rat PBN, two cells were clear outliers on this Burstiness measure, making the decision to group them separately obvious. For the three NST data sets, the distributions of Burstiness scores were skewed, but all had a break...
point at around 12–13, which allowed us to separate those cells that showed the greatest degree of bursting, and which provides an approximate guide for defining B cells in future work.

We are aware that choosing a certain Burstiness score as a threshold could be seen as arbitrary. Furthermore, the lack of bimodal distributions raises the issue of whether the NST neurons that we classified as B-cells are truly a unique "type," or whether all NST neurons burst to varying degrees. However, such complications often occur with categorization; indeed, the common practice of classifying gustatory neurons based on their response profiles has been criticized on similar grounds (Woolston and Erickson 1979; Di Lorenzo and Lemon 2001).

We dealt with this issue by examining short-interval firing along a continuum, as well as comparing cell types. These two approaches yielded similar results in most cases (i.e., if there were larger responses to a stimulus in B cells relative to non-B cells, then there was a significant positive correlation between response magnitudes to that stimulus and the percentage of short intervals).

Thus, bursting in the NST can be viewed either as a feature of only certain neurons, or as a property displayed by all neurons to varying extents, but in both cases there is a relationship between firing patterns and taste-evoked response sizes. Additional work will be needed to resolve the best method for defining B cells and to determine whether bursting is modifiable in the NST, as it is in other brain regions (Woolston and Erickson 1979; Di Lorenzo and Lemon 2001). We dealt with this issue by examining short-interval firing along a continuum, as well as comparing cell types. These two approaches yielded similar results in most cases (i.e., if there were larger responses to a stimulus in B cells relative to non-B cells, then there was a significant positive correlation between response magnitudes to that stimulus and the percentage of short intervals).

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However, our data still support a relationship between taste quality and bursting, given that bursting neurons tended to have particular response profiles. For example, when NaCl is applied in a B6 mouse, there are especially large firing rate increases in NST N-cells, with these neurons’ high degree of bursting maintained during the response. In contrast, when sucrose is applied in the same animal, there are large responses in NST S-cells that rarely fire with short intervals, and small responses in those neurons that show the most bursting. When these findings are considered in light of the evidence that bursting enhances the likelihood of postsynaptic firing (see Introduction), it means that the impact of NST responses to NaCl in B6 mice is likely larger than is reflected by the cells’ mean firing rates. Thus the function of bursting by NST cells may be to amplify the postsynaptic impact of particular taste stimuli, and the variation between our NST data sets may reflect the fact that the animals differ in their need to enhance the gustatory responses to particular compounds.

Of the relationships that we observed between short-interval firing and response sizes, one stands out as being especially useful for the animals, namely, the positive correlation between sucrose responses and short-interval firing in 129 mice, which may help to compensate for their low peripheral sensitivity to sweeteners. This strain is characterized by a form of the T1R3 taste receptor that is ineffective at binding sugars (Nie et al. 2005), and 129 mice have small taste-evoked responses to sweeteners in the CT and NST compared with B6 mice (Inoue et al. 2001; McCaughey 2007). However, there is a discrepancy between these findings and the results of short-term licking tests, in which 129 mice lick mid-to-high concentrations of sweeteners to the same extent as B6 mice (Dotson and Spector 2004; Glendinning et al. 2005). Our findings may help to resolve this issue, because we observed that sucrose responses were large in B cells of 129 mice, but small in B cells of B6 mice. We suggest that the robust short-term intake of sweeteners by 129 mice is due, at least in part, to a significant positive correlation between short-interval firing and sucrose response size in NST neurons. That is, the 129 cells that evoke the largest responses to sucrose also tend to be the ones that fire with the largest percentage of short intervals and therefore should be the ones with the highest postsynaptic impact. In B6 mice, in contrast, there was not a positive correlation between bursting and sucrose response size, and the cells that exhibited the most short-interval firing tended to evoke large responses to HCl and NaCl, rather than to sucrose.

Consideration of Bursting May Help to Understand Gustatory Coding

Some investigators have proposed that perceptions of a given taste quality arise due to the activity of a single neural subtype, which forms a “labeled-line,” whereas others have suggested across-neuron coding in which responses are considered only in the context of cell populations (Erickson 2000; Spector and Travers 2005). It has been difficult to support the purest version of labeled-line coding, given that neurons in the NST and other areas tend to be broadly tuned, with few of them evoking taste-quality-specific responses (Lemon and Smith 2006).

In nongustatory systems, bursts have been proposed to act as “feature detectors” that serve to enhance perceptual contrast or help to detect novel and important stimuli (Marsat and Pollack 2012; Rivadulla et al. 2003). For example, counting only spikes preceded by short ISIs in the visual cortex of macaque monkeys results in sharpening of receptive field maps compared with counting all spikes (Reich et al. 2000). We therefore examined whether considering only spikes that followed short intervals would help to clarify gustatory coding debates simply by yielding more narrowly tuned cells. We found that entropy and noise-to-signal ratios were smaller in B6 mice and rats if NST responses were calculated in terms of short intervals per second, rather than spikes per second. Thus the standard way of calculating the tuning of NST cells (i.e., using all spikes, which vary widely in their contributions to postsynaptic action potentials) may underrepresent their specificity, at least in terms of their effect on postsynaptic cells.

However, several caveats apply to this interpretation. First, our method of counting only intervals less than 5 ms likely overemphasizes the importance of ISI duration, as widely-spaced spikes presumably still have some impact on postsynaptic firing, even if it is less than that of bursts. Second, even though the breadth-of-tuning differences between the two methods of counting spikes were highly significant in B6 mice and rats, their absolute magnitude was modest. Certainly, our reanalysis using only short-interval spikes did not change the tuning of NST cells to the extent that they became taste quality-specific; thus the same difficulties with a pure labeled-
line code exist as mentioned earlier. Third, the tuning of NST neurons in 129 mice was not affected by counting only short-interval spikes rather than all spikes. It may be that the “default” purpose for bursting is to decrease the breadth-of-tuning of cells, but that there are also other possible functions that can override this default, such as a need to amplify a weak incoming signal (e.g., small peripheral responses to sucrose in 129 mice).

Gustatory coding debates have also been informed by quantifying the information within the temporal sequence of taste-evoked responses in NST and PBN cells, with the finding that spike timing plays an important role in the neural coding of taste quality (DiLorenzo and Victor 2003; Rosen et al. 2011). We were not concerned here with the role of bursts in coding taste quality, although it is a valuable topic for future work. Burst firing in the NST represents one specific way in which spike times can deviate from a random pattern, and our results indicate that it occurs independently of firing with longer intervals. Thus separate consideration of short intervals may assist future work on information coding in NST cells. However, our data suggest that there would be little justification for considering short intervals separately for rat PBN cells.

Summary

In conclusion, we propose that classification of gustatory cells based on firing patterns is useful, at least for the NST, where bursting is common. ISI distributions are readily available to researchers upon counting action potentials, even when looking at periods in which no taste stimuli are applied. Quantifying short-interval firing by gustatory cells may also enhance considerations of how neurons stimulate action potentials in their targets, relative to spike-counting metrics that ignore temporal patterns of firing.

Our results suggest that the prevalence of bursting in the NST tends to be similar across kinds of rodents, although there is variation in terms of which neurons exhibit bursting. The purpose of bursting by NST cells is still unclear, but it occurs primarily in neurons with certain response profiles, and the neurons that are favored with bursting patterns likely have a high ability to drive their postsynaptic targets. Thus bursting may serve to amplify the impact of particular taste stimuli, with the kind of stimulus depending on an animal’s needs. Such amplification would be especially useful for sweeter responses in 129 mice because it provides a way for these animals to partially compensate for having poor receptor binding for sugars in the periphery. Alternatively, bursting may act to narrow the tuning of NST cells and thus enhance the contrast between compounds with different taste qualities.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: J.-P.B., M.G.T., and S.A.M. conception and design of research; J.-P.B. and S.A.M. performed experiments; J.-P.B. and S.A.M. analyzed data; J.-P.B., M.G.T., and S.A.M. interpreted results of experiments; J.-P.B., M.G.T., and S.A.M. edited and revised manuscript; J.-P.B., M.G.T., and S.A.M. approved final version of manuscript; S.A.M. prepared figures; S.A.M. drafted manuscript.

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