Sense of Taste in a New World Monkey, the Common Marmoset: 
Recordings From the Chorda Tympani and Glossopharyngeal Nerves

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INTRODUCTION

Callithrix jacchus jacchus is a small New World monkey belonging to the infraorder platyrrhina, which shared ancestry some 38 millions years ago with the catarrhina group to which humans, apes and Old World monkeys belong (cf. Fig. 3 in Glaser et al. 1995). Since then all platyrrhina and catarrhina species have undergone a great deal of differentiation so that extant primates of these two suborders differ in many aspects (Hershkovitz 1977; Nowalk 1991).

C. jacchus is a member of the Callitrichidae family. This family is among the most omnivorous or opportunistic feeders of living primates. Its normal diet consists of large amounts of fruit, leaves, buds, blossoms, green shoots, tree sap, and gums chewed from the bark of twigs and a high percentage of insects and small vertebrates, including eggs (cf. Hershkovitz 1977). Marmosets, like many other arboreal animals, relish the sweet sap or gum produced by trees. They will gnaw on bark, strip or bite off twigs and chew on them. These constituents often contain a high level of tannins, which have a bitter or astringent taste to humans. Thus marmosets can be characterized as omnivorous with a very diverse diet.

In taste studies, a taste quality is often represented by a single compound. Thus sucrose represents sweet taste quality, NaCl represents salt, one of quinine salts represents bitter, and citric or hydrochloric acid represents sour. In our recent studies, we have strived to use a large number of compounds. The main reason for this is that we wanted to address the question of quality and coding in taste not the particular taste of a given compound. To do this, we have to use many compounds whose only common denominator is that they share some taste quality(ies). In doing this, we are well aware of the fact that taste qualities may be limited to the conceptual world of humans.

In regard to the sweet and bitter taste quality, it has shown that the two NG fibers responding to NaCl, we recorded neither suppression by amiloride nor OFF responses. Comparison of marmoset data with the responses in specific groups of taste fibers. Particularly, we have strived to use a large number of compounds. The main reason for this is that we wanted to address the question of quality and coding in taste not the particular taste of a given compound. To do this, we have to use many compounds whose only common denominator is that they share some taste quality(ies). In doing this, we are well aware of the fact that taste qualities may be limited to the conceptual world of humans.

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responding predominantly to bitter stimuli, and S fibers, responding predominantly to sweeteners, were always present; the specificity of the taste fibers, according to human criteria, increased with decreasing phylogenetic distance from human; and the compound that elicited activity only in Q fibers was always rejected and the compound that elicited activity only in S fibers was always liked (Danilova et al. 1998a,b, 1999; Hellekant et al. 1996, 1997a, 1998). However, which compounds give a response in Q and S fibers differ between species. There is a definite phylogenetic component in the ability to taste different compounds as has been shown by many investigators in many species perhaps most convincingly in primates (Brouwer et al. 1973; Diamant et al. 1972; Glaser 1999; Glaser et al. 1995, 1996; Hellekant 1994; Hellekant and Danilova 1996; Hellekant et al. 1974, 1976, 1981, 1985, 1996; Hellekant and Van Der Wel 1989; Hellekant and Walters 1993; Nofre and Tinti 1996).

Sweet or bitter tastes are also segregated on the level of taste receptor cells. They are encoded by activation of different subsets of taste receptor cells within the same taste bud (Nelson et al. 2001). Sweet receptors (T1R) never coexpressed with bitter receptors (T2R) in mice taste buds. Thus there are evidences that peripheral taste sensations are channeled through different sets of taste fibers already at the level of the taste buds.

To further test our idea on taste coding and phylogeny in taste, we present the first article in a series of three from the common marmoset. There is already some information available on sense of taste in marmosets. Some 20 years ago we presented whole chorda tympani nerve recordings and behavioral data to five sweeteners, acesulfame-K, aspartame, D-tryptophan, glycine, xylitol, and thaumatin, in addition to the four basic standards (Hellekant et al. 1981). In that study, we showed that marmosets were unable to taste aspartame and thaumatin. Here we greatly extend that study by presenting single fiber recordings from two taste nerves, the chorda tympani proper (CT) and glossopharyngeal nerves (NG).

Thus the current study is a logical continuation of our investigations of coding in peripheral taste. We wanted to see if a relationship similar to the one outlined in the preceding text exists in a New World monkey. In doing so, we will address both phylogenetic differences in taste by demonstrating that some compounds that taste to us do not taste to the marmoset and reveal if the same major dichotomy into behaviorally positive taste fibers and behaviorally negative taste fibers exists in a platyrrhina primate, i.e., a New World monkey, as we previously observed in Old World primates.

Some of these data have been presented at ISOT XII (Danilova et al. 1998a).

METHODS

Ten male common marmosets (C. jacchus jacchus) weighing 300–380 g were used to obtain neural recordings of the chorda tympani proper and glossopharyngeal nerves. They were part of another project which forced us to euthanize the animals by intravenous injection of pentobarbital sodium (120 mg/kg).

Surgical procedures

Anesthesia was initiated with a mixture of alphaxalone and alphadolone acetate (Saffan) 0.9 ml/animal im. The animals were then intubated and maintained intravenously with pentobarbital sodium in a concentration of 10 mg/ml as needed. Body temperature, heart, and respiratory rates were continuously monitored. Fluid was replenished with intravenous 5% dextrose in lactated Ringer’s solution. We have described the dissection and related methods in two recent overviews (Danilova et al. 2001; Hellekant and Roberts 1995).

Stimuli

Table 1 presents the stimuli and their concentrations used in both nerves. The compounds were chosen to represent the human taste qualities salty, sour, bitter, or sweet. With regard to the artificial sweeteners, we used concentrations equisweet for humans. All compounds except QHCl, which for solubility reasons was dissolved in distilled water, were dissolved in artificial saliva (Hellekant et al. 1997a).

Stimulation

The tastants were delivered to the tongue with an open flow system, Taste-O-Matic, controlled by a computer (Hellekant and Roberts 1995). It delivered the solutions at given intervals, over a preset time, under conditions of constant flow and temperature (33°C). Stimulation time was 5 s. During the CT experiments, the flow was directed over the fungiform papillae. During the NG recordings, care was taken to include the foliate and vallate taste areas. Between stimulations the tongue was rinsed for 55 s with the artificial saliva.

Recording technique

In both nerves, whole nerve and single taste-fiber recordings were obtained. The nerve impulses were recorded with a PAR 113 amplifier, monitored over a loudspeaker and an oscilloscope and fed into a recorder (Gould ES 1000) and into an IBM computer via a DAS-Keithley interface. For whole nerve recordings, the nerve impulses were processed by a smoothed absolute value circuit integrator and changed to a DC potential whose amplitude was related to the nerve impulse frequency, here called the summed response. This signal and a code related to each tastant on the tongue were fed to the computer. The summed response was sampled before, during and after stimulation and displayed on a monitor. The computer also controlled the stimulation times and the order of stimuli.

For single fiber recordings, the nerve was desheathed and teased into fine strands. Each strand was placed on a silver wire electrode held by a micromanipulator. An indifferent electrode was positioned in nearby tissue. The activity of single fibers was recorded with an impulse-amplitude analyzer. It had a window with adjustable upper and lower levels and triggered a pulse when a nerve impulse exceeded the lower but not the upper level. These pulses were processed by the computer. Custom-made software controlled stimulus delivery and stored pulse interval data together with information on the presented stimulus (Hellekant and Roberts 1995). The identity of the stimulus, its order, the level of nerve activity before, during, and after each stimulation as well as other parameters of importance were continuously presented on the computer screen and printed out during the experiment.

Data analyses

As a quantitative measure, we used the integrated area of response during stimulation. The area under the trace was calculated and expressed in arbitrary units. The magnitudes of the summed responses were obtained by deducting the area of spontaneous nerve activity from that during stimulation.

The measure for a single fiber response was the total number of impulses during 5 s of stimulation minus the number of spontaneous impulses recorded during 5 s of the prestimulus period. A fiber was...
TABLE 1. List of solutions used in electrophysiological experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentrations*</th>
<th>Potency in Human Compared with Sucrose</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salts</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.1 M</td>
<td></td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>NaCl + Amiloride</td>
<td>0.1 M + 0.5</td>
<td></td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>LiCl</td>
<td>0.1 M</td>
<td></td>
<td>Mazur et al. (1970)</td>
</tr>
<tr>
<td>KCl</td>
<td>0.1 M</td>
<td></td>
<td>Brussel et al. (1975)</td>
</tr>
<tr>
<td><strong>Acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>40</td>
<td></td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bitter compounds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QHCl</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denatonium benzoate</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aristolochic acid</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sweeteners</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.3 M</td>
<td></td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Acesulfame-K</td>
<td>13.8</td>
<td>140 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Alitame</td>
<td>0.3</td>
<td>2,955 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Ampame</td>
<td>11.9/17.8</td>
<td>50 × (2)</td>
<td>Mazur et al. (1970)</td>
</tr>
<tr>
<td>ASME</td>
<td>6.9</td>
<td>140 × (2)</td>
<td>Brussel et al. (1975)</td>
</tr>
<tr>
<td>Aspartame</td>
<td>5</td>
<td>196 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Brazzein</td>
<td>0.16</td>
<td>2,000 × (2)</td>
<td>Ming and Hellekant (1994)</td>
</tr>
<tr>
<td>CAM</td>
<td>0.18</td>
<td>1,500 × (2)</td>
<td>Nofre and Tinti (1987)</td>
</tr>
<tr>
<td>CAMPA</td>
<td>0.028</td>
<td>15,000 × (2)</td>
<td>Nofre et al. (1996)</td>
</tr>
<tr>
<td>CCGA</td>
<td>0.21</td>
<td>7,000 × (2)</td>
<td>Nofre et al. (1989)</td>
</tr>
<tr>
<td>CGA</td>
<td>0.77</td>
<td>2,700 × (2)</td>
<td>Nofre et al. (1989)</td>
</tr>
<tr>
<td>Cyanosuasan</td>
<td>2.5</td>
<td>650 × (2)</td>
<td>Tinti et al. (1982)</td>
</tr>
<tr>
<td>Scyclamate</td>
<td>9.9</td>
<td>31 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>DMGA</td>
<td>0.027</td>
<td>120,000 × (2)</td>
<td>Nofre et al. (1990)</td>
</tr>
<tr>
<td>D-Phenylalanine</td>
<td>0.1 M</td>
<td>7 × (2.2)</td>
<td>Solms et al. (1965)</td>
</tr>
<tr>
<td>D-Tryptophan</td>
<td>19.5</td>
<td>35 × (0.376)</td>
<td>Shallenberger (1993)</td>
</tr>
<tr>
<td>Dulcin</td>
<td>1.59</td>
<td>250 × (2)</td>
<td>Paul (1922)</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.3 M</td>
<td>1.28 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>MAGAP</td>
<td>0.055</td>
<td>20,000 × (2)</td>
<td>Nofre and Tinti (1994)</td>
</tr>
<tr>
<td>Monellin</td>
<td>0.03</td>
<td>3,000 ×</td>
<td>Morris and Cagan (1972)</td>
</tr>
<tr>
<td>NC-00174</td>
<td>0.23</td>
<td>200,000 × (2)</td>
<td>Nagarajan et al. (1996)</td>
</tr>
<tr>
<td>NC-00351</td>
<td>0.022</td>
<td>30,000 × (3)</td>
<td>Nagarajan et al. (1996)</td>
</tr>
<tr>
<td>NHDHC</td>
<td>0.49</td>
<td>905 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Saccharin</td>
<td>1.6</td>
<td>440 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>SC-45647</td>
<td>0.12</td>
<td>28,000 × (2)</td>
<td>Nofre et al. (1990)</td>
</tr>
<tr>
<td>Stevioside</td>
<td>0.62</td>
<td>120 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Suosan</td>
<td>1.1</td>
<td>950 × (2)</td>
<td>Petersen and Müller (1948)</td>
</tr>
<tr>
<td>Super-aspartame</td>
<td>0.23</td>
<td>3,900 × (5)</td>
<td>Nofre and Tinti (1987)</td>
</tr>
<tr>
<td>TGC</td>
<td>0.17</td>
<td>3,000 × (2)</td>
<td>Tinti et al. (1981)</td>
</tr>
<tr>
<td>Sucralose</td>
<td>0.5</td>
<td>635 × (5)</td>
<td>Dubois et al. (1991)</td>
</tr>
<tr>
<td>Xylitol</td>
<td>0.82 M</td>
<td>0.97 × (5)</td>
<td>Schiffman and Gatlin (1993)</td>
</tr>
</tbody>
</table>

* Concentrations are in mM unless noted otherwise.

considered to be responsive to a stimulus if the nerve impulse rate during the first 5 s of stimulation was larger than 2 times the SD of the spontaneous activity of the fiber.

The four basic stimuli, NaCl, citric acid, QHCl, and sucrose, were used to categorize each fiber by its best stimulus (Frank 1973). The breadth of tuning (H) was calculated according to the formula $H = -K p_i \log p_i$ where $K$ is scaling constant (1.6) and $p_i$ is the proportional response each of four basic stimuli (Smith and Travers 1979).

To detect if there is an organization of the taste fibers, we used hierarchical cluster analysis (SYSTAT for Macintosh). It is a multivariate procedure for detecting natural groupings in data. Hierarchical cluster analysis takes into consideration all responses of a taste fiber. Thus characterization of fibers with many different compounds thoroughly describes the response profiles and helps to find similarities between clusters. The result is usually presented as a dendrogram. Here intercluster similarity was measured using the Pearson correlation coefficients and cluster analysis proceeded according to the average linkage method.

Responses of the fibers belonging to the same cluster were first evaluated by two-way ANOVA on ranked data. This was followed by pairwise comparison of stimuli using Fisher’s least significant differences. Probability <0.05 was considered to be significant. To compare populations of similar fiber groups in CT and NG, we used a Pearson’s $\chi^2$ test.

To visualize similarities between different compounds, we used multidimensional scaling (MDS) analysis (SYSTAT for Macintosh).
MDS computes coordinates of points in a multidimensional space where each point represents a particular stimulus. As a result of MDS, we have a map where the closeness between points reflects similarities between stimuli.

RESULTS

Whole CT and NG nerve responses

Figure 1 presents typical recordings of the whole CT (A) and NG (B) nerve activity. All stimuli elicited a response except NaCl in the NG. Both phasic and tonic components of the responses are visible in both nerve recordings. Another feature in both nerves is the off responses when the citric acid was removed from the tongue by the artificial saliva after stimulation. The same feature was recorded in the CT after NaCl but not in the NG. Interestingly, ethanol was an effective stimulus in the CT at a concentration as low as 5%, while its response was relatively unremarkable in the NG. One difference between the two nerves is that the responses to sucrose were larger than to QHCl in the CT and opposite in the NG.

Single fiber recordings

Figure 2 shows an example of the recordings from single CT taste fiber MA97M18E. We present here only a part of the recordings from this unit. As we can see, this fiber responded to sweeteners. Some high-potency sweeteners, including SC45647 and super-aspartame, elicited good responses in this fiber, whereas others, for example NHDHC, did not stimulate this fiber (not shown). This fiber did not respond during stimulation with NaCl and citric acid and QHCl. However, NaCl and citric acid elicited strong off responses after stimulus was removed. Some of these features will be discussed later.

We recorded responses of 49 CT and 41 NG taste fibers.

**FIG. 1.** Summated chorda tympani (CT, A) and glossopharyngeal (NG, B) nerves activity during taste stimulation of the tongue in a marmoset. The thick horizontal line at the bottom of each recording indicates the onset and end of stimulation. Stimulation time in both nerves was 5 s.
Figure 3 shows overviews of the responses to all compounds in all CT (A) and in NG (B) fibers. The stimuli were arranged along the x axis in order of salt, sour, bitter, and sweet, whereas the fibers were arranged in order of best response to NaCl, citric acid, QHCl, and sucrose along the y axis. The area of the circles represents the impulse activity over the first 5 s of stimulation with the spontaneous activity before each stimulation subtracted. The legend included in Fig. 3 relates circle area with nerve responses. Absence of a mark shows that data are missing. The average spontaneous activity of the 49 CT fibers was 12.4 ± 1.4 and 8.3 ± 5.3 (SD) imp/5 s in the NG nerve. The most striking feature was that the proportion between the number of different fiber types and size of responses was so similar in both nerves; thus the salts gave hardly any responses, the response to acids was modest, whereas bitter and sweet compounds gave large responses.

A closer viewing and a comparison of Fig. 3, A and B, show several features of interest. Both nerves contained a large proportion of QHCl- and sucrose-best fibers. The difference between fibers responding to sweet and bitter is very evident with no overlap between fiber types. Salts gave weak responses; 4 of 49 (CT) and 2 of 41 (NG) responses to NaCl met the criterion. There was only one NaCl-best fiber (MA97F18H in Fig. 3A) in the CT. In the NG, we did not find any NaCl-best fibers.

Table 2 presents results of calculating the breadth of tuning using the best-stimulus classification. The breadth of responsiveness (H) ranges from 0 to 1. It is 0 when a fiber responds to only one of four basic stimuli and 1 when a fiber responds to all four basic stimuli (Smith and Travers 1979). Two CT fibers were not included in calculation of breadth of tuning because they were not tested with all four basic stimuli. Table 2 shows that the S fibers were most narrowly tuned followed by the Q fibers.

Hierarchical cluster analysis

The responses of the CT as well as NG fibers were subjected to hierarchical cluster analysis. We used the responses of 40 CT fibers to 33 stimuli. We did not include in the analysis the responses of nine CT fibers (MA96D18K, MA96D18J, MA96D18N, MA96D10I, MA96D10C, MA96D03C, MA97F18A, MA96D03D, MA96D03I) because they were tested with less than a half of the stimuli. For analysis of NG data, we used the responses of 38 NG fibers (except MA97F11G, MA97J21D, MA97J28E) to all 43 stimuli. The results are represented as dendrograms in Fig. 4. Listed on the left side of the dendrograms is each fiber’s number and response category based on its response to the four standard solutions.

The analysis distinguished three major clusters in both CT and NG: H, Q, and S clusters. The distributions of the Q and S clusters supports the observation in the preceding text, that is, they were very similar in both nerves. Figure 5 illustrates the distribution of the fiber types and shows that the Q and S clusters were almost equally populous in both nerves. The major difference was observed with respect to the H fibers, where the analysis identified 10 H fibers in the CT versus 1 in the NG.

Average response profiles

Figure 6 shows the average response profiles of these three clusters in the CT and the NG. Because we found the same set of clusters in both nerves, we will present in the following each cluster rather than each nerve using subscripts as identification of the nerve. Thus for example, S_CT represents the S fibers in the CT. H clusters. The H_CT cluster consisted of nine citric acid-best fibers. Fiber MA96D18N, responding best to acids and not included in cluster analysis, was used to calculate the average.
FIG. 3. An overview of the response profiles of 49 CT (A) and 41 NG nerve (B) taste fibers of the marmoset. The area of the circles represents impulse frequency over the first 5 s of stimulation. Open circles represent inhibition. Absence of a mark shows that data are missing. The stimuli were arranged along the x axis in order of salt, sour, bitter, and sweet. The fibers were arranged along the y axis in groups: NaCl-, citric acid-, QHCl-, and sucrose-best fibers.
response profile (Fig. 6A). Their average spontaneous activity was 19.3 ± 5.3 imp/5 s.

These fibers were predominantly responsive to acids. Thus citric acid elicited responses in all HCT fibers, whereas aspartic and ascorbic acid stimulated 70% of them. HCl stimulated only two fibers. The response to citric acid can be characterized as tonic, only two fibers showed a phasic component. The sustained level of activity was maintained even after the stimulation, during the first 2–3 s of rinsing.

In general HCT fibers did not discharge during stimulation with other taste qualities (Fig. 6A). These fibers, however, reacted to bitter and salt stimuli in an unusual way. Seventy
percent of H CT fibers showed inhibition of their activity during stimulation with bitter stimuli, mainly QHCl, in a manner shown in Fig. 7. Similar effects were observed during stimulation with salts (described further). Among sweeteners only xylitol elicited significant responses in 70% of H CT fibers, while sucrose and fructose gave small responses in accordingly 40 and 20% of these fibers.

In the NG we encountered only one H NG fiber (Fig. 6D). It strongly responded to all acids. With regard to the other stimuli, only responses to KCl and xylitol met the criterion for a response.

Q CLUSTERS. The average response profile of 15 Q CT fibers (13 QHCl-best and 2 citric acid-best) plus 5 fibers (MA96D18K, MA96D18J, MA96D10I, MA96D10C, MA96D03C), included into this group because they responded best to bitter stimuli, is shown in Fig. 6B. Their average spontaneous activity was 11.3 ± 1.8 imp/5 s.

The average response profile of the Q NG cluster is shown in Fig. 6E. This group consisted of 19 QHCl-best fibers with addition of 2 fibers (MA97F11G, MA97J21D) responding best to bitter stimuli. Their average spontaneous activity was 7.4 ± 1.1 imp/5 s.

Both the Q CT and the Q NG fibers were predominantly responsive to bitter compounds, although their responses to the same set of bitter compounds were quite different. Thus responses to tannic acid and sucrose octaacetate (SOA) were significantly larger in the Q CT fibers, while the responses to denatonium and caffeine were significantly larger in the Q NG fibers. Further, the temporal profiles of Q NG responses to QHCl, caffeine, and SOA were more phasic than those of the Q CT. Finally, OFF responses to one or several stimuli including denatonium, caffeine, aristolochic acid and amphetamine were recorded in 90% of Q NG fibers and only in 30% Q CT fibers.

Among stimuli of other taste qualities acids stimulated both Q CT and Q NG, although to a much lesser extent. There was, however, a significant difference between two nerves in responsiveness to citric acid. All Q CT fibers responded to it. In contrast, only 12 of 19 (57%) Q NG fibers responded to citric acid.

TABLE 2. Breadth of responsiveness for best-stimulus classes of marmoset CT and NG fibers

<table>
<thead>
<tr>
<th></th>
<th>Chorda Tympani (CT)</th>
<th></th>
<th>Glossopharyngeal Nerve (NG)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Fibers</td>
<td>Entropy (H)</td>
<td>No. of Fibers</td>
<td>Entropy (H)</td>
</tr>
<tr>
<td>NaCl-best</td>
<td>1</td>
<td>0.76</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Citric acid-best</td>
<td>12</td>
<td>0.54 ± 0.07</td>
<td>2</td>
<td>0.49 ± 0.32</td>
</tr>
<tr>
<td>QHCL-best</td>
<td>16</td>
<td>0.52 ± 0.05</td>
<td>20</td>
<td>0.36 ± 0.06</td>
</tr>
<tr>
<td>Sucrose-best</td>
<td>18</td>
<td>0.25 ± 0.05</td>
<td>19</td>
<td>0.41 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE.
acid, and the responses were significantly smaller than in $Q_{CT}$. Notice that the responsiveness of the $Q$ clusters to acids contrasted with inhibition of activity in the $H$ clusters by bitter stimuli.

Some sweeteners (acesulfame-K, ampare, d-phenylalanine, NC00351, NC00174, saccharin, stevioloside, and xylitol) stimulated the $Q$ clusters. All these sweeteners, except ampare, were more effective in $Q_{CT}$ fibers than $Q_{NG}$. They elicited strong responses in 60–93% of $Q_{CT}$ and small responses in 14–55% of $Q_{NG}$. The relationship was reversed in the case of ampare which gave robust on and off responses in 92% of $Q_{NG}$ and significantly smaller ON responses in 43% of $Q_{CT}$ fibers.

S Clusters. Figure 6C shows the average response profile of the $S_{CT}$ cluster consisting of 15 sucrose-best fibers, 1 NaCl-best fiber, plus 3 additional sucrose-best fibers (MA97F18A, MA96D03D, MA96D031) not subjected to cluster analysis. The sweeteners were arranged in order of ascending responses. The average spontaneous activity of these fibers was $9.9 \pm 1.5\text{imp/s}$. The $S_{CT}$ cluster responded only to sweeteners (except for the single NaCl-best fiber MA97F18N) and therefore was most narrowly tuned of all clusters (Table 2). Some sweeteners, such as brazzein, monellin, cyclamate, NHDHC, DMGA, and CAMPA, did not stimulate the $S_{CT}$ cluster. Salts, acids, and bitter compounds did not elicit responses in this cluster during stimulation. However, citric and ascorbic acids, QHCl, and salts elicited off responses in 80% of the $S_{CT}$ fibers.

The average response profile of the $S_{NG}$ cluster is shown in Fig. 6F in which the compounds are listed in the same order as in Fig. 6C. It includes 18 sucrose-best fibers plus fiber MA97J28E not included into cluster analysis and responding best to sweet stimuli. Their average spontaneous activity was $9.5 \pm 5.5\text{imp/s}$. The responses of the $S_{NG}$ fibers to sweeteners were very similar to that of $S_{CT}$ and the correlation coefficient between the responses to sweeteners in two nerves was 0.94.

As in CT, $S_{NG}$ was the most narrowly tuned cluster in the NG. The responses to salts, acids and bitter did not meet the criterion of a response except for citric acid, which elicited small responses in 70% of the $S_{NG}$ fibers. This fact reflects in the value of the breadth of tuning (Table 2), which is larger than that of $S_{CT}$. Only 20% of $S_{NG}$ showed off responses. Thus in comparison with $S_{CT}$, a larger percent of $S_{NG}$ fibers responded to citric acid and smaller percent showed off responses after acids, QHCl, and salts.

Responses of CT fibers to salts

Among a total of 90 fibers from both nerves there was only one NaCl-best fiber in CT. Its response to the mixture of amiloride and NaCl was diminished by 50%. Thus it behaved in the traditional way of an N fiber. Among the remaining 89, only 5 fibers met the criterion for a response to NaCl.

We found, however, that 17 of 49 CT fibers showed a complex temporal profile of the activity as a result of stimulation with NaCl, LiCl, and KCl. One or more of the following features were observed: a transient on response followed by inhibition of activity during the remaining stimulation time and then a strong off response after stimulation. Among the H fibers, two fibers showed all three features, four fibers showed the inhibition and the off response, one fiber had only the off response. Two Q fibers and eight S fibers demonstrated inhibition and off responses (an example of an S fiber is shown in Fig. 2). Figure 8 (top) presents examples of this pattern.

The same three features were observed with LiCl and KCl. Fourteen of these 17 fibers showed an off response after KCl and LiCl. From this follows that the most constant feature displayed in all these fibers was the off response after NaCl, LiCl, and KCl.

Relevant to this observation is that the activity after sodium saccharin, sodium cyclamate, and potassium acesulfame showed the same pattern. Similarly the acids elicited off responses in these fibers.

We also studied the effect of amiloride on response to NaCl. Amiloride did not affect the initial transient on responses (if such response occurred) but always eliminated off responses. These effects of amiloride on the off responses after NaCl are shown on Fig. 8 (bottom).

In the NG, the effects of salts and amiloride were different from those in the CT. Only two NG fibers responded to NaCl, LiCl, and KCl and then with a small tonic response with no signs of off response. Amiloride did not affect these small responses.

Multidimensional scaling of stimulus relationship

To investigate the relationship among stimuli, we performed multidimensional scaling (MDS) analysis. It computes coordinates of points in a space. The distances between these points reflect dissimilarities between stimuli. The result of MDS is a map showing dissimilarities between stimuli.

Here, the MDS analysis of the data from 39 CT and 38 NG fibers with 42 stimuli produced the result shown in Fig. 9. The distribution was calculated with the use of Pearson correlation coefficients between the stimuli. The Kruskal stress value was 0.14. The three-dimensional plot shows a large group of sweeteners positioned separate from the rest of stimuli. Some sweeteners are positioned between this group and other taste stimuli. Bitter stimuli are positioned farthest from the sweeteners.

**DISCUSSION**

This study is the first single taste fiber study of a New World primate. To obtain knowledge of species differences and to
address the question of taste coding, we used more than 40 compounds representing four of the human taste qualities. In addition to using a large number of tastants, we recorded from both the CT and NG nerves, a feat rarely done even in regular laboratory species. In the following we will summarize, discuss, and relate our findings in terms of human taste qualities. In doing so, we are aware of the limitation of applying these concepts to animals.
Responses to sweeteners

Sweeteners elicited good responses in the S clusters of both nerves. The S clusters, especially $S_{CT}$, were most narrowly tuned among the six clusters (Table 2). This makes marmosets similar to other nonhuman primates where the S fibers comprised the most specific cluster (Hellekant et al. 1997a,b). The small $H$ value of $S_{CT}$ reflects the findings that the $S_{CT}$ did not respond to stimuli of other taste qualities.

FIG. 6. (continued)
Many of the compounds considered sweet by humans and stimulating S fibers in rhesus and chimpanzee stimulated also S fibers in marmosets. There were, however, some differences. Thus marmoset’s S fibers did not respond to cyclamate, NHDHC, brazzein, and monellin (Fig. 6, C and F). This together with the finding that they were neither preferred nor rejected in TBP tests show that marmosets do not taste these compounds (Danilova et al. 1998a). This indicates that marmosets lack taste receptors for these compounds. In contrast, in rhesus monkey and chimpanzee, cyclamate, NHDHC, brazzein, and monellin stimulated S fibers and were preferred in behavioral experiments (Hellekant et al. 1997a,b).

It is tempting to speculate over the development of the ability to taste the sweet proteins monellin, thaumatin, and brazzein. It is known that African monkeys as well as humans like to eat brazzein fruits. This may reflect evolutionary environmental factors. The plants producing these sweet proteins grow only in Africa where catarrhina live, not in the Americas where platyrrhina primates are found. We think that these plants took advantage of the existence of a particular receptor type that triggers a hedonically positive response in catarrhina primates. By developing sweet proteins, such as brazzein, the plant gained sweetness at less energy cost than by the production of large amounts of sugars. From the point of view of the primate, it consumes the fruit because these proteins elicit a taste similar to sugars that is innately linked to energy source.

With regard to the question as to whether catarrhina primates gained the ability to taste sweet proteins or platyrrhina primates lost it, observations in other species may hint of an explanation. Neither brazzein, monellin, nor thaumatin taste sweet to hamster, pig, rat, and mouse (Hellekant 1976). This suggests that catarrhina primates have gained this ability.

Considering the artificial sweeteners cyclamate, NHDHC, and aspartame, we cannot involve evolutionary factors. The ability to taste them may be explained by a coincidental similarity with other sweeteners. For example, the ability to taste aspartame seems linked to the ability to taste thaumatin.

We have found earlier that DMGA and CAMPA, sweet to humans, in hamsters stimulated S fibers and were preferred (Danilova et al. 1998b). If the finding in hamster applies to all nonprimates, it might suggest that platyrrhina primates lost their ability to taste the sweetness of DMGA and CAMPA rather than a gain by catarrhina primates and hamsters.

No matter what the cause is, identification and comparison of the ability to taste sweeteners could be a new tool to uncover
pathways of primate evolution as we suggested in an earlier study for New and Old World primates (Glaser et al. 1978). Several laboratories have identified two families of taste receptors T1R and T2R (Hoon et al. 1999). The T1R family binds to sweeteners (Bachmanov et al. 2001; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Sainz et al. 2001).
Responses to acids

Acids affected all types of fibers in the CT and NG. In H fibers, acids elicited sustained responses during stimulation and the activity was maintained during 2–3 s of rinsing after the stimulation. Most of the Q fibers also responded to acids, especially to citric acid, with sustained activity during stimulation and a few seconds after stimulation. In many S fibers, we observed a small transient activity at the beginning of the stimulation with citric acid and then an OFF response during rinsing after the stimulation (Fig. 2).

These features were reflected in the summed responses (Fig. 1). OFF responses to acids have also been observed in whole CT recordings of hamsters and rats (DeSimone et al. 1995; Hettinger and Frank 1990) in several nonhuman primates (Hellekant et al. 1981) as well as in isolated taste receptor cells of rat (Lin and Kinnamon 2002).

These broad effects of acids in marmoset taste fibers are not surprising in view of the multiple mechanisms implicated in sour taste. Thus it has been suggested that protons permeate amiloride-sensitive sodium channels (Stevens and Lindemann 2000). Acid-sensing ion channels (ASIC) act as pH-sensing Na channels (Lin and Kinnamon 2002). Lyall and coworkers presented evidence that a decrease in intracellular pH is the proximate stimulus in sour taste (Lyall et al. 2001). Some, or all of the preceding mechanisms may be responsible for the effects of acids recorded here in marmosets.

Responses to salts

Our behavioral data show that marmosets can taste NaCl (to be published). Because N fibers have been implicated in the ability to taste NaCl, the lack of a substantial number of N fibers presents a problem. On the other hand, 35% of the CT fibers reacted to NaCl, LiCl, and KCl with inhibition of activity during stimulation, followed by an OFF response. This OFF response was diminished or eliminated by amiloride.

These characteristics may be responsible for the marmoset’s ability to taste NaCl and can be linked to one of the best-described mechanisms of salt transduction, the permeation of sodium ions through amiloride-sensitive sodium channels (ASSC) (cf. Herness and Gilbertson 1999). First, Gilbertson et al. demonstrated that NaCl at concentrations of 75 and 140 mM caused self-inhibition of sodium permeability in rat ASSC (Gilbertson and Zhang 1998b). Although the time course reported is considerably slower than the one observed here, it is possible that the 100 mM NaCl we used caused self-inhibition and rebound of activity (OFF response) of ASSC when the tongue was rinsed with artificial saliva containing 10 mM Na ions.

Second, ASSC are sensitive to amiloride. Here all the OFF responses to NaCl were amiloride sensitive. Third, Li and protons permeate ASSC. Here our fibers responded similarly to LiCl and displayed OFF responses to acids. Finally, ASSC with high K permeability have been reported in frog taste cells (Avenet and Lindemann 1988) and in “vallate-containing epithelia” in hamsters (Gilbertson and Zhang 1998a). This parallels our observation that K affects these fibers in a similar way as do Li and Na.

As mentioned, we found one NaCl-best fiber in the CT whose response to NaCl was diminished 50% by the addition of amiloride to the NaCl solution. This suggests that an additional mechanism is involved in the Na transduction. In contrast, we recorded neither suppression by amiloride nor OFF responses in the two NG fibers responding to NaCl. This parallels findings in rats, that functional ASSC are absent in vallate papillae (Doolin and Gilbertson 1996).

Responses to bitter stimuli

It is generally thought that bitter taste dominates on the back of the tongue and sweet on the tip. One reason for this is the fact that in many species (rhesus monkey, hamster, mouse, and rat) the NG contains a larger proportion of Q fibers than S fibers and vice versa in the CT nerve (Danilova et al. 1998b; Frank 1991; Hanamori et al. 1986; Hellekant et al. 1997a; Ninomiya and Funakoshi 1989). In many species the ratio of Q fibers in the CT and NG (QNG/QCT) is considerably larger than 1; for example, 6 in rhesus monkey and mouse and 4.5 in rat (Danilova et al. 1998b; Frank 1991; Frank et al. 1983; Hanamori et al. 1986; Hellekant et al. 1997a; Ninomiya and Funakoshi 1989). Here we found that the CT and NG have approximately the same proportion of Q and S fibers. We calculated QNG/QCT to be 1.3, which is about the same as in pig, 1.5 (Danilova et al. 1999). If, as we think, Q fibers connect with T2R-expressing cells, then these taste cells are distributed throughout the tongue. On the other hand, there are probably regional differences in distribution of bitter receptors or in sensitivity of these receptors because we found differences between response profiles of the QCT and QNG.

Compounds that are bitter in humans and elicit only Q fiber activity in marmosets were rejected in behavioral experiments. This supports our idea of an innate connection between Q fibers and hedonically negative reactions. It also suggests a connection between T2R and Q fibers.

Concerning species differences in bitter taste, we had to use a 100-times-higher concentration of denatonium in marmoset than in chimpanzee to evoke a response in Q fibers. The fact that there was no major difference in the stimulating ability of QHCl between chimpanzee and marmoset, however, suggests
that this differences is related to the compound and therefore a specific member of the T2R family and not to a general difference in bitter tasting ability.

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REFERENCES


GILBERTSON TA, ROPER SD, and KINNAMON SC. Proton currents through amiloride-sensitive Na+ channels in isolated hamster taste cells: enhancement by vaspessin and cAMP. Neuron 10: 931–942, 1993.


