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Neuronal cell types and taste quality coding

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Abstract

Over the past 25 years, there have been two opposing views of how taste information is represented in the activity of gustatory neurons. One view, the across-fiber pattern (AFP) theory, postulates that taste quality is represented by the pattern of activity across the afferent population. Stimuli with similar tastes produce similar patterns of activity. The other view is that activity in a few distinct neuron types codes taste quality in a "labeled-line" fashion. Neurons responding best to sucrose, for example, would represent "sweetness," and those responding best to NaCl would code "saltiness." Some of these neuron types appear to have a biological significance, such as the NaCl-best cells, which receive input about sodium stimuli exclusively from an amiloride-sensitive epithelial ion channel. However, the relatively broad tuning of these neurons makes it unlikely that they are capable of unambiguously coding information about taste quality. Rather, these neuron types play a critical role in establishing unique AFPs that distinguish among taste stimuli. The relative activity across these cell types represent taste quality, much like the patterns of activity across broadly tuned photoreceptors code information about stimulus wavelength.

Keywords: Neuronal cell types; Taste quality coding

1. Introduction

The earliest electrophysiological experiments on single chorda tympani (CT) taste fibers by Carl Pfaffmann [37,38] demonstrated that these peripheral axons often responded to stimuli representing more than one of the familiar taste qualities of salty, sweet, sour, and bitter. These human descriptions of the tastes of sodium chloride (NaCl; salty), sucrose (sweet), hydrochloric acid (HCl; sour) and quinine hydrochloride (QHCl; bitter) have come to dominate our thinking about gustatory information processing and, indeed, clearly shaped early on our ideas about the neural coding of information in this system. It had been Pfaffmann's expectation that taste fibers would respond to one of these "basic" taste stimuli, and that activity in particular neurons or neuron types might code information about different taste qualities. However, these cells were multiply sensitive to different-tasting stimuli and the response of any one fiber was ambiguous with regard to either intensity or quality. That is, a fiber that responded to both NaCl and sucrose might respond equivalently to these stimuli at the appropriately chosen concentrations. Therefore, the activity in that one cell alone could not unambiguously represent taste quality [38].

2. The across-fiber pattern (AFP) theory

To provide an explanation of how taste quality is represented in the nervous system, Pfaffmann [39] proposed an "across-fiber pattern" (AFP) theory, in which taste quality is represented by the relative amounts of activity across the afferent population. In this formulation, if fiber A responds more to NaCl than to sucrose, and fiber B responds more to sucrose than to NaCl, then when activity in A is greater the stimulus is NaCl, and when activity in B is greater the stimulus is sucrose. The absolute amount of activity in each fiber can then represent stimulus intensity—it is only the pattern of activity across the fibers (or central neurons) that is necessary and sufficient for representing stimulus quality. The AFP idea was further championed by Robert Erickson, who gave it a quantitative basis with the introduction of across-fiber correlations as a measure of stimulus similarity [9]. Erickson and his colleagues went on to show that the degree of interstimulus correlation could be used to determine the neural response function for a particular neuron and provide some insight into the neural relationships among the stimuli, resulting in the first description of a "taste space" based on these stimulus relationships [17]. The across-fiber correlations among a number of salts were shown to predict rather strongly the behavioral similarities among them [9,31].

In the 1970s and 1980s, Erickson published a number of theoretical articles based on the AFP idea. In these, he em-

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phasized the notion that sensory information does not reside in the activity of a single cell, but is represented in many systems by the pattern of activity across the responding neural population. He first made the connection between the existence of broadly tuned cells, as in vertebrate photoreceptors or taste fibers, and the coding of information by patterns in nontopographic sensory modalities, such as color vision or taste [10]. Where the activity of cells does not have to represent spatial or topographic information in a point-to-point manner, broadly tuned neurons can serve to code a variety of different kinds of afferent information in their relative patterns of activity. However, even topographic modalities might employ population coding (see article by Miguel Nicolelis in this volume). Erickson postulated that such a pattern code was evident in a number of sensory and motor systems [11,13–15]. The AFP theory accounted well for the neural representation of taste quality, providing a framework for understanding how quality remains constant as stimulus intensity is varied [21].

3. Gustatory neuron types and labeled lines

Although the AFP theory was generally accepted as the explanation for quality coding in taste, the idea of neuron types and their implication for sensory coding in this system was lurking in the background. Many investigators had attempted to classify gustatory neurons, but Frank's [18] description of fiber types in the hamster CT nerve was the first to show the systematic organization of sensitivities across these neurons. Unlike the rat, the anterior tongue of the hamster has a good sensitivity to sweet-tasting stimuli, and a careful examination of the profiles of sensitivity of CT fibers showed that they could be categorized into groups, defined by their "best" stimulus, much like auditory nerve fibers by their best frequency. Frank [18] described neurons responding best to sucrose (S-best), NaCl (N-best), and HCl (H-best) in the hamster CT; only 1 of 79 fibers responded best to QHCl (Q). Later, Q-best fibers were described in both the hamster [23] and rat [19] glossopharyngeal nerve. There was a good deal of homogeneity in the response profiles of fibers within a type, but they were not identical or as uniform as, for example the absorption spectra of vertebrate cones.

Using a similar classification of squirrel monkey CT fibers, Pfaffmann suggested that the activity in particular fiber types might represent stimulus quality in a "labeled-line" fashion [40,41]. This was a marked reversal of his earlier AFP idea, and was triggered primarily by the fact that activity in S-best fibers predicted the relative preference behavior of monkeys toward sucrose and fructose better than did activity in the whole CT nerve [40]. At this point, a controversy was born that has been difficult to resolve because the data available from gustatory afferent neurons can be accounted for under either theoretical framework and there has been no way to rigorously test either theory (see 50).

The best-stimulus classification was applied to neurons at the second- and third-order brain stem taste nuclei of the hamster, and these cells, like fibers of the CT nerve, could also be classified by their best stimulus [54,55]. However, taste-responsive neurons in the hamster brain stem were significantly more broadly tuned than CT fibers [49,54,55]. An analysis of the signal-to-noise ratio in the various best-stimulus neuron types suggested strongly that these cells were much too broadly responsive to function effectively as labeled lines [54]. However, it was apparent that each best-stimulus group of cells contributed a large amount of activity to the response to its "best" stimulus. Van Buskirk and Smith [55] were the first to show that a particular cell type (S-best) played a critical role in defining the similarities among the across-neuron patterns for like-tasting stimuli (sugars). Without the contribution of the most sucrose-responsive cells the great majority of which were S-best, the across-neuron patterns evoked by sucrose and fructose were not at all correlated. Thus, even in the early 1980s it was apparent that some neurons were playing a stronger role than others in defining similarities and differences in the AFPs.

In 1979, Woolston and Erickson [57] correctly noted that any classification scheme imposed by an investigator could result in rather arbitrarily defined neural groups. For the first time in taste, these investigators introduced the concept of numerical taxonomy to the classification of neurons, applying hierarchical cluster analysis to the classification of neurons in the nucleus of the solitary tract (NST) of the rat. Based on responses to anterior tongue stimulation, there was no clear evidence for the existence of neuron types in the rat NST. However, when similar techniques were applied to responses of cells in the hamster NST or parabrachial nuclei (PbN), relatively distinct groups of cells emerged, which corresponded very well to the best-stimulus classifications [51]. A similar analysis of hamster CT fibers also showed well-defined neuron types [20]. Part of the reason for the difference between rats and hamsters was the distinct class of S-best CT fibers in the hamster, which are not prevalent in the rat. Differences between N- and H-best neurons are much less distinct, even in the hamster, and those are the dominant types of neurons seen in the rat CT nerve or NST following anterior tongue stimulation. Nevertheless, these data from the hamster demonstrated quite clearly that neuron types could be defined in the taste system, even using numerical taxonomic methods.

Thus, the issue of gustatory neuron types became entangled in the controversy over pattern coding versus labeled lines. Obviously, if neuron types were merely a figment of an investigator's imagination, they could not serve to code information as labeled lines. On the other hand, the existence of neuron types is not incompatible with a pattern code. In his earliest formulations of the AFP theory, Erickson based many of his ideas on vertebrate photoreceptors, which are broadly responsive across stimulus wavelength, and which code color information by their relative patterns of response [10,11]. Erickson [12] specifically argued that a pattern code could exist with or without the occurrence of

neuron types. Inherent in these early arguments was the idea that if broadly tuned neurons serve to code stimulus information by patterns, then the system must be synthetic as opposed to analytic [12]. That is, in color vision, a mixture of two colors (e.g., red and yellow) often results in the synthesis of a new sensation (e.g., orange), whereas a mixture in an analytic modality would appear to be a mixture (such as sweet and sour). Although this issue is probably far from settled, Erickson and colleagues published several articles suggesting that taste is not entirely analytic, as is pitch perception, and may be more akin to the synthetic senses [8,12,16].

If the coding of taste quality were similar to that of color vision, then much could be learned from what is already known about vision. With that idea in mind, Smith and colleagues examined the roles played by individual gustatory neuron types in defining the across-neuron patterns for various stimuli in responses of the hamster brainstem [50,51]. Following up on their earlier work on the PbN, these investigators showed that eliminating the response of any one neuron type from the matrix of responses to an array of stimuli had specific effects on the resulting AFPs. For example, the distinct AFPs evoked by sodium salts and by nonsodium salts or acids were dependent upon activity in both N- and H-best neurons. If either of these neural groups was missing from the analysis, these stimuli did not evoke distinct AFPs. Similarly, a particular neuron group (e.g., S-best) was necessary to define the similarities among similar-tasting stimuli (e.g., sugars), as shown in the earlier work [55]. These data demonstrated beyond a doubt that these cell types play a critical role in defining the similarities and differences among the AFPs. Thus, the same cells are important for coding taste quality information, whether they are viewed as a "labeled line" or as an essential part of the across-neuron pattern [50]. So, these investigators suggested that the question of taste coding was essentially a philosophical one unless an experiment could be conducted to determine which is correct.

4. Neuron types are biologically significant

In color vision, the existence of color-blind individuals provides a natural test of the role of cone types in color perception. It is well known that the absence of a single photopigment results in the inability of individuals to discriminate among certain wavelengths, specifically those where the absorption spectrum of that pigment overlaps with that of another ([5]; see also [45,46]). The classification of photoreceptors into types was not based purely on their profiles of responsiveness to stimulus wavelength; it was also dependent upon the expression of different photopigment molecules. But in taste, the classification of peripheral axons or central neurons into types has largely been based on their response characteristics. Independent confirmation can help to validate a cell classification scheme [42], and recent data on the effects of amiloride on taste responses has provided additional biological rationale for gustatory neuron types.

There are a variety of transduction mechanisms employed by the gustatory system [25]. One of the mechanisms used to transduce the response to sodium salts is an epithelial ion channel located on the apical membrane of taste receptor cells that allows the passive movement of Na^+ into the cell, resulting in a direct depolarization ([1]; see [2]). This channel is blockable by the diuretic drug amiloride, which has been shown to reduce the receptor potential and the CT nerve response to sodium salts. Interestingly, input from the amiloride-sensitive channel is funneled predominantly into N-best CT fibers [26,32] and NST neurons [44]. However, S-best neurons of the NST also show an amiloride-sensitive response to sodium salts in both the hamster [3,47] and the rat [53]. Although amiloride-sensitive input is provided to both N- and S-best neurons, the responses of N-best cells to NaCl appear to arise solely from the amiloride-sensitive pathway. When amiloride is added during an ongoing NaCl response, activity of N-best cells is completely reduced to pre-stimulus baseline [3,4,26]. Even though H-best and Q-best neurons and peripheral fibers often show robust responses to NaCl, they are completely unaffected by amiloride treatment [3,4,26]. Thus, the amiloride sensitivity of N-best neurons, along with other data such as the specific effects of sodium deprivation on N-best CT fibers or NST neurons [6,7,27], suggests that this neuron type is biologically significant [28].

On the basis of the specific effects of amiloride on N-best cells in the rat NST, Scott and colleagues [44] have suggested that these cells comprise a "coding channel" for sodium taste in the rat. In recent studies on CT fibers in the chimpanzee, other investigators have also suggested that S-best cells code "sweetness" because of their relatively narrow tuning, especially in comparison to taste fibers in rodents [24]. Both of these lines of investigation overlook the basic problem facing the labeled-line approach: the multiple sensitivity of gustatory afferent neurons, especially those in the central nervous system. In such broadly tuned cells, the activity elicited by stimuli with different taste qualities can be interpreted in one of two ways, which are depicted in Fig. 1.

Either the "side-band" activity in a neuron type (e.g., the response to HCl by N-best cells) represents noise (Fig. 1A) or signal (Fig. 1B). If it represents noise, then there is a very poor signal-to-noise ratio in gustatory neurons [54], making it difficult to conceive of how a signal could ever be detected (Fig. 1A). This problem is especially apparent as the concentration of nonbest stimuli is raised because, as noted by Pfaffmann [38,39], a concentration can usually be found where qualitatively distinct stimuli produce equivalent responses in gustatory neurons (see also the discussion of Fig. 5, below). If, on the other hand, such side-band responses represent signal, then the responses of broadly tuned neurons in the rat NST, for example, would suggest that the "basic" taste stimuli, such as NaCl or HCl, have multiple taste qualities to the rat (Fig. 1B). Behavioral experiments, however, flatly refute this implication. Rats trained to avoid one of the basic taste stimuli do not generalize that aversion

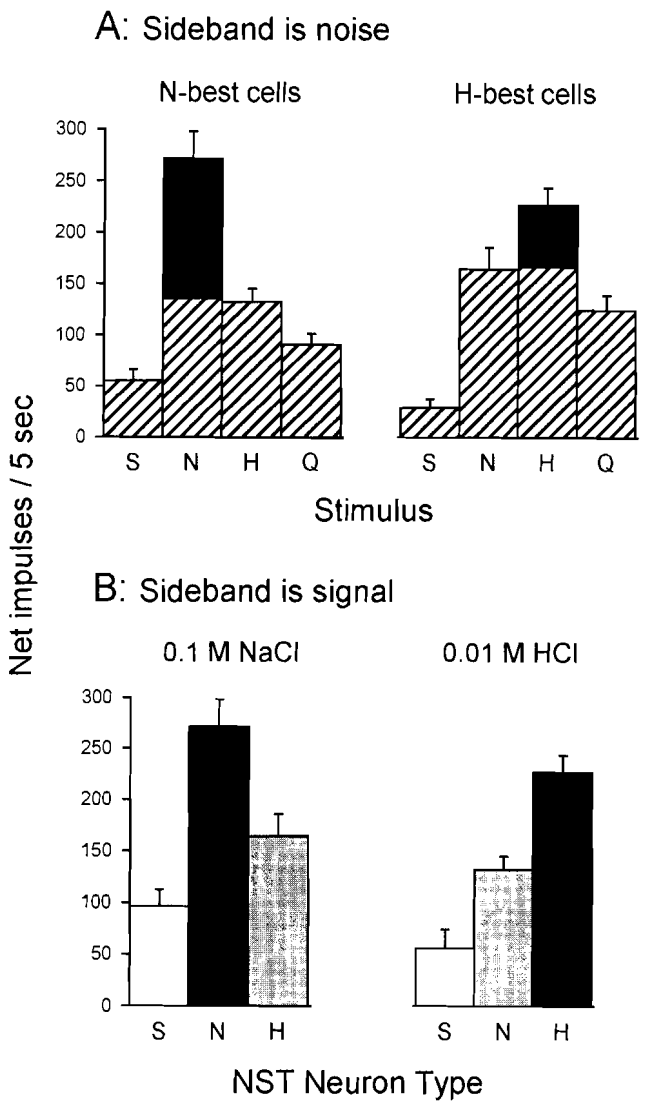

Fig. 1. Schematic diagrams of the implications of a "labeled-line" coding mechanism for the interpretation of the activity in broadly tuned neurons of the rat NST. (A) The mean response profiles for a sample of N-best and H-best neurons in the rat NST. If the activity in a given cell type (N-best or H-best) that is elicited by stimuli other than the "best" stimulus is "noise" (shaded area), then there is very little "signal" (solid area) being provided by these broadly tuned neurons. (B) The mean responses of each of three neuron types (S N, and H-best) in the rat NST to 0.1 M NaCl and 0.01 M HCl. If the response to NaCl in H-best neurons or the response to HCl in N-best neurons is "signal" (gray bars), then NaCl should have a large "acid" taste to rats and HCl should have a large "sodium" taste. Behavioral experiments [33] do not support such a conclusion.

to any of the others [33], even though rats will avoid mixtures of the conditioned stimulus (CS) with other stimuli in proportion to the concentration of the CS in the mixture [48]. Although multiple sensitivity is a greater problem for the rat, chimpanzee CT fibers have not been tested with a range of stimulus concentrations [24], which would be necessary to show that they are not also broadly tuned. Similarly, if the chimpanzee is like other mammalian species, including other primates, its central gustatory neurons are likely to be more broadly tuned than its peripheral fibers [43,55].

The specific effects of amiloride on the response of N-best (and S-best) cells in the NST provides a tool by which the contribution of these cells to the discrimination of sodium salts from other stimuli might be investigated. However, it does not follow that amiloride's disruption of the neural distinction between sodium salts and other stimuli [44,50] or its impairment of a behavioral discrimination between NaCl and KCl [52] is evidence that these cells comprise a labeled line for sodium taste. Such a conclusion would be analogous to concluding that the long-wavelength photoreceptor is a labeled line for "redness." There are several reasons to question such a conclusion. First, amiloride does not only reduce responses in N-best neurons; it has a significant effect on responses to sodium salts in S-best cells as well [3,47]. Further, in the hamster, the substantial response of N-best neurons of the NST to both acids [3] and KCl [4] is blocked by amiloride treatment; KCl responses in the rat NST are also reduced by amiloride ([53]; and see below). In humans, amiloride does not reduce the saltiness of sodium or lithium salts, but rather has a specific effect on their sourness [35,36]. Further, the broad tuning of N-best neurons in the brain stem to sugars and acids raises questions about the signal-to-noise ratio, as discussed above. So we are left with the question of how different neuron types, including the N-best neurons, are involved in the representation of taste quality. These considerations and the data to be presented below suggest that these broadly tuned neuron types represent taste quality by their relative patterns of activity, as suggested by Pfaffmann [38,39] and Erickson [10,11,14,15].

5. Testing the role of neuron types in taste discrimination

To more closely examine the relationship between the effects of amiloride on behavioral and neural discrimination, we conducted a neurophysiological study on rat NST neurons [53] that employed the same parameters used in an earlier behavioral study on the rat [52]. When amiloride was mixed with several concentrations of NaCl and KCl, it disrupted a previously learned two-lever operant discrimination between them in a dose-dependent manner. Amiloride doses ranged from 0 to 100 μM, and a half-maximal suppression in behavioral performance was seen at about 3.9 μM [52]. We recorded the activity of 37 NST neurons in the rat to 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl, and to the three concentrations of NaCl and KCl used in the Spector et al. [52] study (0.05, 0.1, and 0.2 M), mixed with one of three concentrations of amiloride (0, 3, and 30 μM).

The differences in the AFPs across these 37 neurons to 0.1 M NaCl and KCl are depicted in Fig. 2, where the neurons have been arranged along the abscissa into three best-stimulus groups. The first 7 cells on the left were S-best, the

next 21 cells were N- best, and the last 9 cells on the right were H- best, as defined in earlier experiments (e.g., [18]). Mixing amiloride with the stimuli, as in the behavioral experiment (at concentrations of 3 and 30 μM), produced a dose-dependent decrease in the response of the N-best neurons, virtually eliminating the differences in the AFPs produced by these two behaviorially distinct stimuli (Fig. 2C). There was also a small effect on the responses in S-best cells, as shown previously in the hamster NST [3,4]. The response to 0.1 M NaCl was greatly reduced in N-best cells, and the response to 0.1 M KCl was also reduced somewhat by amiloride, as in the hamster NST [4] and rat CT nerve [29]. After treatment with 30 μM amiloride, behavioral discrimination between NaCl and KCl is completely disrupted [52] and the AFPs are virtually identical (Fig. 2C). These results parallel those of Scott and Giza [44] on the rat NST, although these investigators used a much higher dose of amiloride (500 μM). These data and those of Spector et al. [52] are also consistent with those on color-blind individuals, in which the absence of a single photopigment renders them unable to discriminate certain wavelengths along the visible spectrum [22]. These results suggest strongly that activity in N-best (and perhaps also S-best) cells is necessary to establish distinct across-neuron patterns between NaCl and KCl. However, they do not show that activity in the N-best neuron type alone is sufficient for that distinction any more than activity in a single photopigment is sufficient for wavelength discrimination.

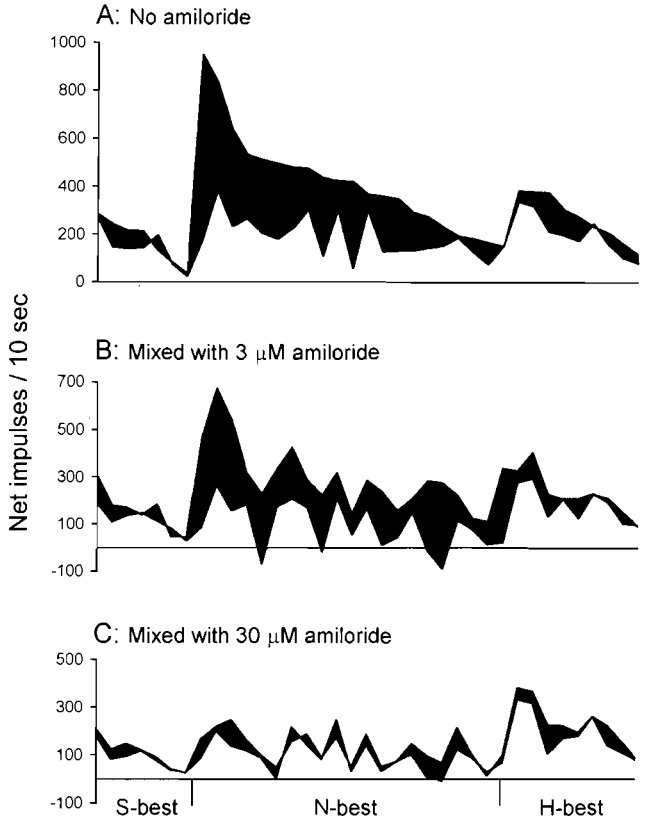

Fig. 2. Differences in the across-neuron patterns of activity in the rat NST evoked by 0.1 M NaCl and 0.1 M KCl when presented alone (A) or mixed with 3 μM (B) or 30 μM (C) amiloride. In general, these cells were more responsive to NaCl than KCl, especially the N-best neurons. The response to NaCl is greatly reduced by amiloride in N-best cells and somewhat reduced in S-best neurons; there is also a reduction of the response to KCl in these neurons, as in the hamster [4]. After 30 μM amiloride, these across-neuron patterns are indistinguishable (C), and rats cannot make a previously learned two-lever operant discrimination between these stimuli [52].

There is no natural condition in taste that is analogous to color blindness. Treatment with amiloride is one way to block or reduce the activity in a certain neuron types (N and S best) and then assess its effects on neural responses and behavior. Responses to NaCl and KCl derive from both an amiloride-sensitive transduction mechanism and an amiloride-insensitive mechanism, the nature of which is not fully understood (see [2]). Ideally, if we could specifically block the amiloride-insensitive transduction mechanism, we could determine whether amiloride-sensitive input alone is sufficient to code the distinction between sodium salts and other stimuli, a necessary requirement to show that amiloride-sensitive cells function as a labeled line for sodium taste (see [28]). There are currently no tools available to effect that condition, although we can mathematically produce a similar result. Suppose we had a drug that could reduce the amiloride-insensitive responses to NaCl and KCl in other cell types to the same degree that amiloride reduces their responses in N-best cells. Then application of that drug could show us the extent to which amiloride-insensitive input to these other cell types contributes to the distinction between NaCl and KCl, or the degree to which amiloride-sensitive activity alone can support that distinction. The results of such a mathematical manipulation are shown in Fig. 3.

In Fig. 3A, the AFPs for 0.05 M NaCl and 0.1 M KCl are shown; these concentrations produce somewhat more equivalent responses in the rat NST than those in Fig. 2. Here, the neurons have been arranged from left to right with the N-best cells on the left and the H- and then S-best cells on the right. With no amiloride treatment, the AFPs for NaCl and KCl are distinct, with an across-neuron correlation of +0.55. Such a correlation represents a clearly behaviorally distinct pair of stimuli (see [9]). The addition of 30 μM amiloride (Fig. 3B) reduces the difference between these patterns, resulting in a correlation of +0.71, which approaches that of pairs of stimuli with similar taste. If the responses to NaCl and KCl in both H- and S-best neurons are reduced mathematically to an extent proportional to the amiloride-insensitive component of their responses, the neural distinction is reduced to a similar degree (Fig. 3C), raising the across-neuron correlation to +0.80.

For this treatment, we assume that the hypothetical blocker of the amiloride-insensitive pathway produces the same effect on H-best cells that amiloride does on N-best cells (i.e., a 75.5% reduction of the response to NaCl using this mixture protocol), whereas it has half that effect (37.75%) on S-best cells. This assumption is based on data from both hamster [3,4] and rat [53], showing that re-

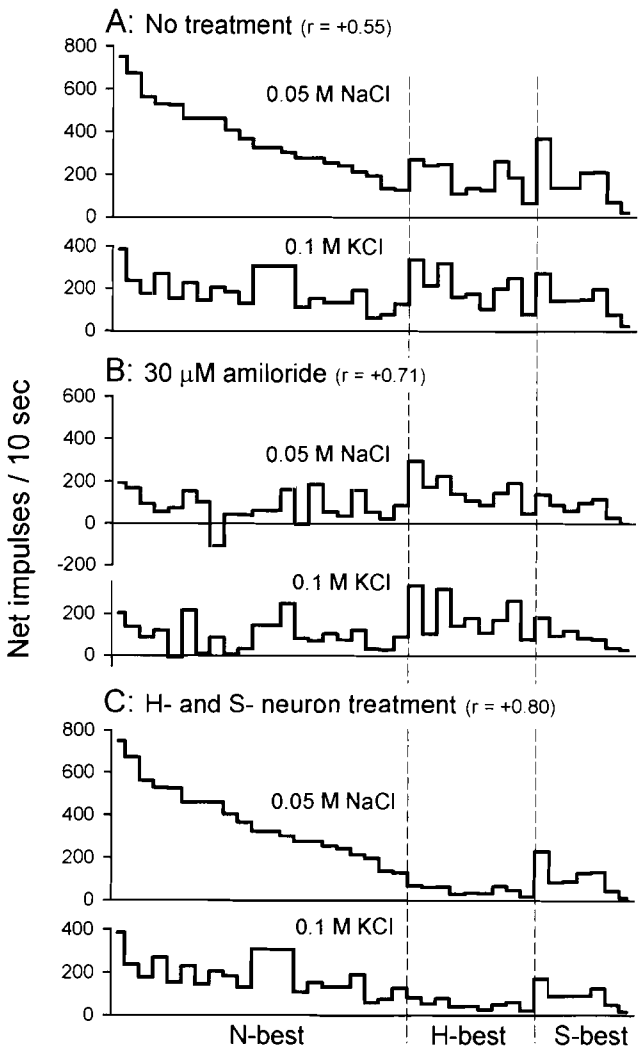

Fig. 3. Across-neuron patterns of activity in the rat NST evoked by 0.05 M NaCl and 0.1 M KCl when presented alone (A), mixed with 30 μM amiloride (B) or after the responses in non-N-best cells have been mathematically reduced to mimic the effects of blocking the amiloride-insensitive pathway (C). In C, the responses to NaCl and KCl were reduced by 75.5% in H-best cells and by half that (37.75%) in S-best cells. The reduction in H-best cells (75.5%) matches the magnitude of the effects of amiloride on NaCl responses in N-best neurons (B) and reflects the proportion of the responses to these stimuli that is not sensitive to amiloride in the H- and S-best neurons ([3,4,53]; see text). Whether the differential responses across neuron types are diminished by amiloride effects on N- and S-best cells or by mimicking a reduction of amiloride-insensitive input to H- and S-best cells, AFPs become less distinguishable, as reflected in the across-neuron correlations.

sponses to NaCl and KCl are completely unaffected by amiloride in H-best NST cells, and that about half of the response to these stimuli is blocked by amiloride in S-best neurons. Although the effect of amiloride is less when it is applied before or mixed with NaCl [44,47,53], as in the present data, the complete inhibition that occurs when amiloride is added to an ongoing NaCl response [3,4,26] suggests that all of the input to N-best cells arises from the amiloride-sensitive transduction pathway. In addition, NaCl and KCl produce comparable responses in the non-N-best neurons [4,53]. Therefore, for this hypothetical treatment, we reduced the responses to both NaCl and KCl in H-best neurons by 75.5% (as in the mixture protocol with amiloride, Fig. 3B) and in S-best neurons by 37.75%, mimicking the effect of a specific blocker of the amiloride-insensitive pathway.

What remains after this hypothetical treatment is largely an intensity difference between NaCl and KCl (Fig. 3C); the AFPs are much more similar over all the cells than in Fig. 3A. Thus, it is not the activity in any given neuron type that is creating the distinct AFPs for NaCl and KCl, but the relative activity across neuron types. Any manipulation that reduces the differential responses of different neuron types, whether in N-best cells or in other cells, results in their across-neuron patterns increasing in similarity. Both amiloride-sensitive and amiloride-insensitive inputs to all these cell types are important for maintaining a neural distinction between NaCl and KCl.

A multidimensional taste space can be used to visualize similarities and differences in the AFPs among a number of stimuli simultaneously; the results of a multidimensional scaling (MDS) analysis are shown in Fig. 4. In this solution, the responses to all stimuli under all conditions were analyzed in a single MDS analysis, but for clarity, the stimulus relationships are depicted separately for each treatment. The responses to the three concentrations of NaCl and KCl (0.05, 0.1, and 0.2 M) before and after treatment with 30 μM amiloride are shown in relation to the untreated (standard) responses to sucrose, NaCl, HCl, and QHCl in Fig. 4A and B. The three NaCl concentrations (triangles) are seen to lie very close to the NaCl standard and the three KCl concentrations (squares) lie between NaCl and HCl and QHCl (Fig. 4A). After amiloride, however, all of the NaCl and KCl concentrations are similar to one another, and quite distinct from untreated NaCl (Fig. 4B). On the other hand, the H and S neuron treatment by the hypothetical blocker of the amiloride-insensitive pathway produced AFPs for all of the NaCl and KCl concentrations that were most similar to the NaCl standard (Fig. 4C). So in either case, whether the stimuli were treated with amiloride or the responses of non-N-best cells mathematically altered to mimic blockage of the amiloride-insensitive pathway, several concentrations of both NaCl and KCl produced indistinguishable AFPs and fell together in multidimensional space.

To further demonstrate that activity in N-best neurons alone cannot distinguish unambiguously between NaCl and KCl, data from cells in the hamster NST are shown in Fig. 5. Here, the responses of 16 N-best neurons to NaCl and several concentrations of KCl, which were recorded in a study on the effects of amiloride on KCl responses in the hamster [4], are shown. The cells are arranged along the abscissa according to their response to 0.01 M NaCl. As KCl concentration increases, the responses of these N-best cells to these two salts become more similar; 0.01 M NaCl and 1.0 M KCl show a correlation within this cell type of +0.92. However, the differential response across the other cell

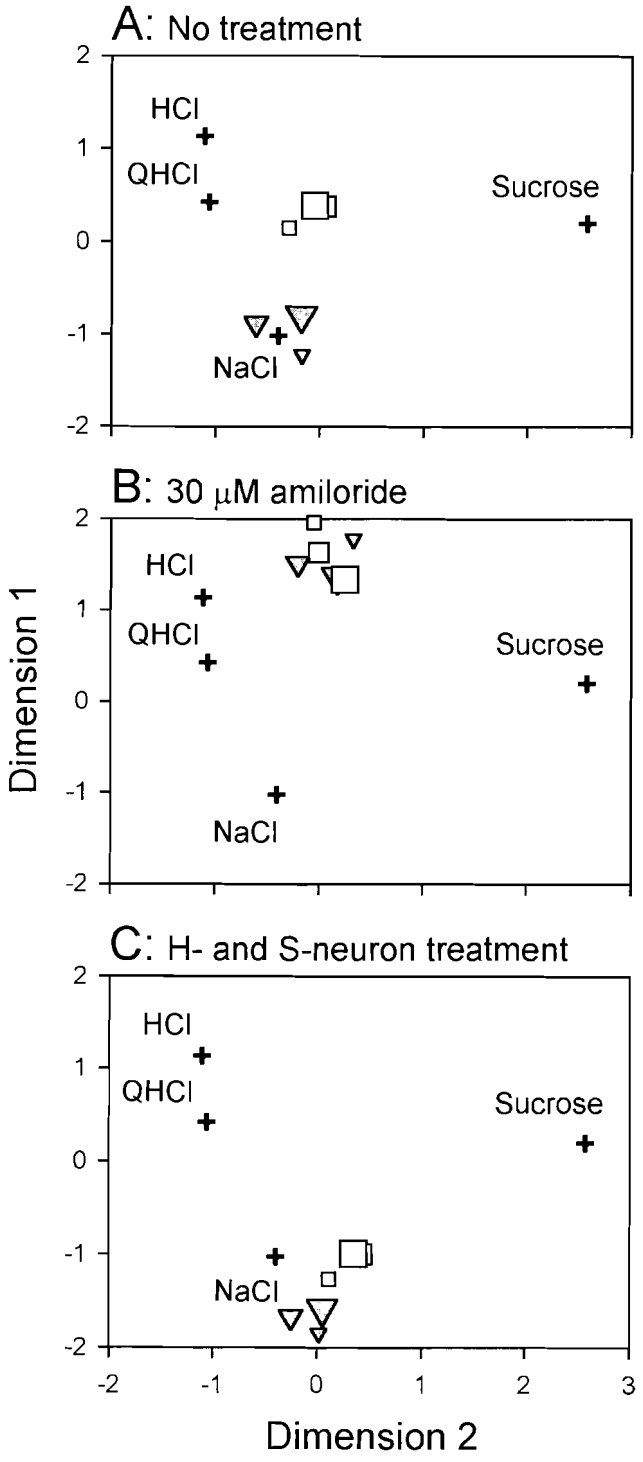

Fig. 4. Multidimensional taste spaces showing the similarities and differences in the AFPs produced by four standard stimuli (0.5. M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.2 M QHCl), indicated by plus symbols, and by three concentrations (0.05, 0.1, and 0.2 M) of NaCl (triangles) and KCl (squares). The size of the triangles and squares indicates the relative concentrations of NaCl and KCl. (A) Stimulus relationships in the absence of treatment. (B) Stimulus relationships after mixing the NaCl and KCl concentrations with 30 μM amiloride, which reduce responses to both stimuli in N- and S-best cells (see Fig. 3B). The standard stimuli were not treated with amiloride. (C) Stimulus relationships after mathematically reducing the responses of non-N-best neurons to mimic the effects of blocking the amiloride-insensitive pathway (see text). A single multidimensional solution was obtained for all stimuli, but the relationships are plotted separately for each treatment condition.

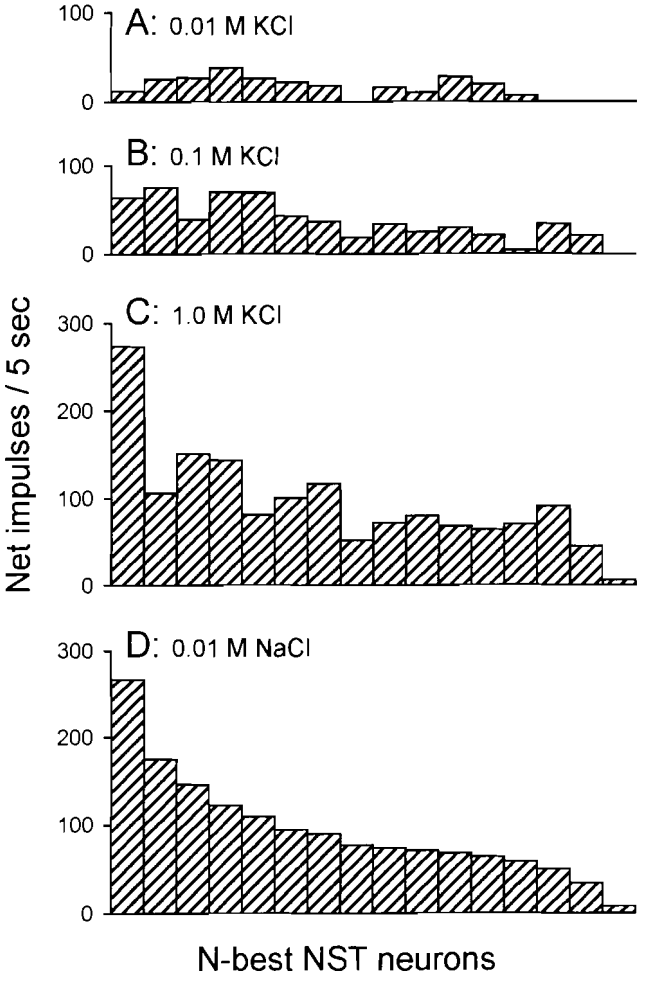

Fig. 5. Responses of 16 N-best neurons of the hamster NST evoked by 0.01 M KCl (A), 0.1 M KCl (B), 1.0 M KCl (C), and 0.01 M NaCl (D) applied to the anterior tongue, which was adapted to distilled water. At the highest concentration of KCl, NaCl and KCl produce similar patterns of activity in N-best neurons ($r = +0.92$), although their overall responses are discriminable because of the differential activity in other neuron types (see [4]).

types, especially the H-best neurons, would allow for discrimination between them. These data show that for a given broadly tuned neuron type, the response to two behaviorally discriminable stimuli, at the properly chosen concentrations, can produce the same response, just as in any broadly tuned single neuron.

In these kinds of analyses, each recorded neuron is treated as though it contributes equally to the neural code for taste quality. Of course, many cells in the NST make only local connections within the medulla, and do not project to the next synaptic relay in the taste pathway, the parabrachial nuclei (PbN; [56]). Therefore, it is conceivable that only some neurons in any sample are directly involved in the representation of taste quality and its projection to higher centers. However, several studies have failed to demonstrate any major differences in the taste responsiveness or breadth of tuning of PbN-projecting neurons in comparison to those that cannot be antidromically activated by PbN

stimulation [30,34]. Thus, we have no reason to assume that we are sampling from NST cells that differ in any significant way from those responsible for relaying information critical for the discriminative aspects of taste.

The arguments presented here directly relate to the neural coding of information about salts. Other classes of stimuli, especially sweet-tasting compounds, could be coded differently. Chorda tympani fibers and NST neurons that are most responsive to sucrose tend to be somewhat more narrowly tuned than N- or H-best cells [20,54], although this narrowness disappears at the level of the PbN [55]. Previous analyses of PbN taste neurons in the hamster have shown that the distinct taste of sweet stimuli is not maintained without a comparison of activity across neuron types. Without the S-best neurons, the AFPs for sweet stimuli are not at all similar and within the S-best cells themselves, sweet stimuli are not distinct from several nonsweet compounds [50]. Neurons that respond best to sweet stimuli often respond well to other qualities, which implies that the ambiguity between quality and intensity discussed above is equally applicable to these neurons. Thus, the arguments presented here for the coding of salt taste are probably relevant to all gustatory qualities.

Conclusions

These data from both the rat and hamster show that although the N-best neuron type is necessary to distinguish NaCl from KCl, it is not by itself sufficient to do so. NaCl and KCl evoke distinct patterns of activity because of their differential effect on more than one neuron type. Because both N- and H-best neurons respond to each of these stimuli and are also modulated by stimulus concentration, the response of any one neuron type alone is ambiguous with regard to both stimulus quality and intensity. This was essentially the same dilemma facing Carl Pfaffmann when he first proposed the AFP theory: the response of any one neuron could not by itself distinguish between two stimuli that activate it. The several considerations raised in this review and these data on the role of neuron types in defining the distinctions between AFPs produced by different stimuli all point strongly to a pattern code as the most viable mechanism for representing taste quality.

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