Chem. Senses 26: 499–505, 2001

Neural Networks Distinguish between Taste Qualities Based on Receptor Cell Population Responses

Brian Varkevisser[1,3], David Peterson[2], Tatsuya Ogura[1,3] and Sue C. Kinnamon[1,3]

[1]Department of Anatomy and Neurobiology and [2]Department of Computer Science, Colorado State University, Fort Collins, CO 80523 and [3]Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, CO 80262, USA

Correspondence to be sent to: Sue C. Kinnamon, Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523, USA. e-mail: sckinna@lamar.colostate.edu

Abstract

Response features of taste receptor cell action potentials were examined using an artificial neural network to determine whether they contain information about taste quality. Using the loose patch technique to record from hamster taste buds *in vivo* we recorded population responses of single fungiform papillae to NaCl (100 mM), sucrose (200 mM) and the synthetic sweetener NC-00274-01 (NC-01) (200 μM). Features of each response describing both burst and inter-burst characteristics were then presented to an artificial neural network for pairwise classification of taste stimuli. Responses to NaCl could be distinguished from those to both NC-01 and sucrose with accuracies of up to 86%. In contrast, pairwise comparisons between sucrose and NC-01 were not successful, scoring at chance (50%). Also, comparisons between two different concentrations of NaCl, 0.01 and 0.005 M, scored at chance. Pairwise comparisons using only those features that relate to the inter-burst behavior of the response (i.e. bursting rate) did not hinder the performance of the neural network as both sweeteners versus NaCl received scores of 75–85%. Comparisons using features corresponding to each individual burst scored poorly, receiving scores only slightly above chance. We then compared the sweeteners with varying concentrations of NaCl (0.1, 0.01, 0.005 and 0.001 M) using only those features corresponding to bursting rate within a 1 s time window. The neural network was capable of distinguishing between NaCl and NC-01 at all concentrations tested; while comparisons between NaCl and sucrose received high scores at all concentrations except 0.001 M. These results show that two different taste qualities can be distinguished from each other based solely on the bursting rates of action potentials in single taste buds and that this distinction is independent of stimulation intensity down to 0.001 M NaCl. These data suggest that action potentials in taste receptor cells may play a role in taste quality coding.

Introduction

The peripheral taste sensory system is responsible for detecting chemicals that represent the five basic taste qualities: salty, sour, bitter, sweet and umami. Taste transduction begins with detection of the appropriate compound by taste receptor cells, which are aggregated into discrete clusters of 50–150 cells called taste buds. Taste buds are housed in connective tissue papillae: the anterior two-thirds of the tongue contains fungiform papillae, each housing one or two taste buds; the posterior tongue contains vallate and foliate papillae, each housing hundreds of taste buds. In taste buds of all papillae the apical microvilli of the taste cells protrude though the taste pore into the oral cavity, where they contact taste stimuli. Binding of a chemical to its corresponding membrane receptor triggers one of several different intracellular mechanisms used for depolarization. In general these can be divided into ionotropic and metabotropic mechanisms, with the former being used for detection of salts and acids and the latter for bitter compounds, sugars and amino acids (Glendinning *et al.*, 2000). In either case depolarization results in the generation and propagation of action potentials along the cell membrane (Roper, 1983), leading to calcium influx and release of transmitter onto gustatory afferent neurons. The presence of action potentials in 'short' primary sensory receptors is unusual and their functional significance remains unclear.

It is generally believed that individual taste cells usually respond to more than one taste quality (Sato and Beidler, 1997). In addition, taste cells may communicate within the taste bud via gap junctions (Bigiani and Roper, 1994, 1995). Evidence thus far has shown that one primary afferent can receive input from several taste cells, though whether or not they need input from this network of taste cells is not known (Miller, 1974; Kinnamon *et al.*, 1988). The relay of information from receptor cell to primary afferent is further complicated by the fact that a single primary afferent can

respond to the application of several different taste stimuli. This observance has resulted in categorizing each primary afferent as 'salt-best' or 'sweet-best' based on the intensity of the response (Pfaffmannn, 1941; Frank, 1973). For instance, a salt-best neuron is one that responds best when activated by salt but may also respond to a slight degree to various other tastes.

In this study we have investigated whether the pattern of action potentials in a single taste bud contains information about taste quality. To address this question we have used the loose patch technique (Avenet and Lindemann, 1991) for recording from single fungiform taste buds *in situ*. This technique allows action currents, reflecting taste cell action potentials, to be recorded from fungiform taste cells in response to several different taste stimuli applied individually over the taste pore. Previously we demonstrated that hamster fungiform taste cells generate trains of action potentials in response to NaCl, acids and sweet compounds (Cummings *et al.*, 1993; Varkevisser and Kinnamon, 2000). Here we compare responses to NaCl and to sweet compounds, based on previous reports indicating that sweet-best and salt-best afferent neurons are more narrowly tuned to their respective stimuli than other primary afferents are to their best stimulus (Contreras and Lundy, 2000). We then used an artificial neural network (ANN) to determine whether population activity in individual taste buds could code taste quality. We hypothesize that: (i) population activity recorded from single taste buds can distinguish between taste stimuli; (ii) rate features of the population activity are sufficient for this distinction.

Materials and methods

Preparation

Golden Syrian hamsters ranging from 4 to 10 weeks of age were killed by CO_2 asphyxiation and cervical dislocation. Their tongues were excised ~4 mm posterior to the fungiform papilla. Experiments were performed using the loose patch technique for recording from taste buds *in situ* (Avenet and Lindemann, 1991). Details of the recording procedure have been described elsewhere (Varkevisser and Kinnamon, 2000). In brief, the technique consists of placing a recording pipette over a single taste pore of a fungiform papilla and recording responses to taste stimuli perfused over the taste pore via an internal perfusion pipette. Responses consist of bursts of action currents, reflecting taste cell action potentials, generated in response to the taste stimulus. The advantages of this system are: (i) taste stimuli are applied directly to the apical membrane, as occurs *in vivo*; (ii) physiologically relevant concentrations of taste stimuli can be used, since the stimulus is restricted to the apical membrane; (iii) the structural organization of the taste bud, including the presence of tight junctions and possible gap junctions, is maintained. In addition, the tongue is not exposed to proteolytic enzymes, which can destroy taste receptors. The principal disadvantage of the technique is that it is not possible to determine the identity of the taste cells that participate in a response. In addition, a recording frequency of 125 Hz may be too slow to capture detailed differences within the bursts and, given the temporal resolution of our stimulus delivery system, we cannot determine exactly when the stimulus reaches the taste bud. Thus, we defined the beginning of a response by the presence of the first burst of spikes following stimulus application.

Concentrations of solutions

The control solution, which also acted as the wash solution, consisted of 30 mM *N*-methyl-D-glucosamine (NMDG) (used to mimic the osmolarity of saliva) and 5 mM HEPES, pH 7.4. All taste solutions were diluted in the control solution. The NaCl concentrations were 0.001, 0.005, 0.01 and 0.1 M. Two different sweet compounds were used: sucrose at 200 mM and NC-00274-01 (NC-01) (NutraSweet Corporation), a high potency artificial sweetener, at 200 μM. The experimental procedure involved three steps: (i) application of taste stimulus lasting, typically, until adaptation of the response; (ii) a wash phase, consisting of the control solution only, lasting >2 min after the signal returned to the previous baseline value; (iii) application of the second taste stimulus. All three taste solutions were used in each experiment. All chemicals were obtained from Sigma Chemical Corp. (St Louis, MO) except NC-01, which was a generous gift of the NutraSweet Corp.

Definition of a response

Baseline noise was present before application of taste stimuli and persisted long after tastants were washed from the preparation. Thus, we describe a response as any activity occurring during the application of a tastant producing spike amplitudes at least 7 SD above the baseline noise. Response time windows were defined by onset of the first spike burst. The response ended with the final spike burst. However, responses often lasted for >30 s, well beyond the time window for detection of the tastant (Halpern and Darlington, 1998). Therefore, we chose to analyze only the first 5 s of the response. Any responses that did not contain at least two spikes within the first second were discarded, as we assumed that the response would be insufficient to encode the taste.

Neural network

Qualitative inspection of the response shown in Figure 1A suggests that a response consists of a sequence of variable amplitude spikes superimposed on some baseline noise. As stated earlier, we assumed that only spikes with amplitudes much greater than the baseline noise were relevant for coding the stimulus. We measured the standard deviation of the response amplitude during the first 100 samples of spike-free data to quantify the level of baseline noise. Then

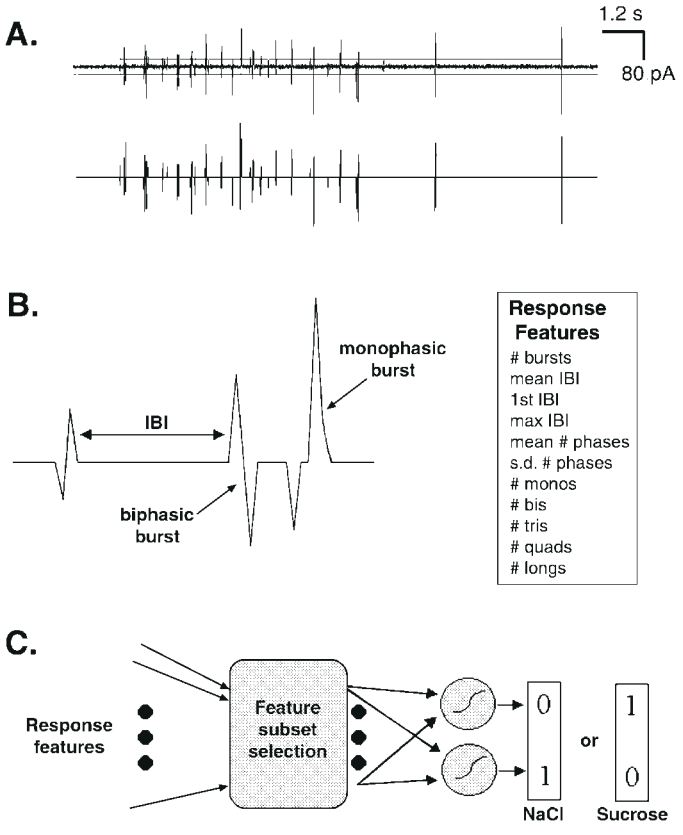

Figure 1 Methods used to quantify taste responses. **(A)** A sucrose response recorded with the loose patch technique; the horizontal lines above and below the top trace represent 7 SD from the baseline. Responses within this window were removed by thresholding, as shown in the bottom trace. **(B)** Individual bursts shown at higher resolution. The window contains four bursts, two monophasic and two biphasic. IBI is the inter-burst interval. In this window the first IBI is also the maximum IBI. Time windows begin at the onset of the first burst. Other definitions of response features include: 1st IBI, value of the first inter-burst interval; mean IBI, average IBI over the window; s.d. # phases, standard deviation of the number of phases; # monos, bis, tris and quads, the total number of monophasic, biphasic, triphasic and quadraphasic phases; # longs, bursts containing more than four phases. **(C)** Artificial neural network used to discriminate between two different taste stimuli. Each discrimination involved a single pair of stimuli, e.g. 0.01 M NaCl versus sucrose.

every sample in the overall response with an absolute amplitude less than 7 SD of the baseline noise was set to 0. This threshold was chosen empirically based on visual inspection of dozens of responses to each of the different tastants applied. We refer to sequences of one or more contiguous spikes as 'bursts' and to each sign change within a burst as a 'phase'. Thus, a burst containing both a positive and a negative spike is referred to as a 'biphasic' burst (see Figure 1B). Note that these bursts reflect activity of the population of taste cells being recorded in the taste bud and should not be confused with fast, repetitive spiking of individual neurons that is more commonly referred to as bursting.

In order to quantitatively compare responses to different tastants we characterized each response by several 'features' (listed in Figure 1B). The variety of inter-burst interval (IBI)-based features are meant to capture both 'rate' and 'temporal' coding in the taste bud population activity. For instance, mean IBI is simply the reciprocal of firing rate, whereas first and maximum IBI are, albeit rather crude, measures of temporal patterning in the activity. Features were computed for 0.25, 0.5, 0.75, 1, 2, 3 and 5 s time windows, all starting at the onset of the first response burst. In other words, we analyzed cumulative time periods rather than moving windows, as in some sensory coding studies (Getz and Akers, 1994). We chose cumulative time periods simply because the information made available to the CNS is cumulative in nature rather than based solely on brief time windows. The disadvantage of using cumulative time periods is that the relative role of response characteristics during different sub-windows can only be indirectly inferred by comparing the response characteristics of multiple cumulative time periods. Features for each response were concatenated to form an input vector for an ANN. We used a neural network merely as a tool to see if the data we gave it was sufficient to distinguish between the different tastes. We did not use a neural network with the intention of modeling the neurobiology of taste coding *per se*. Nor did we attempt to use the neural network to parse single unit activity from the population response. However, neither of these preclude the use of similar networks to model the biological neural network and/or to estimate single unit activity based on the population activity.

We used a simple feedforward neural network with no hidden layer and two sigmoidal output layer units. Preliminary work with a network that included non-linear hidden layer transfer functions showed no incremental benefit compared with the simple linear, no hidden layer network. Thus, any of several traditional linear techniques [e.g. principal components analysis (Lemon and Getz, 2000)] could have been used on this data with similar results. We continued to use the neural network method because of the ease with which we could revert to a non-linear classifier. One disadvantage of the neural network approach, however, is the difficulty of interpreting what the network learns. Our network inputs were simply the individual response features described above for a single specific time window. When we analyzed fewer than the full set of features we used correspondingly fewer inputs to the network. All features were normalized to N(0,1) before presentation to the network.

The network was trained with the Levenberg–Marquardt optimized version of back propagation (Hagan and Menhaj, 1994). We used cross-validation with the data partitioned into randomly selected training, validation and test sets consisting of 80, 10 and 10% of the responses, respectively. Tastant responses were compared in a pairwise fashion. During training we used different binary vectors as the network's target outputs (e.g. 01 for salt and 10 for sucrose). During validation and testing real valued output vectors

were tested for proximity to the binary vectors used during training. The performance metric was simply how well the network could discriminate between the responses to different taste stimuli, i.e. the classification accuracy on the test set.

Results

In this paper we have used the loose patch technique (Avenet and Lindemann, 1991) to record from single fungiform taste buds during stimulation with NaCl, sucrose or NC-01 (an artificial sweetener). From the 87 hamsters tested we obtained 129 responses for the two sweeteners and 183 responses for all concentrations of NaCl. We then used an ANN to determine whether responses to the different stimuli could be distinguished from each other based on certain electrical characteristics of their response, which are listed in Figure 1B. Next, we compared subsets of these features between each of the tastants to determine the major factor in distinguishing between the different tastes. Finally, we varied the concentration of NaCl to determine whether the ability to distinguish between tastes is concentration-dependant and, if so, at what concentration the neural network loses the ability to distinguish NaCl from the two sweeteners.

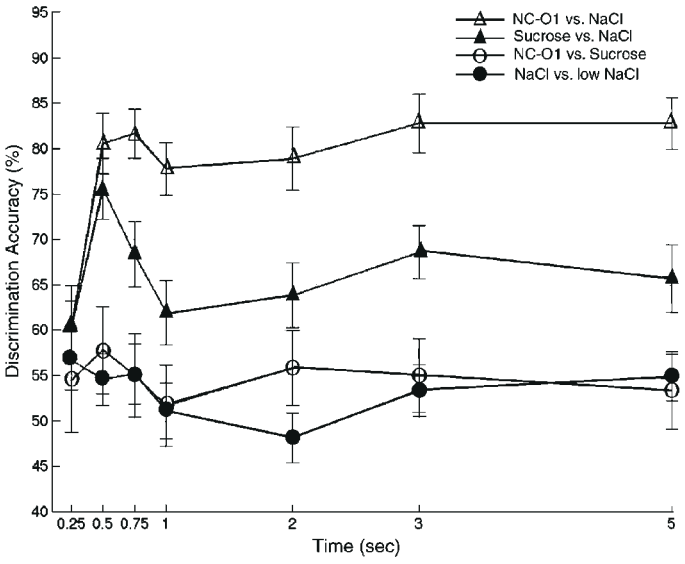

Figure 2 Discrimination accuracy using all features listed in Figure 1. Note that NC-01 and sucrose can each be discriminated from 0.1 M NaCl. For each set of compounds tested the statistical maximum for discrimination occured at 0.5 s. NC-01 cannot be distinguished from sucrose with much greater than chance accuracy, i.e. 50%, and 0.01 M NaCl cannot be distinguished from 0.005 M NaCl.

Discrimination of taste quality using the complete set of features

Pairwise comparisons of NaCl versus NC-01, an artificial sweetener, are shown in Figure 2. The *x*-axis depicts the length of time measured for each response, all beginning with onset of the first spike. The *y*-axis shows the accuracy of the neural network given 30 trials for each test; error bars represent the SEM. Results for NaCl versus NC-01 indicate that the lowest score occurred during the first time interval (0.25 s). This was followed by a sharp increase in the performance of the neural network occurring between the 0.25 and 0.5 s time windows. The performance of the neural network reached its statistical maximum in the 0.5 s time window, at 82%, and continued through the 5 s window.

Comparisons between NaCl and sucrose are also represented in Figure 2. The overall trend is similar to that of NaCl versus NC-01. The lowest score occurred in the 0.25 s time window, with a discrimination accuracy similar to NaCl versus NC-01, while the statistical maximum was reached at the 0.50 s mark, with an accuracy of 76%. Following this point the discrimination accuracy decreased and followed a downward trend to the 1.0 s mark, before leveling off at the ~65% accuracy mark. Discrimination accuracies for sucrose versus NC-01 remained at chance for the entire 5 s tested. These indicate that there is no discernable difference in the patterning of action potentials, using all of the features listed, between sucrose and NC-01. Similar results occurred when comparing two different concentrations of NaCl solutions, 0.01 versus 0.005 M.

Discrimination of taste quality using subsets of features

The success of the neural network depends not on the importance of all of the features combined, but rather it chooses which features have the most success and ignores the others. Thus, a high score is somewhat misleading as it may be due to only one or two of the features given to the neural network. The neural network reduces the influence of those features that are not important in the training phase. In order to determine which features were important for distinguishing between the two tastes we grouped the features into two categories: those describing the firing rate of the response (rate features) and those describing characteristics within each burst (non-rate features). Figure 3 shows the performance of the neural network in deciphering between all three pairwise comparisons when given only those features that correspond to the firing rate of the bursts. The results were similar to those shown in Figure 2. Here the lowest score for all of the comparisons occurred in the first time interval taken. The performance for both sucrose and NC-01 versus NaCl reached a peak of 75% in the 0.75 s window and then leveled off for the duration of the time intervals. Pairwise comparisons between the two sweeteners did score above chance, although this occurred only sporadically. Comparing the two salt solutions showed no improvement over tests utilizing all features (Figure 2). Thus, presenting the neural network only with those features describing the rate of bursting did not hinder the performance of the neural network for comparisons between the sweeteners and NaCl.

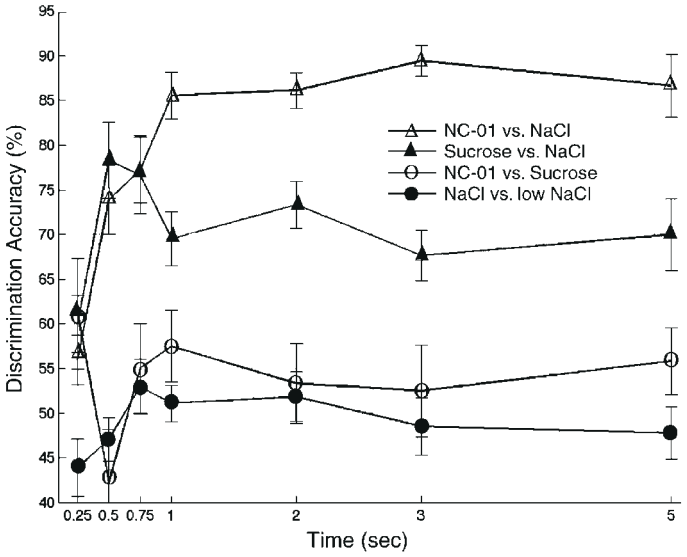

Figure 3 Discrimination accuracy using rate features only (mean IBI and no. of bursts in window). Discrimination accuracies of 75–80% were reached within the 0.5 s time window and the maximum accuracy reached 85% for NC-01 at 1 s. Results for NC-01 versus sucrose and NaCl versus low NaCl remained similar to tests using all features.

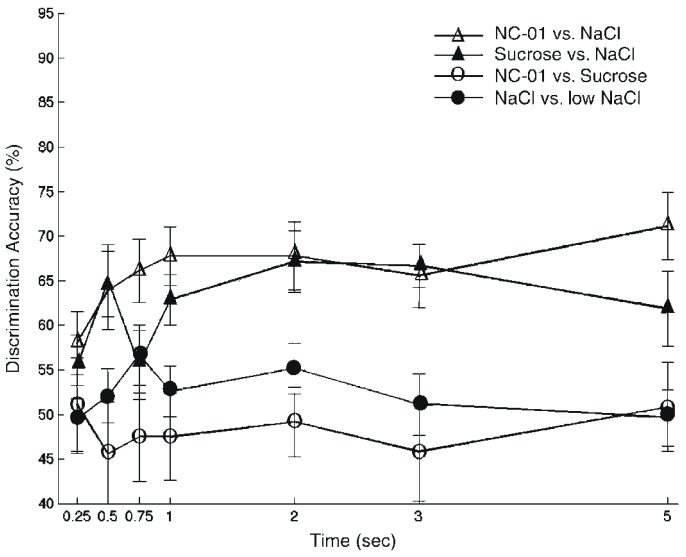

Figure 4 Discrimination using all features except rate features. The result was a large drop in discrimination accuracy at all window lengths tested. Results for NC-01 versus sucrose and NaCl versus low NaCl remained similar to tests using all features.

In fact, the statistical maximums for discrimination accuracy increased for both solutions.

We then presented the neural network with all features excluding those pertaining to the rate of bursting. As shown in Figure 4, the performance of the neural network decreased significantly to a discrimination accuracy of ~70% for both NC-01 and sucrose versus NaCl, which was maintained through most of the time windows tested. Though there was a large drop in results as compared with the performance of the neural network using all features, it should be noted that comparisons remained significantly above chance throughout most of the time windows. Since the results for the non-rate features were poor, a further breakdown and testing of these features was not done.

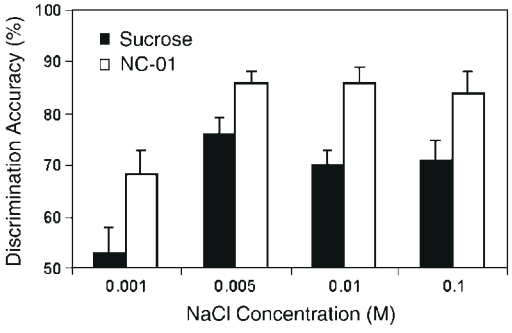

Figure 5 Discrimination accuracy as a function of NaCl concentration using only rate features during the 1 s time interval. Discrimination accuracies remained well above chance through the 0.005 M concentration of NaCl. The largest drop in accuracy occurred at a salt concentration of 0.001 M NaCl.

Dose–response curves

While the data presented thus far suggest that the network can discriminate taste quality using rate features of the response, the role of stimulus intensity must also be considered. This would ensure that any success in the neural network is not due to stimulating the taste receptor cells at a higher intensity for NaCl than for either of the sweeteners. So in the next set of experiments we compared data from each of the sweeteners while varying the concentration of the NaCl solution. We chose to use only rate features in the 1 s time window. As can be seen in Figure 5, four different concentrations of NaCl were each tested against both sucrose and NC-01. The *x*-axis depicts the NaCl concentration tested and the *y*-axis shows the performance of the neural network. There was a high level of discrimination accuracy for both sweeteners versus NaCl at all concentrations with the exception of 1 mM NaCl versus sucrose. Across the 5, 10 and 100 mM NaCl concentrations the performance of the network remained consistent. These data suggest that the network was discriminating quality rather than intensity of stimulus. If the network was discriminating stimulus intensity one might expect performance to degrade gradually as the NaCl concentration was decreased. Although not explicitly tested, a comparison of the sweet responses with the control solution (i.e. 0 M NaCl) should produce 100% accurate discrimination, because by definition the response during the wash included no supra-threshold spikes and would not, therefore, have any non-zero rate feature values.

Discussion

In this paper we have presented data showing that information regarding stimulus quality is present in the firing patterns of taste receptor cells within a single taste bud. More specifically, we have shown that a neural network can discriminate responses to NaCl from responses to either sucrose or NC-01, a synthetic sweetener, at all NaCl concentrations except the very weakest. In addition, the network was unable to discriminate responses to sucrose from responses to NC-01. This suggests that the success of the neural network is due to the differences in taste quality and not simply different chemical solutions. It also suggests that sucrose and NC-01 act on taste receptor cells in a similar fashion. The peak success of the network in discriminating taste quality occurred within the first second of the response. This falls well within the time window needed for detection of the stimulus (Halpern and Darlington, 1998). Further, discrimination tends to fall off in later time windows, suggesting that adaptation to the stimulus may have occurred.

This is the first study that shows that taste stimuli can be discriminated solely based on features relating to the rate of the response and that these features are present in the patterns of action potentials of the taste receptor cells. One theory to explain our data is that there are simply more NaCl-responding taste receptor cells than sweet-responding taste cells. This is, in fact, a possibility, since N-best fibers are more abundant than S-best fibers in the hamster chorda tympani nerve (Frank, 1973). The data on the concentration dependence of the response argues that this is not the basis of the discrimination, since the performance of the net for NaCl versus both sweet stimuli did not vary over NaCl concentrations ranging from 0.005 to 0.1 M. In addition, the network was unable to discriminate responses to high concentrations of NaCl from those to low concentrations, which argues that the pattern of the response is more important than the intensity of the response. Although it is possible that all NaCl-sensitive taste cells are responding to the lowest concentration of NaCl, they are at least doing so at a different rate of response.

It is of interest that at the lowest concentration of NaCl the stimulus could not be discriminated from that of sucrose. This has an interesting psychophysical correlate whereby human subjects received varying concentrations of NaCl and were asked to describe their taste (Bartoshuk *et al.*, 1978). At high concentrations (>0.1 M NaCl) subjects almost unanimously described the solutions as having a salty taste. However, when concentrations were reduced significantly, to ~0.01 M, a substantial number of subjects chose sweet to describe the taste of the NaCl solution given to them.

The hypothesis that information can be found in the form of a 'rate code' isn't new to the chemical senses. Electrophysiological studies have shown that in larval *Manduca sexta* stimulation of the taste receptors by two different bitter compounds resulted in a 2-fold difference in the maximal firing rates of the taste receptor cells (Glendinning *et al.*, 1999). Also, in recent studies examining electrophysiological recordings from both chorda tympani nerve fibers and cortical neurons of the rat gustatory system (Nagai *et al.*, 1992, 1995; Nagai, 2000) an ANN was used to distinguish between different tastants based on the rate of firing of each neuron. The network was successful in distinguishing to a high degree between all four of the taste stimuli tested. The neural network was pruned to eliminate neurons that had a weak firing rate. The absence of these neurons resulted in a significant drop in the ability of the neural network to correctly distinguish sugar from the other four taste stimuli. The results of their studies are consistent with our data showing that responses to taste stimuli can be discriminated based on differences in the rates of firing of the taste receptor cells, i.e. that the average firing rates for the two sweeteners were significantly slower than those for NaCl at all concentrations. The cellular basis for this rate code likely involves differences in the transduction mechanisms for the two types of stimuli: sweet transduction involves G protein-coupled receptors, while NaCl transduction involves direct interaction with ion channels (Glendinning *et al.*, 2000).

That a rate code may encode taste quality at the level of the taste bud has implications for the organization of the taste bud. A typical taste bud contains 50–150 taste receptor cells that are innervated by a small number of nerve fibers, with 3–5 taste receptor cells converging to innervate a single nerve fiber (Kinnamon *et al.*, 1988). With this high degree of convergence some synchronization of activity among taste cells responding to the same stimulus must occur in order for the rate code to be maintained in the afferent fibers. Although the presence of gap junctions has not been verified in mammalian taste buds, gap junctions are clearly present in amphibians (Yang and Roper, 1987). If present in mammalian taste buds, it is possible that gap junctions could provide the electrical continuity required for synchronous activity.

The role of action potentials in taste cells has been a source of spirited controversy since their discovery over 15 years ago. Most primary receptor cells lack action potentials and sub-threshold responses are generally able to mediate release of transmitter from receptor cell to primary afferent neuron. It has been suggested that action potentials code stimulus intensity, but our data would suggest that action potentials also encode stimulus quality. Since both chorda tympani fibers and cortical taste neurons can discriminate between taste qualities based on rate coding, it is possible that the source of this code is the pattern of action potentials in the receptor cells.

Acknowledgements

We thank Dr Takatoshi Nagai for his helpful comments on the

manuscript and Dr Charles Anderson for discussions of the neural network architecture. This work was supported by National Institute on Deafness and Other Communication Disorders grant DC00244 to S.C.K. and by a minority supplement to B.V.

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Accepted January 5, 2001