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VARIATION IN THE GENE *Tas1r3* REVEALS COMPLEX TEMPORAL PROPERTIES OF MOUSE BRAINSTEM TASTE RESPONSES TO SWEETENERS

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Abstract

The gene *Tas1r3* codes for the protein T1R3, which dimerizes with T1R2 to form a sweetener-binding receptor in taste cells. *Tas1r3* influences sweetener preferences in mice, as shown by work with a 129.B6-Tas1r3 segregating congenic strain on a 129P3/J (129) genetic background; members of this strain vary in whether they do or do not have one copy of a donor fragment with the C57BL/6ByJ (B6) allele for *Tas1r3* (B6/129 and 129/129 mice, respectively). Taste-evoked neural responses were measured in the nucleus of the solitary tract (NST), the first central gustatory relay, in B6/129 and 129/129 littermates, in order to examine how the activity dependent on the T1R2/T1R3 receptor is distributed across neurons and over time. Responses to sucrose were larger in B6/129 than in 129/129 mice, but only during a later, tonic response portion (> 600 ms) sent to different cells than the earlier, phasic response. Similar results were found for artificial sweeteners, whose responses were best considered as complex spatio-temporal patterns. There were also group differences in burst firing of NST cells, with a significant positive correlation between bursting prevalence and sucrose response size in only the 129/129 group. The results indicate that sweetener transduction initially occurs through T1R3-independent mechanisms, after which the T1R2/T1R3 receptor initiates a separate, spatially-distinct response, with the later period dominating sweet taste perceptions and driving sugar preferences. Furthermore, the current data suggest that burst firing is distributed across NST neurons non-randomly and in a manner that may amplify weak incoming gustatory signals.

Keywords: taste, gustatory, sugar, nucleus of the solitary tract, bursting

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Introduction

Sugars provide a boon to animals that encounter them in the form of readily available calories, though there can also be negative consequences to excessive consumption of them over the long-term (1). These compounds, as well as other natural and artificial compounds that have similar chemical structures and mimic them, provide a powerful gustatory signal when they enter the oral cavity, resulting in the unique taste quality perception of sweetness. There is also activation of brain areas such as the nucleus accumbens that are involved in pleasure and reward, serving to drive ingestion. At the same time, though, it is important not to portray these events as simple, given that there appear to be multiple taste transduction mechanisms activated by sugars, and that there can be wide variation among different individuals in the extent to which they prefer sweeteners (2).

The gene *Tas1r3* codes for the protein T1R3, which forms a taste receptor for sweeteners when it dimerizes with the protein T1R2 (3). Sequences of *Tas1r3* differ between mouse strains originally identified as "tasters" or "non-tasters", based on high or low sweetener preferences, respectively, in 2-bottle tests (4-7). *In vitro* and electrophysiological work has suggested that the non-tasting strains express a T1R2/T1R3 receptor with low affinity, and this poor function early in taste transduction is propagated as an unusually small neural response to sweeteners in the periphery of these mice (8,9). Reports have varied, though, as to whether this reduced sweetener sensitivity in “non-tasters” does (10) or does not (11) extend to the brainstem.

Subsequent work complicated this initial model, in which sweetener preference is dominated by a single gene that affects peripheral responsiveness. Evidence for *Tas1r3*-independent sweet taste appeared, based on T1R3-knockout mice retaining some sensitivity to sweeteners, especially non-sugars (12-14). In addition, supposedly non-tasting mouse strains (e.g., 129P3/J [129]) were found to match or exceed the intake of tasting strains (e.g., C57BL/6J [B6]) in short-term licking paradigms (15,16). Furthermore, B6 and 129 mice reacted differently when given exposure to sucrose, with only the latter showing an increase in subsequent sweetener preferences, so that they matched B6 mice in later testing (17). These results suggest that central events in 129 mice can compensate for their low peripheral sensitivity to sweeteners.

Additional insight into B6 and 129 mouse sweetener responses was provided by examining the time course of neural firing in the nucleus of the solitary tract (NST), the first central relay for taste information. On a scale of hundreds of milliseconds, independent early (i.e., phasic, 0-600 ms after onset) and later (i.e., tonic, 600-5000 ms) response components were revealed for sweeteners, but not for most non-sweet compounds (10). Furthermore, there were larger responses to sweeteners in B6 than 129 mice during the later, tonic period, but not during the initial phasic period. These data are consistent with a broader conception of gustatory responses as dynamic and consisting of multiple temporal phases, during which different aspects of the stimulus (e.g., quality, palatability) might be encoded (18,19).

B6 and 129 mice have also differed on analyses that addressed temporal firing patterns on a scale of milliseconds; these analyses revealed that many NST neurons often fired with short (< 5 ms) interspike intervals characteristic of burst firing (20). The B6 and 129 strains showed similar overall rates of bursting in the NST, but differed on which neurons tended to fire in bursts. In B6 mice, the cells that burst the most were the ones that gave the largest response sizes to NaCl; in contrast, within 129 mice the bursting cells tended to be those that gave the largest responses to sucrose. It was proposed that this positive correlation between bursting and sucrose response size serves to amplify the weak peripheral sucrose response that inputs to the NST in 129 mice, since burst patterns are especially effective at driving post-synaptic firing (21). Such amplification would provide one means by which 129 mice could partially compensate for possessing a T1R2/T1R3 receptor that binds sweeteners poorly, and it might contribute to their unusually high behavioral sensitivity to sweeteners in some circumstances (as mentioned above).

The strain differences observed previously between B6 and 129 mice could be due to their different sequences of *Tas1r3*, but the strains also differ at many other genes. One way of pinpointing *Tas1r3*'s role has been through the use of a 129.B6-Tas1r3 segregating congenic strain, which has a 129 background, but with a small donor fragment in some of the mice containing the C57BL/6JByJ (B6) allele for *Tas1r3* (22); congenic mice with this B6 donor fragment (B6/129 mice) demonstrated similar sweetener preferences as B6 inbred mice, but higher preferences than their littermates who had a pure 129 background (129/129 mice). In the current experiment, taste-evoked NST responses were measured in B6/129 and 129/129 mice, in order to define the influence of *Tas1r3* on responses to sweeteners and other compounds. The

precise time course of the neural activity was analyzed in order to address issues such as bursting and phasic versus tonic response components. The results indicated that *Tas1r3* influences only the later response portion evoked by sweeteners in the NST. Furthermore, a significant positive correlation was found between bursting activity and sucrose response size in only the 129/129 group, consistent with the hypothesis that burst firing is directed to particular NST cells when it is important for them to amplify responses to particular taste stimuli.

MATERIALS AND METHODS

Subjects

Adult male mice from the segregating congenic strain 129.B6-Tas1r3 were used. Within this strain, littermates differ in genotypes for the *Tas1r3* gene. Mice with the B6/129 Tas1r3 genotype have one chromosome with a *Tas1r3*-containing donor fragment from the C57BL/6ByJ (B6) strain, and the other complete chromosome (including *Tas1r3*) from the 129P3/J (129) strain. Mice with the 129/129 *Tas1r3* genotype have no copies of the donor fragment (and they therefore have 2 copies of the 129 allele for *Tas1r3*). Thus, the B6/129 and 129/129 groups have nearly identical genetic backgrounds, except for the presence or absence of one copy of the *Tas1r3*-containing donor fragment from B6 mice. The donor fragment is less than 194 kb and contains several genes, including *Tas1r3* (23; see 22 for details on maintenance of the congenic strain). Recordings were made of the activity of 37 cells from 13 different 129/129 mice and 42 cells from 17 different B6/129 mice.

Animals were housed individually at 23°C on a 12-h light/dark cycle, and they were given *ad libitum* access to tap water and standard laboratory chow. They were naive to all test solutions at the time of electrophysiological recording, and thus they could not have experienced the post-ingestive conditioning effects that have been reported to follow exposure to sweeteners (17). They ranged from 10–59 weeks of age and weighed 20–31 g on the day of recording. Procedures were approved by the Institutional Animal Care and Use Committees of Monell Chemical Senses Center and Ball State University.

Electrophysiological recording

The methods used for measuring neural activity and applying taste stimuli were as described previously for NST recording in B6 and 129 inbred mice (10). Animals were anesthetized with a mixture of ketamine, xylazine, and acepromazine (90, 20, and 3 mg/kg, respectively, i.p., with additional doses as necessary). A tracheotomy was performed to prevent suffocation, and a fistula was inserted into the esophagus to avoid ingestion of taste solutions. The head was secured in a nontraumatic head holder modeled after that used in rats to avoid injury to the chorda tympani nerve. A section of skull overlying the cerebellum was removed, and the cerebellum was then aspirated to expose the surface of the medulla. Body temperature was maintained at 33-36°C using heating pads, and depth of anesthesia was checked by monitoring breathing rate, heart rate detected by subcutaneous electrodes, and pedal withdrawal reflex.

The activity of single units was isolated using glass microelectrodes filled with 1.6 M potassium citrate and with a tip diameter of 1-5 μM. Cells were identified as being in the NST by the presence of a clear change in firing rate in response to gustatory stimuli. In addition, after the activity of a cell was measured, the electrode position relative to obex was determined. The mean coordinates of the cells that were recorded were 1.7 mm anterior to obex, 1.2 mm lateral to the midline, and 660 μm ventral to the surface of the brainstem, which corresponds to the location of the rostral NST in mouse neuroanatomy atlases (24,25). The signal was amplified, filtered, displayed on an oscilloscope, and stored for off-line analysis with a 20 kHz sampling rate.

Presentation of taste stimuli

When the activity of a single taste-sensitive neuron was isolated, responses were recorded to an array of 13 stimuli that included four compounds that served as prototypical sour (10 mM HCl), salty (100 mM NaCl), bitter (20 mM quinine HCl), and sweet (500 mM sucrose) solutions. Also included in the array were 10 mM disodium 5-inosine monophosphate (IMP), 10 mM citric acid, 100 mM $CaCl_2$, 100 mM NH_4Cl, the sugar maltose at 500 mM, the artificial sweeteners 20 mM acesulfame-K and 1 mM SC-45647, the sweet amino acid D-phenylalanine at 100 mM, and 10 mM NaSaccharin.

In addition, the taste stimulus 100 mM L-proline was originally included in the stimulus array for the experiment, but it was dropped from the array before the experiment was completed, because it proved to be too weak to drive most taste-responsive neurons. In total, it was applied in only 65 neurons of the 79 neurons that were included in the final analyses. The incomplete nature of the L-proline data prevents it from being used in some of the analyses (e.g., multidimensional scaling), and its low responsiveness means that it provided very limited insight into the effect of *Tas1r3* on NST responses. Therefore, the data for it were not included in the manuscript, other than to briefly address the issue of somatosensory contribution to responses (see Discussion. Multiple transduction mechanisms for sweeteners).

Here and throughout the paper, descriptions of taste quality are given for stimuli based on previous work (26-30), with the understanding that they must be inferred in nonhuman species such as mice. The term “sweeteners” is used to refer to the sugar sucrose, based on its status as a prototype, and to maltose, acesulfame, SC-45647, and D-phenylalanine, based on them being treated similarly to sucrose by mice and evoking similar across-neuron patterns of activity (10). However, the label "sweetener" is used with the understanding that all compounds evoke side-tastes, and so labeling a stimulus as such does not denote equivalence with "sweetness"; that is, sweeteners never taste purely sweet, and under some circumstances may have substantial non-sweet components to their quality. This is true to such a large extent for saccharin that this compound was not labeled as a sweetener, given that prior NST recordings in 129 inbred mice indicated a predominantly NaCl-like pattern of responding evoked by this compound (10). The concentrations of stimuli were chosen to replicate prior work and with a goal towards them being of moderate intensity, but also so that they would be effective at evoking responses in mouse NTS neurons.

All stimuli were mixed in distilled water, with the exception of the sugars and D-phenylalanine, to which 10% tap water was added to promote activation of an automatic stimulus onset marker (31). Two milliliters of each stimulus were presented at room temperature and at a rate of 1 ml/s, and the stimulus was not rinsed off until at least 5 s after onset. Stimuli and water rinse were sprayed throughout the entire oral cavity, including the palate, using syringes. Prior to starting the experiments, blue dye was sprayed in test animals in order to confirm that the method delivered solution to the entire oral cavity, including the roof of the mouth and back of

the tongue. Stimulus presentations were separated by at least a minute and were followed by at least 10 ml of deionized water as a rinse in order to return the cell's firing rate to its usual baseline, and then by a syringe full of air in order to clear the line. To avoid adaptation effects, stimuli were given in a semi-random order, in which compounds with similar taste qualities were not presented consecutively.

There were rare instances in which a neuron with both gustatory and tactile sensitivity was isolated, as indicated by a clearly discernable change in firing rate in response to water rinse or air. Such cells were excluded from the experiment. Thus, the responses of the included cells can be assumed to be purely gustatory in nature and to lack a clear somatosensory contribution to their response sizes.

Stimuli were presented multiple times when possible and an average of all presentations used. The use of multiple presentations allows for a test of the variability in responding. There were 125 instances in which a stimulus was applied more than once for a given cell in B6/129 mice, and 160 instances in 129/129 mice. The net responses for the first and second presentations were highly correlated in both B6/129 and 129/129 mice ($r = +0.91$ and $+0.90$, respectively). These results indicate a high degree of stability in the recording preparation.

Data Analysis

Action potentials were counted using the Spike2 software program (Cambridge Electronic Design, Inc.). Interspike intervals (ISIs) were calculated for two purposes: 1) to ensure that there was a clear refractory period, indicating good isolation of a single neuron's activity; and 2) for conducting analyses related to burst firing (as described below). Action potentials were counted for 3 s before (spontaneous period) and 5 s after (evoked period) stimulus onset.

Response sizes to taste stimuli were expressed as net spikes per second, based on subtracting the spontaneous firing rate for the 3 s immediately prior to stimulus application from the evoked firing rate for the 5 s immediately following application, unless indicated otherwise. Neurons were considered to be taste-responsive and included in the experiment if they gave a significant response to at least one of the 13 stimuli. A response was considered to be significant if the absolute value of the net spikes per second exceeded the SD of the spontaneous firing rate

of the cell multiplied by 2.89. This criterion set an α-level of 0.004 (or 0.05/13), two-tailed, to correct for the number of comparisons per cell. Positive responses that met this criterion gave evidence of excitation relative to the spontaneous firing rate, and negative responses that met it gave evidence of inhibition, though the latter is rare in the mouse NST (10). Spontaneous firing rates were compared between the 129/129 and B6/129 groups using t-tests. Net responses were compared between groups using two-way mixed ANOVAs with group and stimulus as factors, followed by post-hoc t-tests when appropriate to compare responses to individual stimuli between groups.

Stimuli were compared to each other within a group by calculating the Pearson correlation coefficients between each one's across-neuron pattern of responding and those of the other stimuli; multidimensional scaling was then performed on the correlation matrix that resulted, and a multidimensional space was generated in which stimuli with similar across-neuron patterns were located close to each other. Corresponding correlations were compared between groups using a Z-test for independent correlation coefficients.

Neurons within a group were compared to each other using cluster analysis based on their profiles of responding across the four prototypical stimuli. The distance measure used was 1 minus the Pearson correlation coefficient between the response profiles of the cells, and an "unweighted pair-group average" amalgamation rule was used. The resulting dendrograms were then examined and subtypes of neurons determined visually. Net responses in subtypes were compared between groups of mice using ANOVAs with group and stimulus as factors, followed by post-hoc t-tests to determine the stimuli on which they differed.

I considered the possibility that the different genetic backgrounds of the two groups could lead to differential development of the NST and a lack of correspondence between similarly-named neural subtypes in 129/129 and B6/129 mice, as observed previously for T1R3-knockout mice (13). In light of this, my strategy was to define neural subtypes and examine them, but to also rely on other measures that did not place NST cells into categories (e.g., comparisons of across-neuron patterns of activity). Furthermore, both categorized neurons and across-neuron patterns were examined to reflect the fact that gustatory data can be viewed from either "across-neuron patterning" or "labeled-line" perspectives, which differ in their dependence on the existence of discrete neural subtypes and on the importance of considering individual neurons in

the context of populations of cells (32,33; see 34 for a further discussion of gustatory coding theories and their relevance to sweet taste perception).

Temporal patterns of net responses were examined for each stimulus by constructing post-stimulus time histograms (PSTHs) in which spikes across the 5-s evoked period were distributed into 100 ms bins, and net values were calculated by subtracting the mean spontaneous firing rate. Separate phasic (0-600 ms after stimulus onset) and tonic (600-5000 ms after onset) response periods were also defined, based on prior work (10). Statistics (e.g., mean responses across all cells, multidimensional spaces) using net responses across these phasic and tonic periods were then conducted as described above for net responses across 5 sec. For two figures responses were grouped into 10 bins of 500 ms each, rather than maintaining the 600-ms length that defined the phasic period, in order to evenly cover the entire 5-sec evoked period; corresponding graphs using eight bins of 600 ms each were also constructed (data not shown), which yielded similar results as for 500 ms bins.

Bursting analyses were conducted as described previously (20). In this prior work, bursting was found to be a property of certain NST neurons, which fired frequently with interspike intervals (ISIs) of less than 5 ms, and it did not depend on stimulation with any particular taste stimulus or on presenting any tastant at all. That is, bursting appears to be a general characteristic of an NST neuron, rather than cells switching from non-bursting to bursting mode when responding to particular stimuli (though such responses have been reported for gustatory neurons in the chorda tympani nerve and parabrachial nucleus; 35-37). Thus, in bursting-related analyses ISIs were counted solely during spontaneous firing, immediately before taste stimuli were given.

For each neuron, ISI distributions during the spontaneous period were plotted across half-millisecond bins. As in prior work, two approaches were used to consider how the prevalence of short intervals during spontaneous activity (i.e., bursting) related to the responsiveness to taste stimuli. First, this relationship was considered along a continuum, without placing neurons into categories (e.g., by calculating Pearson-product moment correlations). Secondly, NST neurons were categorized within each group of mice by calculating a "Burstiness" score for each cell, and then categorized cells as "bursting" (B) or "non-bursting" (non-B) cells based on having large or small burstiness scores, respectively. The two kinds of analyses generally resulted in similar

conclusions. For example, in 129/129 mice there was a significant positive correlation between bursting and sucrose response size, as well as larger responses to sucrose in B versus non-B cells. Thus, only the results of the continuum-based analyses are presented, in the interest of brevity.

In these analyses, the percentage of intervals less than 5 ms during spontaneous firing was correlated with response sizes when the prototypical taste stimuli were applied; a threshold for significance $p <$ of 0.0125 (0.05/4) was used to take into account the use of multiple comparisons across the four prototypical stimuli. These analyses were also repeated with intervals that were 5-10 ms duration; this was done to confirm prior results that the frequencies of intervals longer and shorter than 5 ms are independent of each other (20), and to provide further evidence that there is a specific bursting mechanism that operates only on a scale of less than 5 ms and not over longer intervals.

Statistics were performed using the Systat software package. Values are presented as means ± SEM. A criterion of $p \leq 0.050$ was used for significance, except when noted otherwise.

RESULTS

Mean responses averaged across 5 seconds

The mean (± SEM) spontaneous firing rate in both groups was 10 ± 1 spikes/sec. When mean response sizes across all neurons were compared between the B6/129 and 129/129 groups, the former gave significantly larger responses to the sweeteners sucrose, maltose, acesulfame-K, SC-45647, and D-phenylalanine (figure 1; main effect of group, $F_{1,77} = 8.9$, $p = 0.004$; $t_{77} \geq 2.0$, $p < 0.05$ in post-hoc tests). That is, sweeteners tended to be less effective at driving NST responding in the 129/129 group relative to B6/129 mice, who have one B6 allele for *Tas1r3*. Most of the non-sweet compounds evoked similar response sizes in the groups, with the exceptions of IMP and quinine, which evoked larger responses in B6/129 than 129/129 mice.

The mean responses described above likely have relevance to the perceived intensity of compounds in the mice, but do not provide insight into taste quality perceptions. To address the latter phenomenon, the stimuli were compared on their across-neuron profiles of activity within each group using multidimensional scaling. In the resulting multidimensional spaces based on

the full 5-sec evoked period (figure 2A and B), compounds tended to be grouped based on their taste qualities as described by humans. As in prior analyses of rodent NST data (10,38,39), sour and bitter compounds were grouped together (consistent with evidence that quinine and citric acid are not easily distinguished from each other by mice; 40), but separate from NaCl and sucrose. Both B6/129 and 129/129 mice showed a grouping of sucrose and the other sweeteners that was separate from the remaining compounds. NaSaccharin was located closest to NaCl among the basic stimuli in both groups of mice, as was found previously for 129 inbred mice (10), indicating a profile dominated by its sodium cation; this reinforced the decision to not label it as a sweetener for this experiment (see Methods. Presentation of taste stimuli). This was also the case in both groups for IMP, presumably due to the use of the disodium salt form, and despite behavioral data suggesting a predominantly umami taste quality in B6 and 129 mice (41).

Overall, 129/129 and B6/129 mice had similar results for the multidimensional spaces, suggesting similar taste quality perceptions in the two groups, though D-phenylalanine was between the sweeteners and the sour/bitter compounds in only the B6/129 animals. This difference in placement was confirmed by a finding that the correlation between the across-neuron profiles of D-phenylalanine and quinine was significantly higher in B6/129 than in 129/129 mice (+0.49 and -0.07, respectively; $Z = 2.6$, $p = 0.005$). Saccharin was closer to sucrose and farther from NaCl in B6/129 versus 129/129 mice, but the groups did not differ significantly on the relevant individual correlations (i.e., saccharin versus NaCl and saccharin versus sucrose), which argues against this small difference in placement within the MDS being important.

Rodent NST cells vary in their response profiles and typically are categorized into acid-, salt-, and sugar-oriented subtypes (H-, N-, and S-cells, respectively). This classification was performed on the cells using cluster analysis (figure 3). The percentage of each cell type was approximately similar in the groups, with 26% and 21% of the neurons classified as S-cells, 35% and 51% classified as N-cells, and 38% and 27% classified as H-cells in B6/129 and 129/129 mice, respectively. Each neural subtype was then compared on response sizes between groups of mice, which resulted in a complex pattern of differences (figure 4; see figure legend for statistical values). Responses to the defining stimuli of each cell type were larger in B6/129 mice, with bigger responses to HCl in H-cells, NaCl in N-cells, and sucrose in S-cells. H-cells

also gave larger responses to citric acid and D-phenylalanine in B6/129 than in 129/129 mice, as did N-cells to IMP, sucrose, and SC-45647. In addition, S-cells evoked larger responses to saccharin and SC-45647 in B6/129 compared to 129/129 mice.

Overall, then, there was a patchwork pattern of group differences in neural subtypes, which generally did not provide insight into the previously described differences between B6/129 and 129/129 mice that were found across all neurons, and which did not correspond closely to the known function of T1R3. It is possible that this outcome reflects a complex set of effects on gustatory development and neural connections spurred by variation in T1R3 protein sequence. However, this cannot be assumed, and the appearance of broad group differences may relate more to the complications involved in classifying gustatory cells. For example, the neural subtypes were defined using data *within* each group of animals, rather than shared between them. Thus, it is possible that the similarly-named cell types did not truly correspond to each other between groups, especially when considering that they were defined on their response profiles across the prototypical stimuli, and mean responses to two of these four stimuli (sucrose and quinine) differed between the groups (see figure 1). Additional analyses were conducted using these H-, N-, and S-cell categories (see below). However, in all cases the same issues were examined without categorizing cells (e.g., by looking at correlations between profiles of responding across all neurons).

Temporal patterns of responding compared between groups of mice

The time course of evoked responses across the 5-second evoked period is shown in the post-stimulus time histograms (PSTH's) in figure 5. In general, group differences were not found during the initial response period, but instead were limited to the later part. These data match the results of an earlier study, in which NST activity was measured in B6 and 129 inbred mice; in that experiment the strains gave similar taste responses during an early, phasic response period (0-600 ms after onset), but B6 mice had larger sweetener response sizes during a later, tonic period (600-5000 ms; 10).

Similar phasic and tonic response periods were defined here. The groups did not differ on their NST responses during the phasic period of 0-600 ms after stimulus onset (main effect of group and group × stimulus interaction, n.s.). For the tonic period of 600-5000 ms, though, there

were significant differences similar to those observed across all 5 seconds, with larger responses in the B6/129 group to IMP and the sweeteners sucrose, acesulfame-K, maltose, SC-45647, and D-phenylalanine (main effect of group, $F_{1,77} = 14.1$, $p < 0.001$, $t_{77} \geq 2.4$, $p < 0.02$ in post-hoc tests); unlike the 5-sec comparisons, though, HCl responses were also larger in B6/129 mice, and there was no group difference in response sizes to quinine. The lack of group differences during the phasic period did not occur because there was insufficient time to yield significant taste responses to sweeteners. Many neurons gave robust responses to sucrose at 600 ms after onset, with 35% and 50% of the cells evoking significant responses in the 129/129 and B6/129 groups, respectively.

Next, across-neuron profiles of activity were examined during only the phasic or tonic periods. In the phasic multidimensional spaces (figure 2C and D), salty stimuli were located closer to the sour and bitter stimuli than they had been for the spaces based on 5 sec of activity, as was found previously in B6 and 129 inbred mice (10). However, the sweeteners sucrose, maltose, SC-45647 and D-phenylalanine were still separate from the other stimuli (located close to each other in 129/129 mice and spread farther apart in B6/129 mice), as they had been in the 5-sec spaces (figure 2A and B). In both groups of mice there was a difference in the phasic spaces compared to the 5-sec ones in the location of acesulfame-K, which was closest to the sour/bitter stimuli in the former analysis and to the sweeteners in the latter. Presumably this was due to its potassium cation activating primarily sour- and bitter-responsive neurons during the initial response period that was dominated by *Tas1r3*-independent mechanisms; these results are consistent with the HCl/quinine-like across-neuron profile in the NST reported for acesulfame-K in T1R3-knockout mice, which possess *only Tas1r3*-independent sweet taste transduction mechanisms (13).

The multidimensional spaces based on only the tonic period (figure 2E and F) were generally similar to those based on all 5 seconds, which is not surprising given that the former encompasses 4400 out of 5000 ms of the latter. Saccharin was located next to NaCl in 129/129 mice and grouped with the sweeteners in B6/129 mice. This difference in location was reflected in the correlations between the across-neuron profiles of individual stimuli that were used to generate the tonic spaces. In B6/129 mice, the correlation between the tonic profiles of saccharin and sucrose was +0.58, which was significantly larger than the correlation of +0.08 between

these two stimuli in 129/129 mice ($Z = 2.5$, $p = 0.01$); the two groups had similar tonic correlations between the profiles of saccharin and NaCl, though (+0.49 and +0.53 in the B6/129 and 129/129 groups, respectively).

Temporal patterns of responding within groups of mice

Thus, the presence of the *Tas1r3*-containing donor fragment influenced the tonic, but not phasic, responses to sweeteners. The independence of the two time periods was investigated further by comparing their response sizes to each other *within* each group of animals. In 129/129 and B6/129 mice, the correlations between the phasic and tonic responses to sucrose were only +0.17 and +0.14, respectively, which were not significant (figure 6). The correlations between phasic and tonic response sizes were also non-significant for all of the other sweeteners in both groups and ranged from +0.09 to +0.39. In contrast, correlations between phasic and tonic response sizes were larger than +0.47 and significant for all of the non-sweet compounds, with the exceptions of $CaCl_2$ and quinine in the 129/129 group. In other words, the fact that an NST neuron gives a large response to a sweetener within the first 600 ms does not necessarily mean that it will continue responding robustly after that period, whereas for most non-sweet compounds the same neurons tend to give large responses both before and after 600 ms.

This principle is further illustrated in figure 7, which compares responses between H-, N-, and S-cells within each group of animals for some of the sweeteners. The phasic period of 600 ms was adequate time for many of the cells to give significant responses to sucrose, but this occurred primarily in N- and H-cells. S-cells, which were defined based on their large responses to sucrose across 5 seconds, were characterized by an *absence* of responding to sweeteners immediately after onset; in fact, the neuron that gave the largest tonic response to sucrose in the B6/129 group (more than 80 spikes/sec) failed to give a significant response to the compound during the phasic period (figure 6). Only 13% and 27% of the S-cells evoked significant phasic responses to sucrose in the 129/129 and B6/129 groups, respectively. There were no indications that this occurred simply because 600 ms is too brief a period for NST cells to generate increases in firing above baseline. For example, many cells gave clear responses to acesulfame-K within this period (figure 7). In addition, 50% of the non-S-cells (i.e., the H- and N-cells in both groups) gave significant responses to sucrose during the phasic period. After the first 500-600

ms, the responses of N- and H-cells to sweeteners tended to decline, whereas the responsiveness of S-cells showed a sharp increase, especially in the B6/129 group.

The lack of an initial S-cell response to sucrose and SC-45647 helps to explain why the phasic multidimensional spaces showed these stimuli in a separate location than the salty, sour, and bitter compounds (figure 2C & D), since such non-sweet stimuli *did* tend to evoke significant phasic responses in S-cells. That is, the uniqueness of the phasic across-neuron patterns to sucrose and SC-45647 derives from them evoking small phasic responses in H- and N-cells and *no* phasic response in S-cells; non-sweeteners, on the other hand, evoked phasic responses in all three subtypes of cells. Moreover, the absence of early S-cell responding to sucrose is important, as it suggests that the initial response to sweeteners was *not* primarily somatosensory (i.e., the response did not occur evenly across all NST neurons, as one would expect for a signal related merely to fluid contacting the tongue).

Figure 8 shows the percentages of the total response across all neurons that were evoked within H-, N-, and S-cells at different intervals across the 5-sec evoked period for saccharin, NaCl, and sucrose. Consistent with figure 6, in both groups of mice sucrose showed a dramatic shift after 500 ms, as S-cells suddenly increased their share of the total response and H- and N-cell shares declined. This was not the case for NaCl, however, as N-cells evoked the preponderance of NST activity throughout the entire 5 sec. For saccharin, the largest share of the total NST activity occurred in N-cells in both the B6/129 and 129/129 groups; this was true both initially and during almost all of the later time periods. The groups were also similar in that only a small percentage of the total activity was evoked in S-cells to start with. Subsequently, though, S-cells continued to remain unresponsive to saccharin in 129/129 mice, but in B6/129 mice they gradually increased their share of the total response to saccharin, eventually exceeding the percentage found for N-cells. These data provide greater temporal resolution to the phasic and tonic across-neuron profiles described earlier, in which saccharin's profile was more similar to that of sucrose in B6/129 versus 129/129 mice, but only during the tonic period.

Initially, it may appear that figures 7 and 8, which show that across-neuron profiles to sweeteners were highly time-dependent, contradict figure 2C-F, in which most of the sweeteners were found in similar locations for the phasic versus tonic multidimensional spaces. However, such spaces are defined based on comparing all of the members to each other *within* a particular

space, but absolute locations cannot meaningfully be compared *between* different spaces. Thus, the similar sweetener locations in both the phasic and tonic spaces (e.g., being found on the left side in all spaces) may be a coincidence and should not be taken as evidence for matching across-neuron patterns between the phasic and tonic periods.

Spatio-temporal patterns of responding compared between groups

Earlier, parallels were described between spatial patterns (i.e., across-neuron profiles) and the presumed taste qualities of stimuli. At the same time, there is evidence that *temporal* patterns of activity evoked by taste compounds can contribute to quality perceptions, even in the absence of spatial patterning (42,43). Although the two kinds of taste-evoked patterns have traditionally been described and analyzed separately, both spatial and temporal response distributions must occur simultaneously as an animal samples solutions, and there has been growing appreciation for the need to conceive of gustatory processing in terms of combined spatial and temporal activity (37,44-47). Thus, combined spatio-temporal patterns were created and compared between the groups of mice, in order to more clearly illustrate the full influence of *Tas1r3* on NST responses.

Figure 9 shows heat maps that represent the responses evoked by seven stimuli in 500 ms bins and in terms of the mean response per subtype. These maps confirm that the different basic compounds each evoked a unique spatio-temporal pattern of activity in the NST, with the exception of the patterns for HCl and quinine being similar to each other. In addition, they indicate that the responses evoked by SC-45647 and acesulfame-K differed between the B6/129 and 129/129 groups primarily in terms of the overall response level, rather than in terms of their across-subtype or temporal patterns; in both groups of mice, though, acesulfame-K begins with an initial across-subtype pattern similar to that of HCl before switching to a sucrose-like pattern. The heat maps for saccharin are also informative, in that they show that the compound initially evokes an NaCl-like across-subtype pattern in both groups, before switching to a more sucrose-like pattern in B6/129 but not 129/129 mice. This gradual change over 5 sec for saccharin in B6/129 mice presents a contrast to the results for sucrose, where there was a sudden dramatic shift in how its activity was distributed after 500 ms in both groups of animals.

Burst firing

As in prior work (20), many NST neurons in each group displayed bursting behavior during spontaneous firing, as indicated by a high percentage of interspike intervals (ISIs) less than 5 ms. Figure 10 shows examples of raw voltage traces for a bursting and a non-bursting cell. The overall prevalence of bursting was similar in 129/129 and B6/129 mice. For example, in both groups there were 18 neurons where more than 20% of their total ISIs were less than 5 msec (figure 10C and D). However, the groups differed on how the prevalence of bursting was related to taste-evoked responses upon presentation of sucrose. In 129/129 mice, the correlation between the percentage of intervals less than 5 ms and the size of the sucrose response was +0.56, which was highly significant ($p < 0.001$; figure 10 and table 1). In other words, in 129/129 mice knowing the prevalence of bursting during spontaneous activity, prior to application of taste stimuli, allows one to predict the size of the response if sucrose were applied, with the most bursting occurring in those cells that give the largest sucrose responses. There were no other significant correlations between the percentage of intervals less than 5 ms and response sizes to the prototypical stimuli in either group, including for sucrose in B6/129 mice (table 1).

There were also no significant correlations when intervals of 5-10 ms were used instead (table 1), nor were the percentages of intervals that were 0-5 ms and 5-10 ms significantly correlated with each other in 129/129 or B6/129 mice ($r = +0.04$ and +0.15, respectively). Thus, the results were consistent with prior data indicating that intervals of 0-5 ms result from a special bursting-related mechanism that is not involved in firing with longer intervals (20). Presumably, a currently unidentified mechanism found in some NST cells can automatically generate a new action potential immediately following a previous one (e.g., as is proposed to occur through back-propagation of spikes; 48).

DISCUSSION

Multiple transduction mechanisms for sweeteners

The finding of larger NST responses to sweeteners in B6/129 versus 129/129 mice demonstrates the powerful influence of the *Tas1r3* sequence, on which the two otherwise-similar groups differed. At the same time, the results also suggest that sweeteners must activate multiple

transduction mechanisms, since phasic (before 600 ms) and tonic (600-5000 ms) sweetener-evoked responses were independent of each other, as found previously (10). The current data also indicate that *Tas1r3* sequence is only a partial determinant of sweetener response size, given that the overall response size to sweeteners in the B6/129 group was generally lower than that observed previously in B6 inbred mice, and that the percentage of neurons defined as S-cells in B6/129 mice (26%) was lower than that found before in B6 inbred mice (55%). Thus, as a result of adding the donor fragment containing the B6 allele for *Tas1r3* onto the 129 genetic background, there is an increase in neural sensitivity to sweeteners that is large, but not large enough to increase it to the level found in B6 inbred mice. This is consistent with genes other than *Tas1r3* being partially responsible for the larger sensitivity to sweeteners in B6 vs. 129 inbred mice, though more work will be needed to confirm this and identify the genes in question.

The current data progress beyond earlier findings by directly evaluating the role of *Tas1r3* in the phasic and tonic response periods. Phasic responses to sweeteners were similar in the groups, despite their genetic differences at *Tas1r3*, so they must occur solely through *Tas1r3*-independent mechanisms. In contrast, the larger tonic responses to sweeteners in B6/129 versus 129/129 mice make this response component *Tas1r3*-dependent, likely deriving from group differences in peripheral events. In 129/129 mice there should be less effective binding of sweeteners to T1R2/T1R3 in taste buds, resulting in low levels of intracellular signaling and release of neurotransmitter onto peripheral gustatory nerves. Confirmation of reduced peripheral sensitivity to sweeteners in 129/129 relative to B6/129 mice has been found in recordings from the chorda tympani nerve, which projects to the NST (22). The long latency of the *Tas1r3*-dependent response may relate to T1R2/T1R3's activation of G-protein-mediated cascades, rather than direct passage of sweeteners through a channel. However, responses to quinine are also G-protein-mediated, but have a short latency. Thus, additional work will be needed to determine why the *Tas1r3*-dependent sweetener response takes so long to influence the NST.

There is no way to know what taste quality is generated by the phasic response to sweeteners (i.e., although it is "sweetener-evoked," it may not involve perceptions of "sweetness"; see "Methods. Presentation of taste stimuli" for discussion of this issue), nor is it possible at present to identify which *Tas1r3*-independent mechanisms are responsible for it. Glucose transporters are expressed in taste bud cells and mediate T1R3-independent sweet taste.

However, these proteins are co-expressed with T1R3 (49,50), whereas the current data implicate differentially-expressed mechanisms (i.e., proteins *not* expressed in T1R3-containing taste bud cells), given the independence of the phasic and tonic across-neuron profiles. A similar reason argues against umami receptors mediating the phasic sweetener responses, given that umami and sweet compounds evoke similar across-neuron profiles in the rodent brainstem (51,52).

Another possibility is that the phasic component represents non-gustatory information (e.g., ionic contributions) that allows for detectability but not recognition of a stimulus. Arguing against possibility, though, is the fact that phasic and tonic response periods were independent almost exclusively for sweeteners and not for non-sweet compounds. That is, for almost all of the non-sweet stimuli, the response size during the phasic period closely tracked the size during the tonic period, suggesting that similar information is carried during the two phases. I also considered whether the phasic response component involves primarily a somatosensory (e.g., tactile or thermal), rather than gustatory, response, given that some NST cells receive trigeminal input (53) and that gustatory cortex neurons are thought to display an early somatosensory-related response component (18). However, this prospect is unlikely, given that: 1) neurons that had noticeable tactile sensitivity were excluded from the experiment (as described in Methods. Presentation of taste stimuli); 2) similar results were observed for 500 mM sucrose and 1 mM SC-45647, despite their large difference in viscosity; 3) phasic responses to sweeteners were not uniform across all NST cells, but varied across H-, N-, and S-cells, with the latter showing a lack of responding.

In addition, the taste stimulus 100 mM L-proline was originally included in the stimulus array for the experiment, but dropped before completion due to its ineffectiveness at driving neurons (see Methods. Presentation of taste stimuli). However, the limited proline data can be helpful in addressing whether the phasic responses of most NST neurons had a significant somatosensory component. Out of those 65 neurons in which it was applied, only 15 neurons (i.e., 23% of the total) gave a phasic response that was significantly larger than the baseline firing rate. Moreover, this 23% represents the maximum number of neurons that might have responded to pure water, since the 100 mM L-proline solution contained not only water, but also molecules of the taste compound proline. The low percentage that resulted from this analysis helps to confirm that the neurons that were included in the experiment did not generally have a

somatosensory contribution to their phasic responses. Nonetheless, it is not possible to completely rule out the possibility that there was a somatosensory contribution to the phasic portion of the responses in some neurons.

In addition, the phasic response to sweeteners did not appear to represent salty, sour, or bitter side-tastes, given the distinct locations of sweeteners and non-sweet compounds in the phasic multidimensional spaces. Another possibility is that the phasic response evoked by sweeteners is mediated by transduction mechanisms for one or more non-traditional tastes that are distinct from the traditional five basic categories, such as "starch" taste activated by multioligosaccharides (54). However, the absence of such stimuli from the current study makes it impossible to address this issue at present. Regardless, sweeteners likely caused *some* kind of taste quality perception within 600 ms, even if it cannot be definitively identified, given that they evoked significant phasic responses in many NST cells. Certainly, this time period is adequate for behaving rodents to discriminate some taste compounds from each other (55-57).

Comparisons with prior behavioral data

In general, the group differences in tonic, but not phasic, neural responses provided a close match with prior behavioral results, in which B6/129 mice preferred sucrose, maltose, acesulfame-K, SC-45647, D-phenylalanine and saccharin to a larger extent than did 129/129 mice (22). Although the tonic response size to saccharin did not differ between the groups, saccharin's tonic across-neuron profile was more similar to that of sucrose in B6/129 mice; this result parallels prior differences in saccharin's across-neuron profile between B6 and 129 mice (10), as well as between groups of rats with discrepant saccharin preferences (58). The more sucrose-like profile of saccharin in B6/129 mice suggests an influence of *Tas1r3* sequence on perceived *quality* (i.e., purity of sweet taste), whereas the larger mean responses across all cells to sucrose, maltose, acesulfame-K, SC-45647, and D-phenylalanine suggest an influence on perceived *intensity*.

The mean response across all neurons was larger for all stimuli in B6/129 relative to 129/129 mice, and these differences rose to the level of significance for a few of the non-sweeteners that were equally preferred by 129/129 and B6/129 mice in prior work, including quinine (in 5-sec responses only), HCl (in tonic responses only), and IMP (in both measures).

This raises the issue of whether variation is *Tas1r3* affects the size of sweetener responses or of NST responsiveness more generally (or, indeed, of whether it affects both, but to different degrees). Arguing against the second possibility is the fact that all five of the sweeteners showed significant group differences in mean responses sizes, whereas for the salty, sour, and bitter taste stimuli, only one of the representative compounds showed a significant group difference (IMP and saccharin are categorized as salty stimuli here, based on the multidimensional spaces; see figure 2). Nonetheless, the data do not rule out the possibility that variation in *Tas1r3* sequence results in generalized effects on responsiveness, or effects on additional taste qualities beyond sweetness, through unknown mechanisms.

Considerations of responses *within* each group of mice also suggest that tonic activity provided a closer match with prior behavioral data than did phasic activity. For example, in the spaces based on tonic activity, the artificial sweetener acesulfame-K was located closer to sucrose than it was to HCl or quinine in both groups of mice. In contrast, in the spaces based on only phasic activity this compound was located closer to HCl and quinine than it was to sucrose. This outcome occurred even in B6/129 mice, who prefer acesulfame over water (22), suggesting that their behavior is driven primarily by the later tonic response portion, during which acesulfame evokes a sucrose-like across-neuron profile.

Spatio-temporal codes as a basis for taste quality perception

The exact process by which perceptions of taste quality are generated in the brain (i.e., gustatory coding) remains to be determined, though there are likely important roles for multiple factors, including specific neural subtypes, comparisons across large populations of cells, and dynamic changes in firing rates. Although there remain mysteries about the neural basis for taste perceptions, presumably there must be *some* measurable set of characteristics of taste-responsive neurons that vary in direct relationship to the kind of stimulus that is applied. That is, salt must taste primarily salty and sugar taste sweet because these compounds differ in how they cause gustatory neurons to change their firing rates after application. The NST is likely not sufficient, or even dominant, in this process, and other regions (e.g., gustatory cortex) obviously play large roles. Nonetheless, the NST is an obligatory relay, and all other taste-sensitive neurons in the brain depend on it to provide a discriminatory neural signal related to which compounds are present in the mouth.

In the current work, application of sucrose resulted in two unique consequences in the NST that distinguished it from the non-sweet compounds: 1) a *lack* of responding in S-cells during the phasic period; and 2) large responses in S-cells during the tonic period. It is possible that sucrose's primarily sweet taste depends on *both* of these unique neural consequences. However, there are several reasons why this is unlikely. First, this interpretation would require neurons to serve different roles at different times, which is incompatible with general conceptions of how taste quality perceptions are created. Second, it would require some kind of improbable "homunculus" that somehow keeps track of stimulus onset and "knows" when to expect a shift in the evoked across-neuron pattern; moreover, it would need to keep track of this process differentially for sweeteners and non-sweet compounds, given that only sweeteners showed this shift to activating different NST cells after 600 ms. Third, it is clear that every individual compound, even ones that serve as so-called "prototypes", evokes multiple taste qualities, and some compounds even evoke quality perceptions that change drastically over time (e.g., ones with a bitter aftertaste). Thus, there is no reason to assume that every effect of sucrose application on NST firing rates is associated with sweetness perception.

In light of these factors, it is difficult to say at present what kind of taste quality perception is generated by the phasic sweetener response portion. One possibility is that the quality is neither sweetness, nor any of the other four canonical basic tastes. On the other hand, there is evidence that the *tonic* response is related to sweet taste perceptions, with levels of activity differing between the two groups of mice in a way that is consistent with their different behavior toward sweeteners. I acknowledge that the phasic sweetener response cannot simply be dismissed, given the high percentage of H- and N-cells that responded significantly during only the phasic period, but the data argue against this 600-ms interval providing a strong influence on behavior; this period may be adequate to guide intake of non-sweet compounds, though, given evidence for more rapid detection of NaCl than sucrose in both mice and humans (57,59,60).

A true understanding of how taste quality perceptions are generated will likely require considering evoked responses as complex spatio-temporal patterns. Among the stimuli used here, this issue is especially relevant to acesulfame-K and NaSaccharin. The former evoked large responses during the phasic period, but developed a sucrose-like across-neuron profile only during the later period. Saccharin also showed a large time-dependence of its across-neuron

profile in the B6/129 group, with a predominantly NaCl-like profile gradually changing to a more sucrose-like profile. Further work will be needed to determine the time periods most relevant for examining taste quality perceptions within this model of across-neuron patterns that can shift over time. The influence of earlier neural activity must eventually become lost as new activity is generated, and considering extremely long intervals fails to take into account the dynamic nature of perception. However, looking at extremely brief periods of activity (several milliseconds) yields across-neuron patterns that are not stable enough across successive intervals to allow individual neurons to maintain a consistent role in gustatory coding. There are also additional factors beyond the scope of the current work (e.g., anesthesia state, anticipatory effects, the method by which stimuli are delivered) which likely affect how taste perceptions evolve over hundreds of milliseconds to seconds.

Role of burst firing

I characterized distributions of interspike intervals of cells in 129/129 and B6/129 mice here, and previously in rats and in B6 and 129 inbred mice (20), and between the two experiments several facts are clear: 1) a subset of rodent NST neurons often fire in bursts with interspike intervals of less than 5 ms; 2) these bursting mechanisms are not randomly distributed across NST cells, but instead tend to occur in neurons with certain response properties; and 3) *which* NST cells show bursting behavior varies across groups of animals. Here, the overall prevalence of bursting was similar between 129/129 and B6/129 mice, but the groups differed on whether bursting was related to sucrose response sizes. Only the 129/129 group had a significant positive correlation between the percentage of intervals less than 5 ms and the size of the sucrose response. Thus, the presence or absence of the *Tas1r3*-containing donor fragment affected how bursting-related mechanisms were distributed across NST cells with different response properties.

Between the current experiment and the earlier study, a high degree of bursting was observed in the most sucrose-responsive neurons in only two out of five groups (129 and 129/129 mice), and these were the two groups that are known to have poor sweetener binding to T1R2/T1R3 and a weak peripheral nerve response to sweeteners. It is possible, of course, that this is a coincidence. However, it is also possible that the mechanism that causes bursting in NST cells is directed to cells that receive weak peripheral gustatory signals that would benefit

from amplification, as suggested previously (20). This should cause the few NST cells that *do* respond robustly to sweeteners in these mice to be especially effective at driving their post-synaptic targets and compensate for the animals' poor peripheral responsiveness. That is, it should augment the sweetener responses of the cells that *follow* the NST in 129/129 mice; in contrast, in B6/129 mice (here) and in B6 mice (previously) there were large responses in the NST to sucrose, but primarily in non-bursting cells that may have a limited ability to drive their targets. The net effect of these bursting distributions should be to bring 129/129 mice closer to B6/129 mice in their sweetener responsiveness for areas that follow the NST, compensating for the former's poor peripheral responses to sweeteners. Additional work will be needed to test this hypothesis more directly. Bursting in the NST may also serve additional functions, such as sharpening breadth-of-tuning and enhancing synchronized firing, as suggested by work in non-taste systems (61,62).

Perspectives and significance

Single-unit recordings using 129/129 and B6/129 mice from the segregating congenic strain 129.B6-Tas1r3 provided insight into how the taste-evoked activity generated by the T1R2/T1R3 receptor is distributed across neurons and across time in the NST. Binding of sweeteners to this receptor resulted in a neural signal directed primarily to a subclass of S-cells, but only after a delay of approximately 500-600 ms. Prior to this time, sweeteners activated unknown taste transduction mechanisms that must be expressed in different taste bud cells than T1R3, and which generated neural signals distributed primarily to N- and H-cells in the NST. Thus, the relative contributions of these *Tas1r3*-dependent and -independent mechanisms varied at different times. The diversity and complexity of neural responses evoked by sweeteners (i.e., compounds that are treated similarly to sucrose by mice) suggested that NST responses are best characterized in terms of spatio-temporal patterns of activity, in which the neurons that fire the most can change across periods of hundreds of milliseconds. Even briefer time periods (less than 5 ms) are also important to observe, as they are relevant to bursting behavior by NST neurons, which likely has an impact on the effectiveness with which the cells are able to drive their targets. Only the mice with low sweetener sensitivity (i.e., the 129/129 group) exhibited a significant positive correlation between amount of bursting and sucrose response size. This may be part of a general property of the NST to amplify taste responses to certain compounds,

719 depending on an animal's peripheral gustatory sensitivity. Regardless, it will be worthwhile to
720 expand considerations of bursting in the taste system, given that the true impact of neurons
721 occurs in their ability to drive their post-synaptic targets, which cannot be fully determined by
722 merely counting the number of action potentials across periods of several seconds.

723

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Figure captions

Figure 1. Responsiveness to sweeteners was larger in the NST of B6/129 than 129/129 mice. Mean (± SEM) responses across all neurons and averaged across the entire 5-sec evoked period are shown for the 129/129 (open bars, n = 37) and B6/129 (filled bars, n = 42) groups. Statistics were as follows: main effect of group, $F_{1,77} = 8.9$, $p = 0.004$. Abbreviations: HCl, 10 mM HCl; Na, 100 mM NaCl; Q, 20 mM quinine HCl; Suc, 500 mM sucrose; IMP, 10 mM disodium 5 -inosine monophosphate; Ci, 10 mM citric acid; Ca, 100 mM $CaCl_2$; NH, 100 mM NH_4Cl; Ace, 10 mM acesulfame-K; Mal, 500 mM maltose; Phe, 100 mM D-phenylalanine; SC, 1 mM SC-45647; Sac, 10 mM NaSaccharin. *, $p < 0.05$, 129/129 vs. B6/129 in post-hoc tests.

Figure 2. Comparisons of across-neuron patterns of NST responding to each stimulus within each group of mice showed groupings consistent with presumed taste quality when based on the full 5-sec evoked period (A and B). However, distinctions between across-neuron profiles of different stimuli were clearer for tonic than for phasic responding. Two-dimensional spaces generated using multidimensional scaling based on only the first 600 ms of evoked activity are shown for the 129/129 (C) and B6/129 (D) groups. Within each group, across-neuron patterns of NST activity during the phasic period alone showed less distinctions between stimuli than had been the case for entire 5-sec evoked period, but most of the sweeteners were still grouped apart from non-sweet stimuli. Acesulfame-K was located closest to the sour and bitter stimuli in both groups, in contrast to the spaces based on 5-sec responses, where it was located closest to the sweeteners. Spaces based on across-neuron patterns during tonic activity (E and F) were generally similar to those based on the full 5 seconds. Abbreviations: HCl, 10 mM HCl; Na, 100 mM NaCl; Q, 20 mM quinine HCl; Suc, 500 mM sucrose; IMP, 10 mM disodium 5 -inosine monophosphate; Ci, 10 mM citric acid; Ca, 100 mM $CaCl_2$; NH, 100 mM NH_4Cl; Ace, 10 mM acesulfame-K; Mal, 500 mM maltose; Phe, 100 mM D-phenylalanine; SC, 1 mM SC-45647; Sac, 10 mM NaSaccharin.

Figure 3. Three subtypes of neurons were identified in each group using cluster analysis. The resulting dendrograms are shown for 129/129 (top) and B6/129 (bottom) mice. Cells were

compared with each other based on their profiles of responding across the prototypical stimuli, and the branch points defining S-, N-, and H-cells are indicated by the appropriate letter.

Figure 4. The groups of mice differed on responses to stimuli within neural subtypes. Mean net responses across H- (top), N- (middle), and S-cells (bottom) are shown for the 129/129 (open bars) and B6/129 (filled bars) groups. In 129/129 there were 10 H-cells, 19 N-cells, and 8 S-cells, and in B6/129 mice there were 16 H-cells, 15 N-cells, and 11 S-cells. Statistics were as follows: H-cells, main effect of group, $F_{1,24} = 5.4$, $p = 0.03$, group × stimulus interaction, $F_{12,288} = 2.1$, $p = 0.02$; N-cells, group × stimulus interaction, $F_{12,384} = 2.8$, $p = 0.001$; S-cells, main effect of group, $F_{1,17} = 4.6$, $p = 0.048$, group × stimulus interaction, $F_{12,204} = 2.6$, $p = 0.004$. Abbreviations: HCl, 10 mM HCl; Na, 100 mM NaCl; Q, 20 mM quinine HCl; Suc, 500 mM sucrose; IMP, 10 mM disodium 5 -inosine monophosphate; Ci, 10 mM citric acid; Ca, 100 mM $CaCl_2$; NH, 100 mM NH_4Cl; Ace, 10 mM acesulfame-K; Mal, 500 mM maltose; Phe, 100 mM D-phenylalanine; SC, 1 mM SC-45647; Sac, 10 mM NaSaccharin. *, $p < 0.05$ in post-hoc tests, 129/129 vs. B6/129.

Figure 5. Group differences in NST responsiveness were clearer for the later (tonic) response portion than for the earlier (phasic) portion. Post-stimulus time histograms (PSTH's) show mean (± SEM) responses across all cells in 100-ms bins for the 129/129 (grey lines) and B6/129 (black lines) groups. Results are shown for nine representative stimuli. Abbreviations: HCl, 10 mM HCl; Na, 100 mM NaCl; Q, 20 mM quinine HCl; Suc, 500 mM sucrose; IMP, 10 mM disodium 5 -inosine monophosphate; Ace, 10 mM acesulfame-K; Mal, 500 mM maltose; Phe, 100 mM D-phenylalanine; SC, 1 mM SC-45647.

Figure 6. Sucrose response sizes for the phasic and tonic response periods were independent of each other. Across-neuron profiles of activity evoked by 500 mM sucrose are shown for the 129/129 (left) and B6/129 (right) groups, based on response sizes during the phasic (0-600 ms, top) or tonic (600-5000 ms, bottom) periods. Responses that were significantly different from

zero are indicated by filled bars; in almost every case these significant differences indicated excitation, though one neuron in 129/129 mice showed a significant inhibitory response. For both time periods, neurons are ordered based on phasic response size, in descending order, within each group of mice. Correlations between phasic and tonic response sizes were non-significant for both 129/129 and B6/129 mice ($r = +0.17$ and $r = +0.14$, respectively).

Figure 7. Within each group of mice, temporal patterns of NST responding to sweeteners varied across types of cells with different response profiles. Post-stimulus time histograms (PSTH's) show mean responses across the entire 5-sec evoked period within H-cells, (blue), N-cells (green), and S-cells (red) for the 129/129 (left) and B6/129 (right) groups. Results are shown for sucrose (top), acesulfame-K (middle), and SC-45647 (bottom).

Figure 8. Taste stimuli varied in how their NST responses were distributed across neural subtypes over time. Shown are the percentage of the total number of spikes generated by saccharin (top), NaCl (middle), and sucrose (bottom) within the H-cells, (blue), N-cells (green), and S-cells (red) across 500 ms time bins. Patterns across time were similar for the 129/129 (dashed lines) and B6/129 (solid lines) groups for sucrose and NaCl. Saccharin, in contrast, gradually increased its share of the total response within S-cells over the 5-sec evoked period in B6/129 mice, but not in 129/129 mice. Note that four time bins for sucrose in 129/129 H-cells had slightly inhibitory mean response sizes, which resulted in negative percentages that are difficult to interpret; these analyses were also conducted after taking the absolute values of net response sizes, which eliminated negative values, and similar results were obtained (data not shown).

Figure 9. Responses to taste stimuli in the NST can be characterized as spatio-temporal patterns that vary between stimuli and can be affected by *Tas1r3* sequence. Heat maps show how mean response sizes within H-, N-, and S-cells varied across 500-ms time bins in the 129/129 (left half of each map) and B6/129 (right half) groups for representative stimuli. The map for each

stimulus is drawn with a different scale in order to emphasize response *patterns* across subtypes and time (thought to be related to taste quality), rather than the overall response level (thought to be related to taste intensity).

Figure 10. A subset of NST neurons showed bursting during spontaneous activity, as indicated by a high percentage of interspike intervals less than 5 ms. Raw voltage traces are shown at the top for two representative neurons that varied in whether they did (A) or did not (B) show a high degree of bursting, even though their mean spontaneous firing rates were similar. At the bottom, scatterplots show the relationship between the percentage of the total interspike intervals that were less than 5 ms and the response size to sucrose in the 129/129 (C) and B6/129 (D) groups. The membership of each neuron in the three neural subtypes is indicated by the kind of symbol (H-cells = square; N-cells = circle; S-cells = triangle). The correlation between these two variables was significant in 129/129 ($p = +0.56$) but B6/129 ($r = +0.02$) mice. Note that the two X-axes are on different scales, based on the larger sucrose responsiveness of the B6/129 group.

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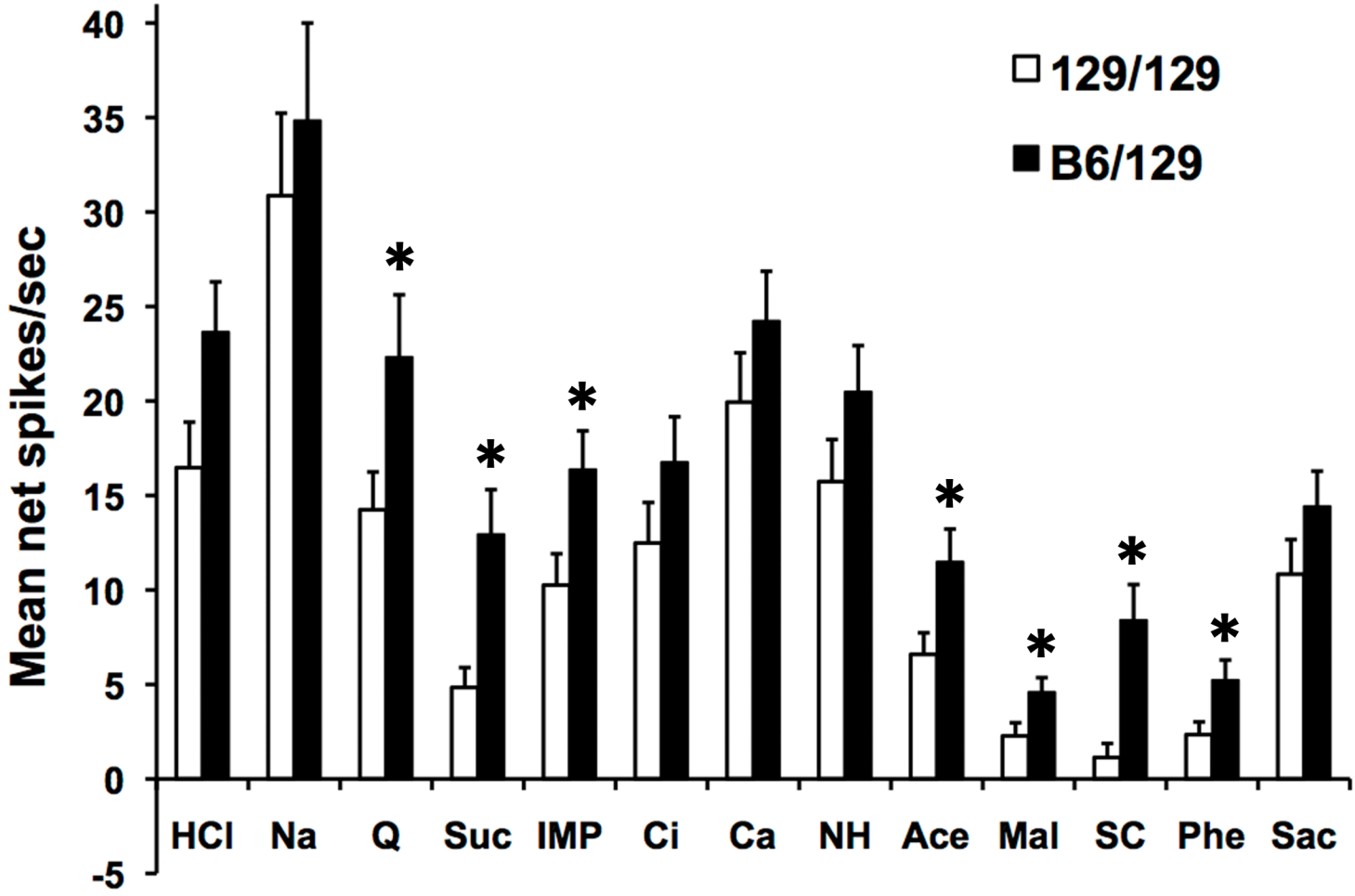

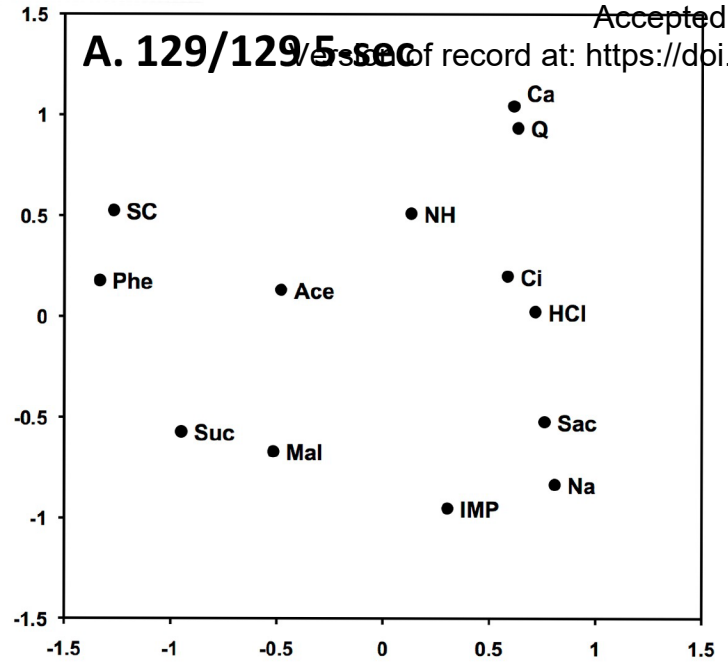

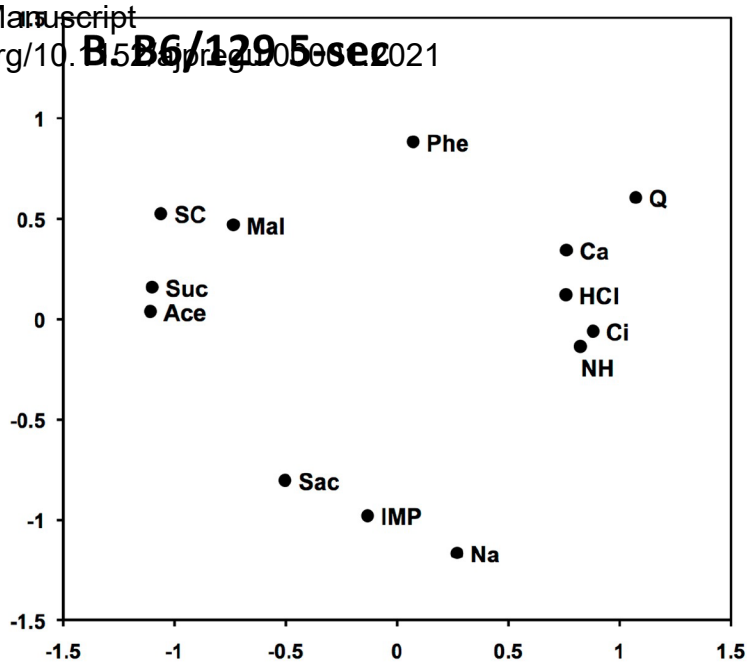

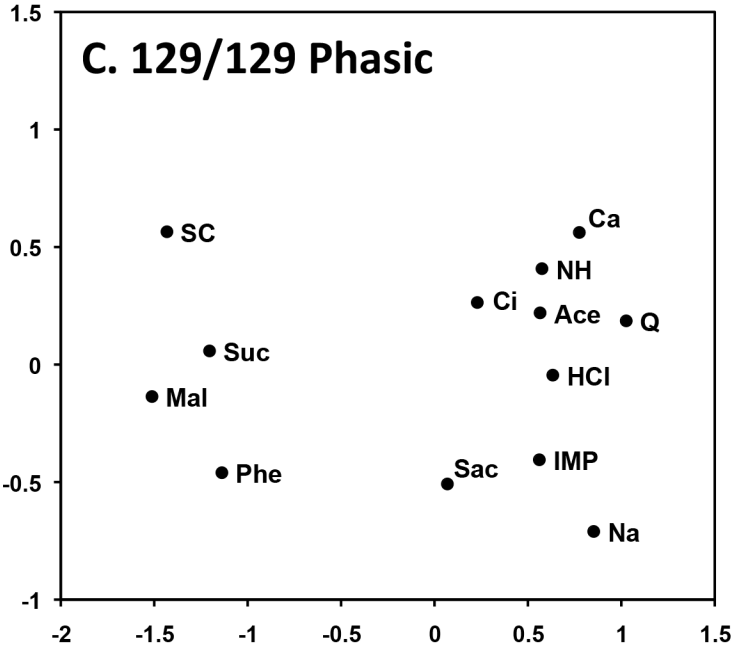

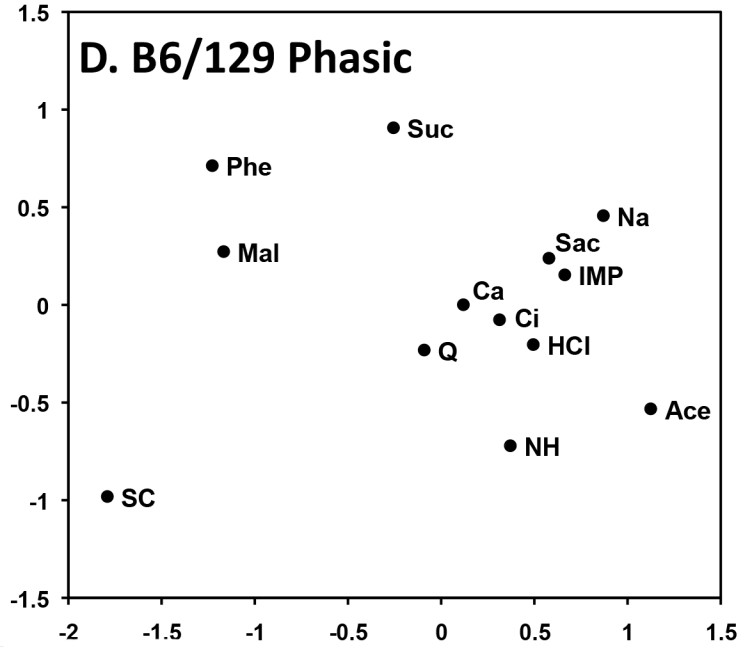

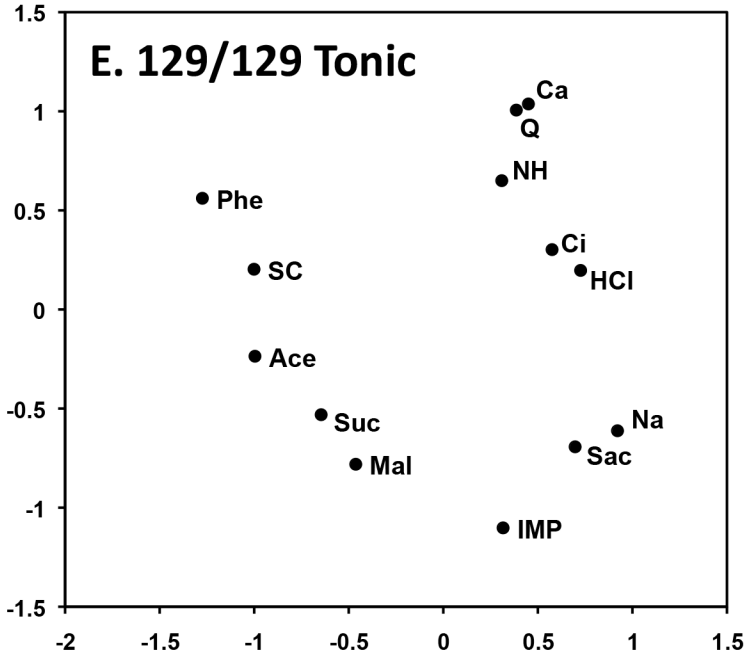

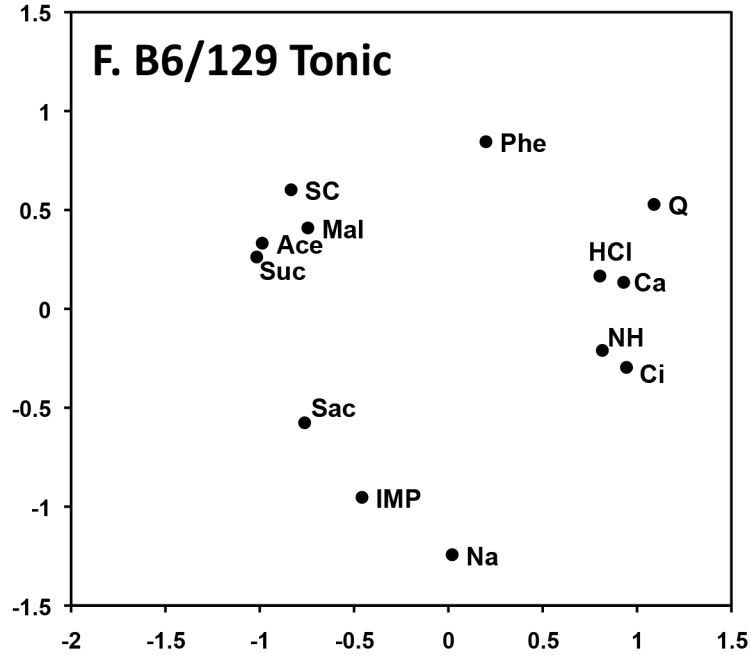

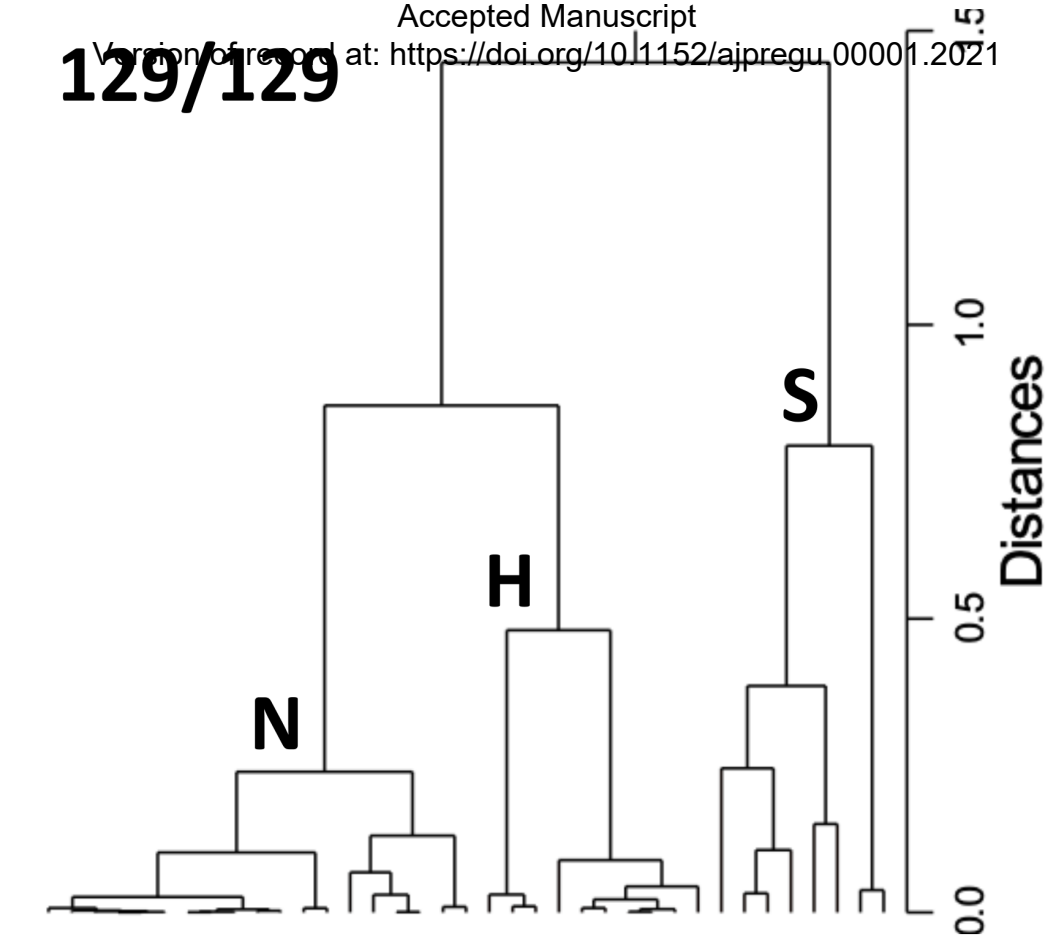

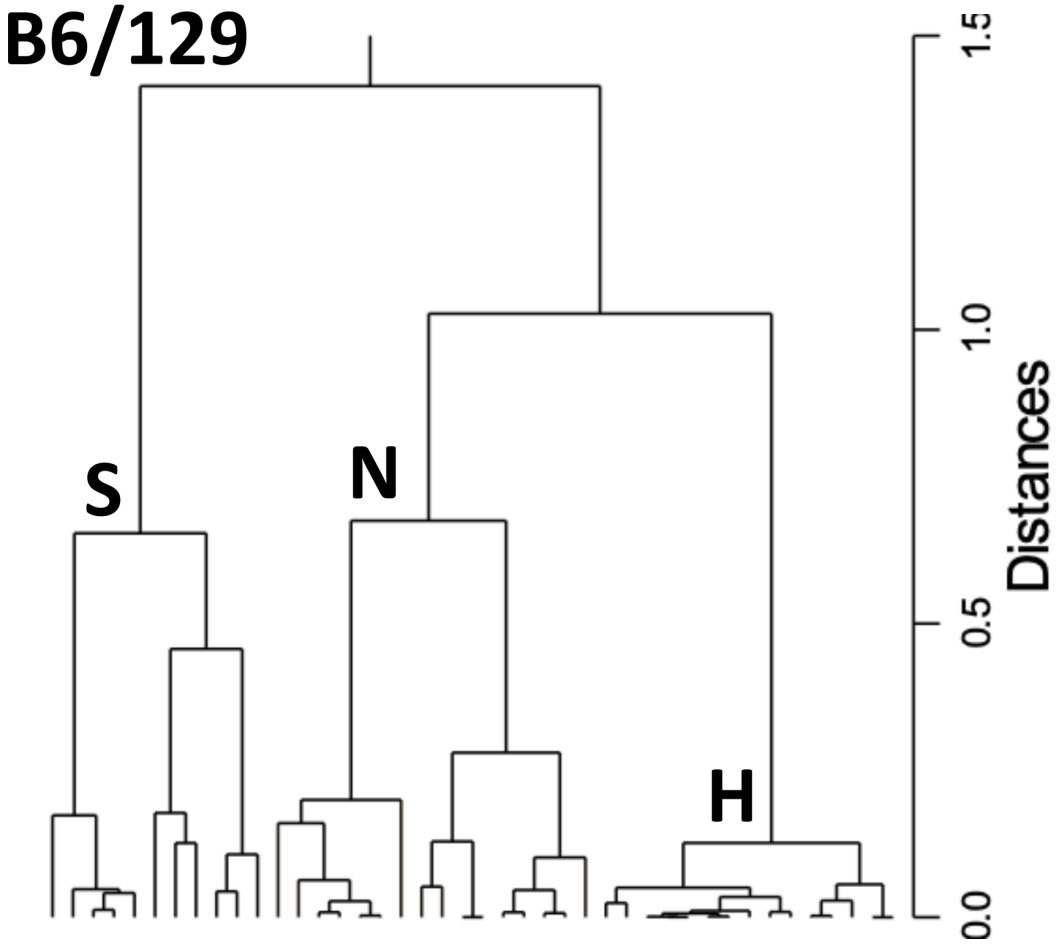

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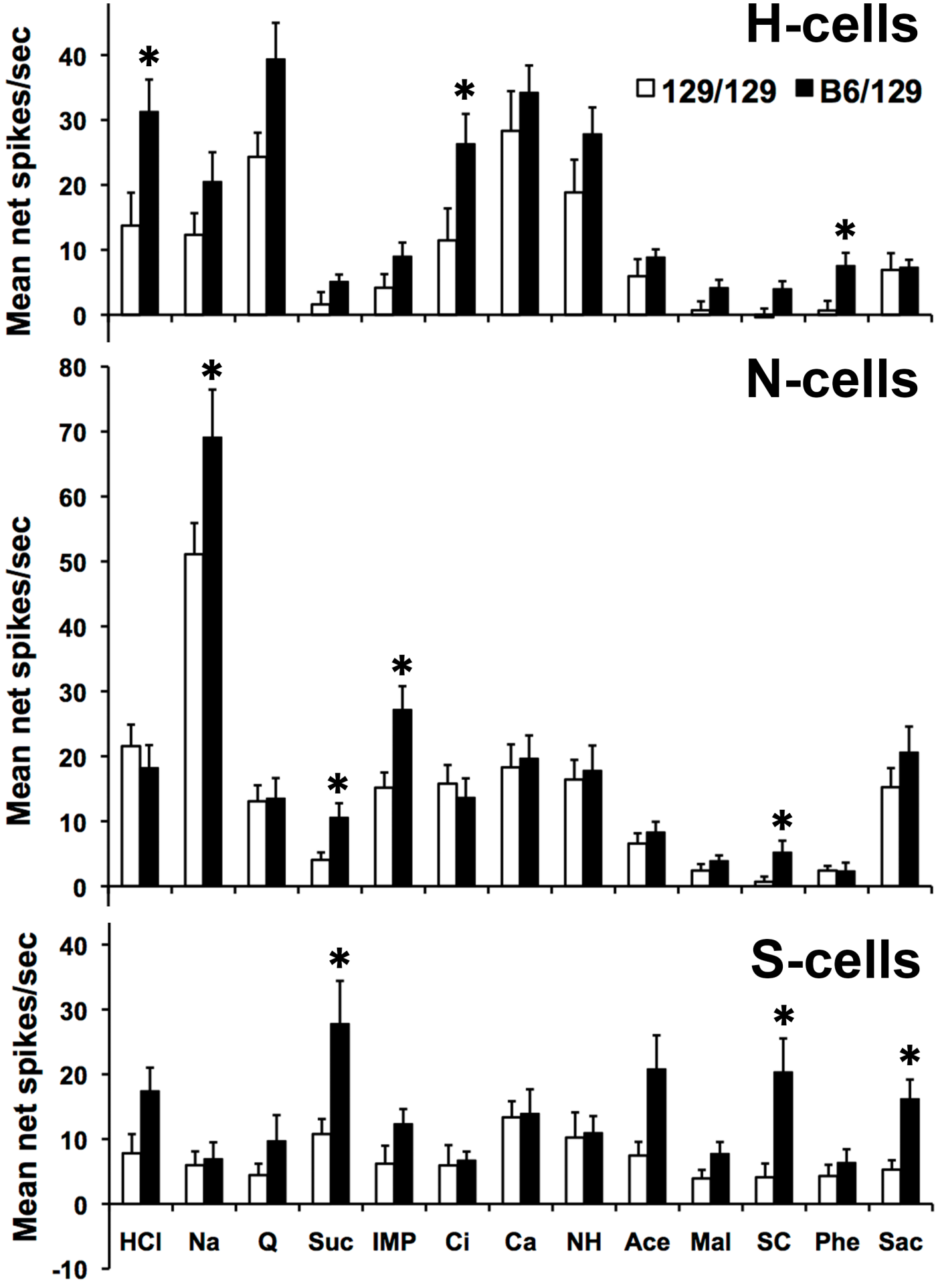

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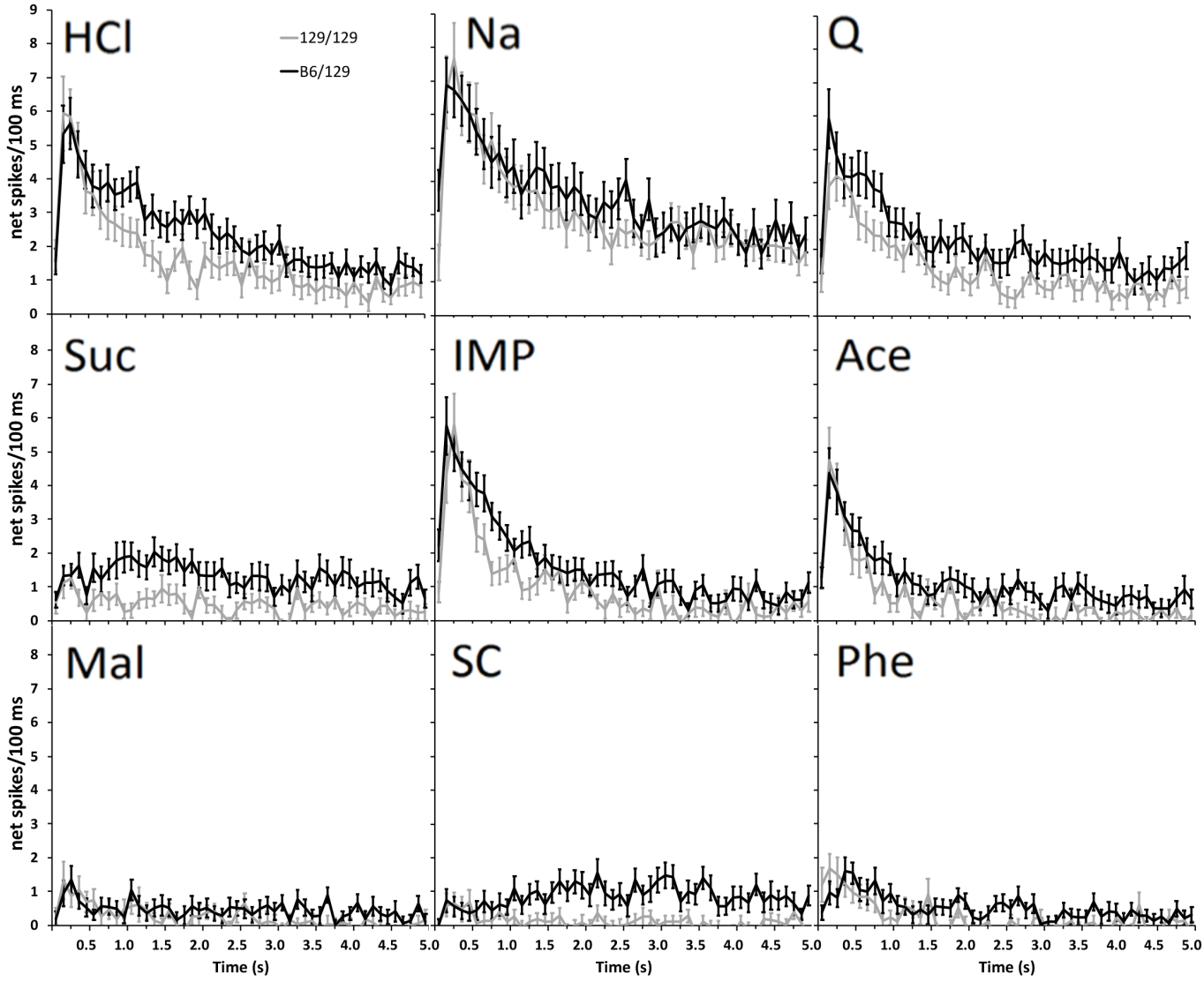

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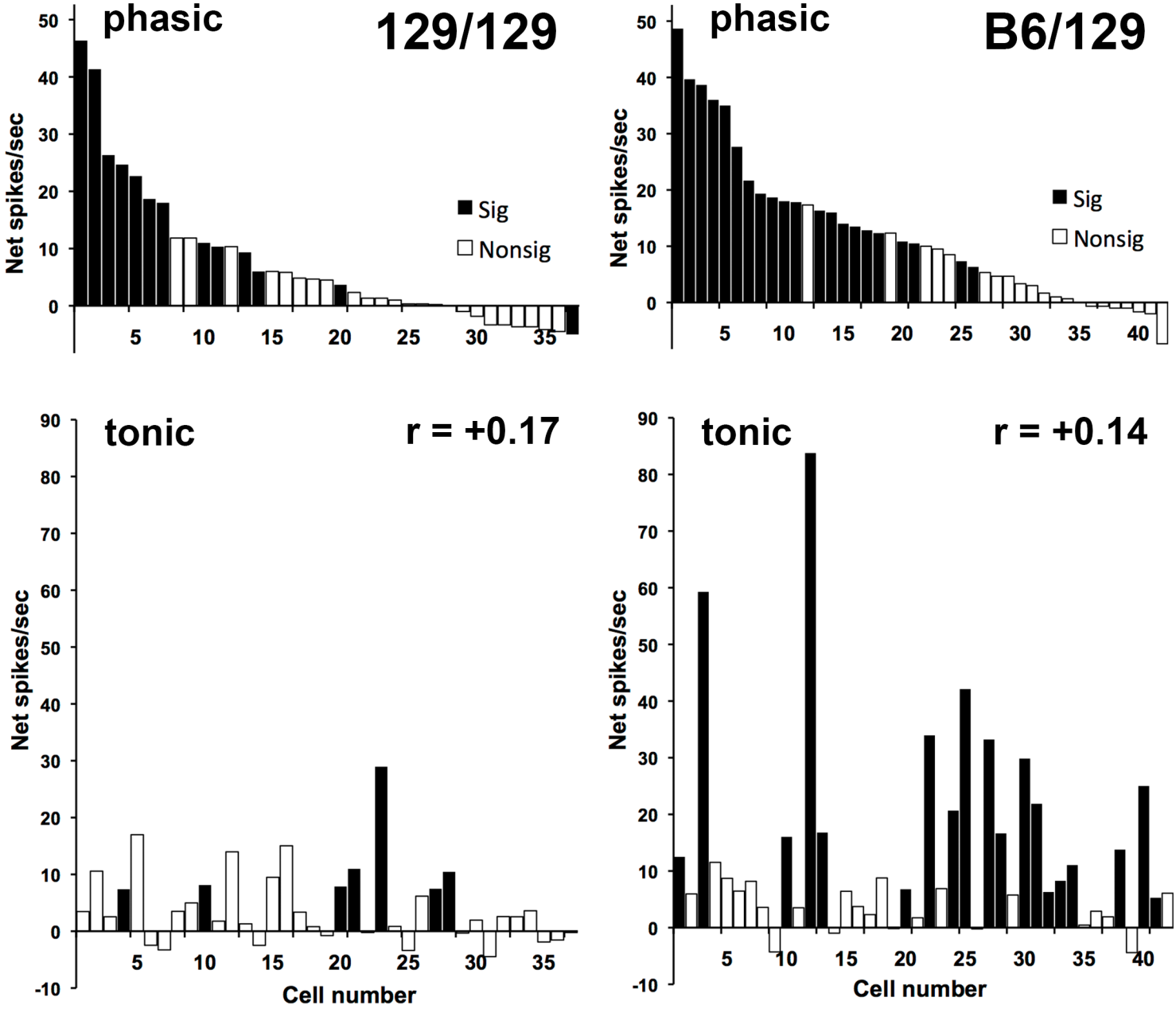

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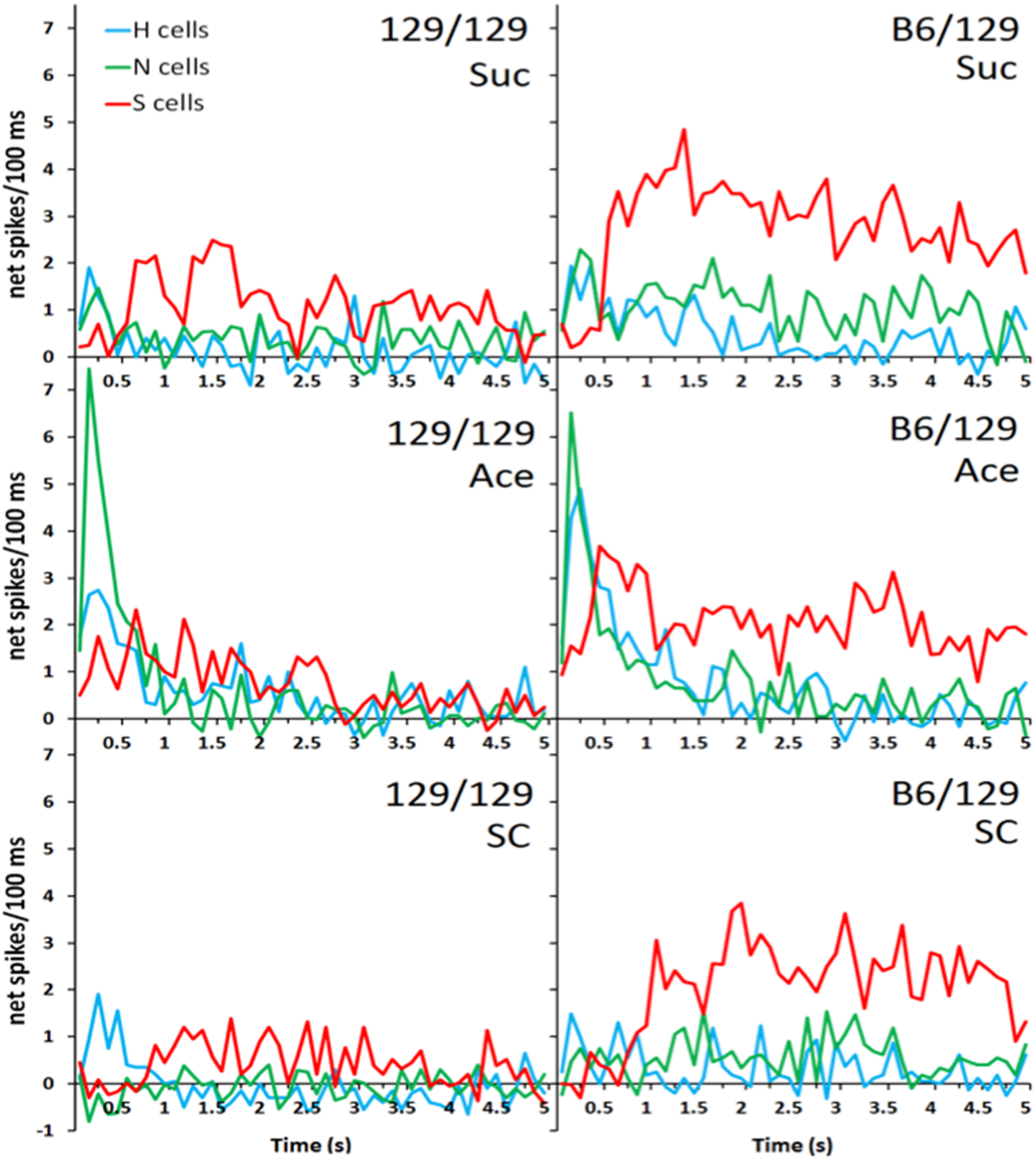

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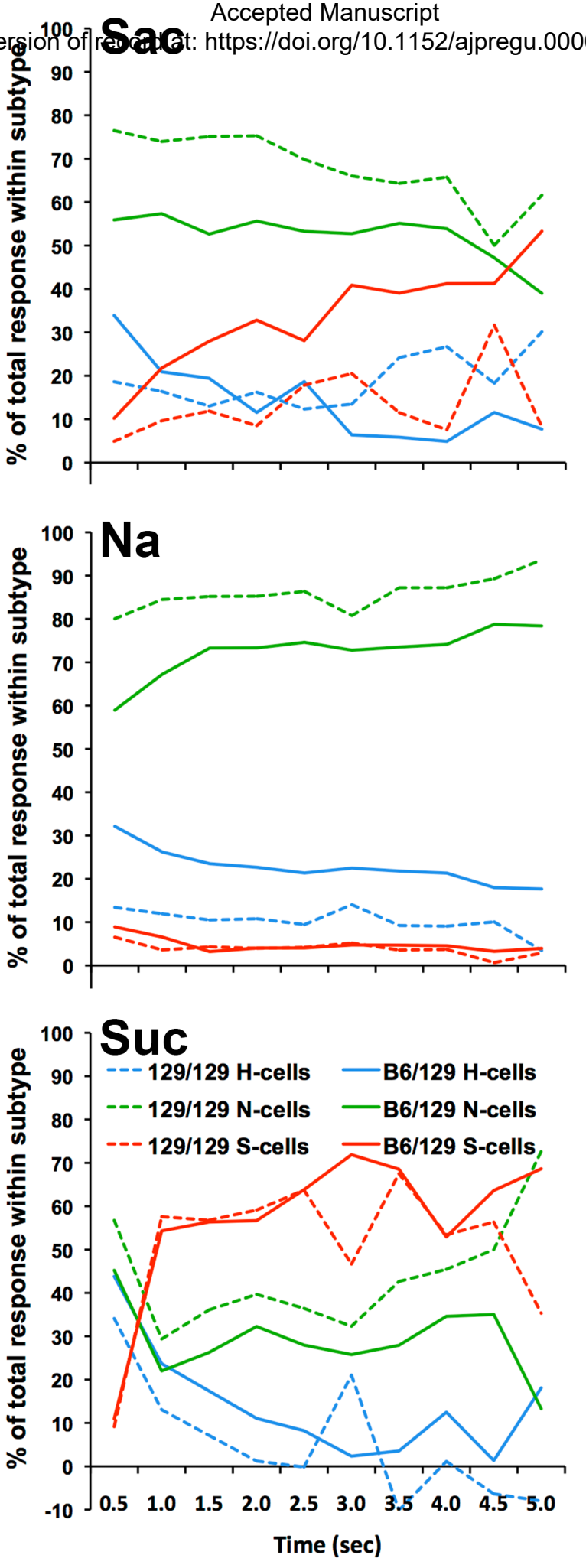

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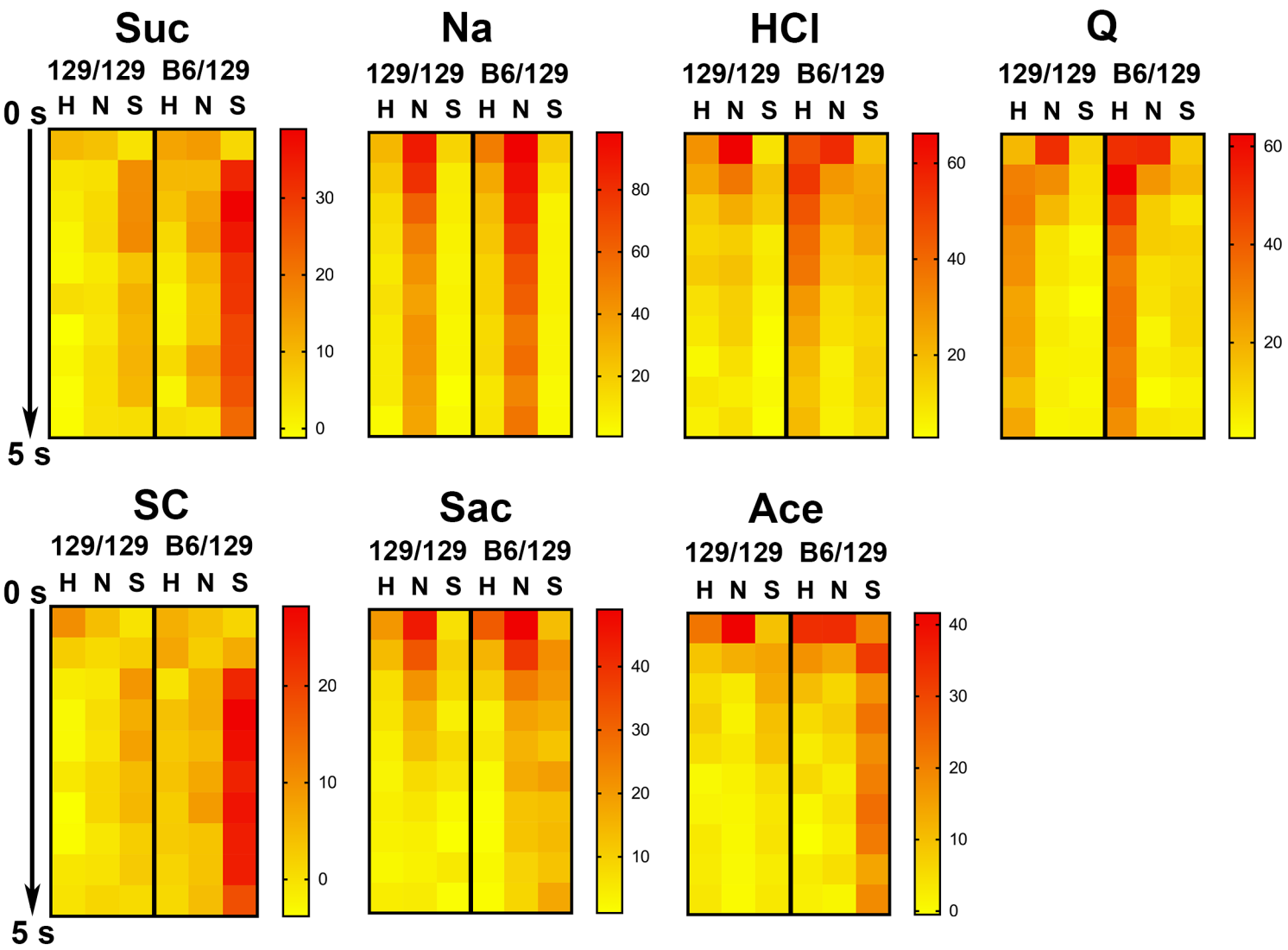

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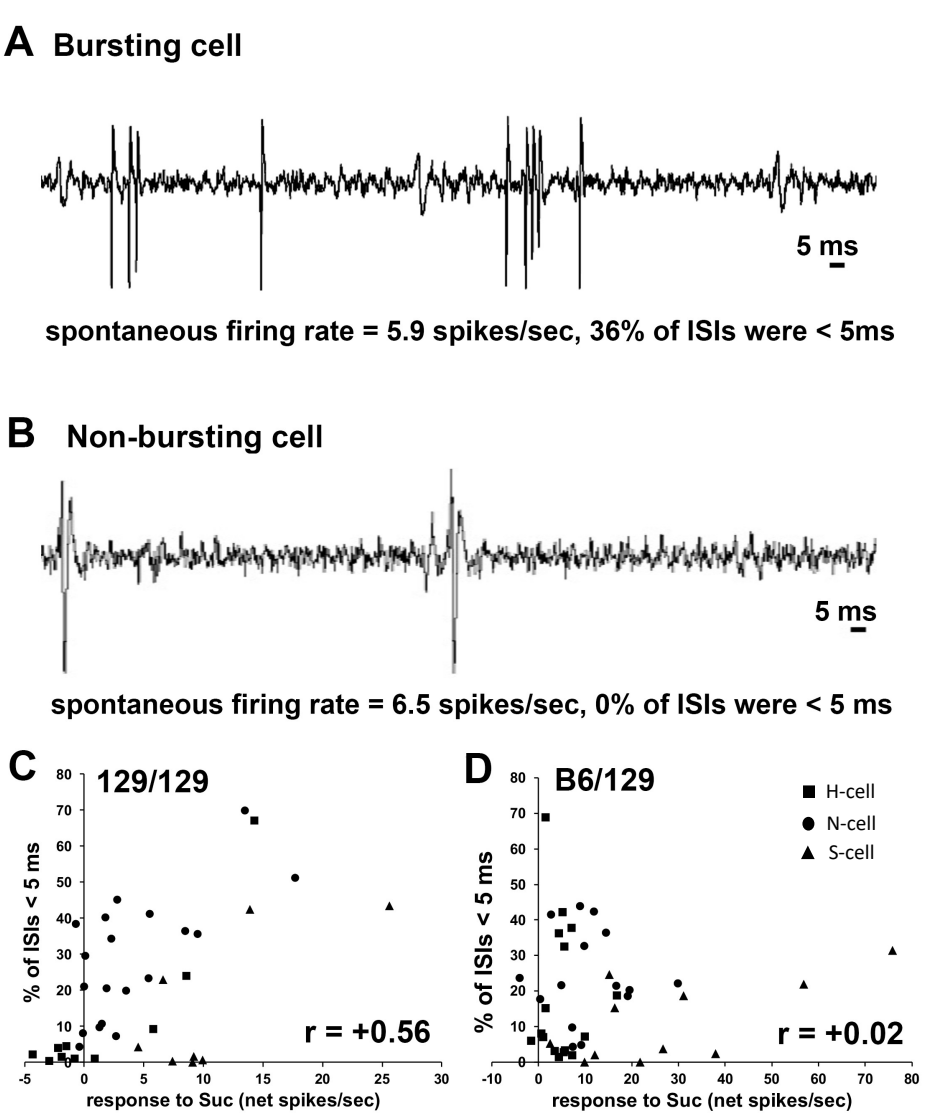

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Table 1. Correlations between response sizes to the prototypical stimuli (in net spikes/s) and the percentage of total interspike intervals that fell within a certain range (either 0–5 ms or 5–10 ms) in 129/129 and B6/129 mice. *, $p < 0.0125$.

	129/129		B6/129	
Stimulus	0-5 ms	5-10 ms	0-5 ms	5-10 ms
HCl	+0.30	+0.13	-0.03	+0.31
NaCl	+0.40	+0.37	+0.31	+0.25
Q	+0.06	-0.06	-0.04	-0.01
Suc	+0.56*	-0.04	+0.02	+0.26