Sense of Taste in a New World Monkey, the Common Marmoset.
II. Link Between Behavior and Nerve Activity

Viktoria Danilova and Göran Hellekant
Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, Wisconsin 53706

Submitted 9 December 2003; accepted in final form 1 April 2004

INTRODUCTION

The common marmoset, Callithrix jacchus jacchus, is a South American monkey increasingly used in neuroscience, reproductive biology, infectious disease, drug metabolism, toxicology, and behavioral research (Mansfield 2003; Zulike and Weinbauer 2003). It is phylogenetically less related to humans than Old World monkeys, such as macaques, but in spite of that is often used as an animal model of humans. We have explored its sense of taste in a few previous studies (Danilova et al. 1998a, 2002; Hellekant et al. 1981). In the latest study, we recorded the responses of single taste fibers in the two major nerves supplying the tongue, the chorda tympani (CT) and glossopharyngeal (NG) nerves. Hierarchical cluster analysis identified three clusters of taste fibers: S fibers, responding predominantly to sweeteners, Q fibers, responding predominantly to bitter compounds, and the H fibers responding predominantly to sour compounds.

Our studies in primates as well as in non-primates suggest that acceptance or rejection of a compound is influenced by how much it stimulates S or Q taste fibers (Brouwer et al. 1983; Danilova et al. 1998b; Hellekant et al. 1997a, 1998). It seems that this theory is presently receiving support by the finding of separate sets of receptors responding to either compounds, which we human call sweet and generally like or bitter and generally reject (Nelson et al. 2001; Zhang et al. 2003; Zhao et al. 2003). These receptors, identified in mice and humans, belong to two families of receptors T1Rs and T2Rs and are localized in different cells of the taste buds. Therefore already separated information on the taste quality has the potential to be transmitted through different taste fibers.

Here we hypothesize that if sweet and bitter tastes are conveyed in different sets of nerve fibers, then we should be able to relate a species intake of a compound to the distribution of activity in its S and Q fibers. We demonstrated this in hamster (Danilova et al. 1998b). We also tested this hypothesis in a recent study using S fiber responses as a measure of sweetness (Jin et al. 2003). We found a high correlation (0.76) between the S fiber responses to 28 sweeteners in rhesus monkeys and psychophysical results in humans for the same array of compounds. Here we tested if activity in S fibers was related to intake and activity in Q fibers to rejection using two-bottle preference (TBP) and conditioned taste aversion (CTA) techniques. Although it is implicit that consumption is also influenced by many other factors, including postigestive factors, learning experience, and homeostasis of an animal, we concentrated on the role of S and Q fibers in this study.

A short summary of the results with the TBP method has been published earlier (Danilova et al. 1998b).

METHODS

All animals were housed in the Wisconsin Primate Research Center. The University of Wisconsin’s Animal Care and Use Committee approved all experiments performed with these marmosets.

TBP experiments

A total of 21 adult marmosets of both sexes were used as subjects. Three animals were tested as individuals and 18 as pairs. No distinction between the consumption of the two in a pair was attempted. Thus
the consumption of each pair was considered as one measurement. Table 1 shows the number of cages tested and presentation time for each stimulus.

During the experiments, the automatic water system was turned off, and the animals were presented with two bottles: one with water and the other one with the stimulus. The bottles were presented for 15 min beginning from the moment when the animals had tasted both solutions. If the animals showed no preference, the bottles were left on the cage for 24 h. To eliminate preference for side, the positions of the bottles were switched on the next day, and the bottles were again presented for a 15-min or 24-h period. The concentrations of the 35 sweeteners and 5 bitter stimuli tested are shown in Table 1.

The preference ratio was calculated as the amount of a test solution consumed divided by the total amount of liquid consumed. Thus equal consumption from both bottles resulted in a preference ratio of 0.5; consumption from only the stimulus bottle resulted in a preference ratio of 1. The preference ratios were averaged for 2 days. The results were first assessed using ANOVA. Then a two-tailed t-test was used to compare mean preference ratios with 0.5. A $P < 0.01$ value was considered as a significant level.

### Table 1. List of solutions used in behavioral experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in TBP Experiments</th>
<th>No. of Animals Tested and Duration of the TBP Test*</th>
<th>Concentration in CTA Experiments†</th>
<th>Concentration in Experiments with Lickometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl M</td>
<td>—</td>
<td>—</td>
<td>0.01; 0.025; 0.05; 0.07; 0.1; 0.15</td>
<td></td>
</tr>
<tr>
<td>LiCl M</td>
<td>—</td>
<td>10, 40, 60</td>
<td>0.05; 0.07; 0.1</td>
<td></td>
</tr>
<tr>
<td>KCl M</td>
<td>—</td>
<td>15, 50, 70</td>
<td>0.01; 0.05; 0.07; 0.1</td>
<td></td>
</tr>
<tr>
<td>Acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid, mM</td>
<td>—</td>
<td>30, 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid, mM</td>
<td>—</td>
<td>15, 50, 70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid, mM</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrochloric acid, mM</td>
<td>—</td>
<td>3, 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitter compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QHCl, mM</td>
<td>5</td>
<td>1 (24)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Denatonium benzoate, mM</td>
<td>1</td>
<td>2 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aristolochic acid, mM</td>
<td>0.01</td>
<td>1 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOA, mM</td>
<td>1.1</td>
<td>7 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannic acid, mM</td>
<td>0.02, 0.2, 0.5, 2</td>
<td>9 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweeteners‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ace-K, mM</td>
<td>13.8</td>
<td>10 (15)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Alitame, mM</td>
<td>0.3</td>
<td>10 (15)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Ampame, mM</td>
<td>11.9</td>
<td>10 (15)</td>
<td>11.9, 17.8</td>
<td></td>
</tr>
<tr>
<td>ASME, mM</td>
<td>6.9</td>
<td>10 (15)</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Aspartame, mM</td>
<td>5</td>
<td>9 (15)</td>
<td>5, 10</td>
<td></td>
</tr>
<tr>
<td>Brazzein, mM</td>
<td>0.0015</td>
<td>11 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM, mM</td>
<td>0.18</td>
<td>10 (15)/8 (24)</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>CAMPA, mM</td>
<td>0.028</td>
<td>10 (15)/9 (24)</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>CCGA, mM</td>
<td>0.21</td>
<td>10 (15)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>CGA, mM</td>
<td>0.77</td>
<td>11 (15)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Cyanoosuan, mM</td>
<td>2.5</td>
<td>10 (15)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Cyclamate, mM</td>
<td>9.9</td>
<td>11 (15)</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>DMGA, mM</td>
<td>0.027</td>
<td>8 (15)/8 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Phenyllalanine, M</td>
<td>0.1</td>
<td>8 (15)/8 (24)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>D-Tryptophan, mM</td>
<td>0.01</td>
<td>11 (15)</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>Dulcin, mM</td>
<td>1.59</td>
<td>11 (15)</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td>Fructose, M</td>
<td>0.3</td>
<td>10 (15)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Glucose, M</td>
<td>0.5</td>
<td>10 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine, M</td>
<td>0.89</td>
<td>11 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose, M</td>
<td>0.6</td>
<td>10 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGAP, mM</td>
<td>0.055</td>
<td>10 (15)</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Monellin, mM</td>
<td>0.003</td>
<td>11 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC-00174, mM</td>
<td>0.23</td>
<td>10 (15)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>NC-00351, mM</td>
<td>0.22</td>
<td>10 (15)/8 (24)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>NHDHC, mM</td>
<td>0.49</td>
<td>7 (15)/8 (24)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Saccharin, mM</td>
<td>1.6</td>
<td>11 (15)/11 (24)</td>
<td>1.6, 3.2</td>
<td></td>
</tr>
<tr>
<td>SC-45647, mM</td>
<td>0.12</td>
<td>11 (15)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Stevioside, mM</td>
<td>0.62</td>
<td>11 (15)/8 (24)</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Sucralose, mM</td>
<td>0.5</td>
<td>10 (15)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Sucrose, M</td>
<td>0.3</td>
<td>11 (15)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Sucrononic acid, mM</td>
<td>0.942</td>
<td>10 (15)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Suosan, mM</td>
<td>1.1</td>
<td>10 (15)</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Super-aspartame, mM</td>
<td>0.23</td>
<td>11 (15)/11 (24)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>TGC, mM</td>
<td>0.17</td>
<td>10 (15)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Xylitol, M</td>
<td>0.82</td>
<td>10 (15)</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

*Number in parenthesis shows a duration of the TBP tests (15, bottles were presented for 15 min; 24, bottles were left for 24 h). When both 15-min and 24-h tests were performed, Fig. 1 presents results of the 24-h test. †Sucrose 0.3 M was used as a conditioned stimulus. ‡Potencies of the sweeteners in humans compared with sucrose were presented in (Danilova et al. 2002).
Conditioned taste aversion experiments

Twenty-four naive adult marmosets of both sexes, housed as pairs, were used as subjects. They were separated only during the conditioning (~1 h) and the tests (2–10 h). The animals were first trained to drink water from a lickometer, a device that measures consumption as a number of licks from 16 different bottles mounted in a carousel. Each lick broke an infrared beam positioned between the animal and the bottle in use and triggered one count by the computer. The first lick started a timer that limited the presentation to 30 s. On completion of training the animals drank only from the lickometer.

The marmosets were then offered a 0.3 M sucrose solution (conditioning stimulus). After consumption of the sucrose, 3 ml 0.3 M LiCl (unconditioning stimulus) was injected intramuscularly within 30 min. After a 1-day rest, we tested if the animals were successfully conditioned. If the consumption of sucrose was suppressed to a level <10% of water consumption, the animals were considered conditioned. If their consumption of sucrose was not suppressed, LiCl was administered again. No animal needed more than two injections. The control animals underwent the same routine but with NaCl injections.

We obtained data from 13 control and 11 conditioned animals. In each series, the marmosets were tested with three cycles of presentations of 16 taste stimuli, including distilled water and sucrose. The presentation order within cycles was randomized. We ran four series of experiments: two different series with a single concentration of 30 sweeteners, one concentration series with 3 sweeteners and one series of acids. Because multiple presentations of sucrose and other sweeteners attenuate the reflex, each conditioned animal participated only in two series, whereas most of the control animals participated in every series.

Because the level of drinking differed between animals, the consumption of sweeteners was normalized to a “standard.” For each animal, we considered the average number of licks of water during three cycles as the standard and assigned it the value 100%. Then for each marmoset, consumption of all stimuli was expressed in percent of this standard. The data were then averaged for the control and conditioned animals.

Using a t-test for independent samples, we analyzed the difference in consumption by control and conditioned animals. A P < 0.01 value was considered as a significant difference in the statistical analysis.

Lickometer tests with salts

Five naive marmosets were tested in this series. The lickometer and the training procedure were the same as in the previous CTA series. The marmosets were offered six concentrations of NaCl, three of LiCl, and four of KCl. As controls, 20 mM citric acid, 0.1 M sucrose, 2.5 mM QHCl, and water were included. Each stimulus was presented three times in random order. The consumption of stimuli was also expressed as percent of water consumption.

RESULTS

Results of TBP tests

Figure 1 presents the result of the TBP tests with the compounds arranged in an order of increasing preference. The stimuli can be divided into three groups. The first group was composed of compounds whose preference ratios were significantly lower than 0.5, which means that they were rejected. These included, as expected, five bitter compounds but also two compounds generally considered as sweeteners, d-phenylalanine and steviol.

The preference ratio of the compounds in the second group did not significantly differ from the 0.5 level, which means that they were neither preferred nor rejected. This group included N-(4-azido phenyl)-N’(diphenylmethyl)guanidinocacetic acid (NC00351); N-3,5-dichlorophenyl-N’-(S)-a-methylbenzylguanidinaceacetate (DMGA); neohesperidin dihydrochalcone; ampame (L-aspartyl-(R)-a-methylphenethylamine (NHDHC); saccharin; brazzein; aspartame; ampame; N-4-cyanophenylcarbamoyl-(R,S)-3-amino-3-(3,4-methylenedioxyphenyl) propionic acid (CAMPA); N-4-cyanophenylcarbamoyl-(S)-a-methylbenzylguanidinaceacetate (DMGA); neohesperidin dihydrochalcone; ampame; super-aspartame; L-aspartyl-(R)-a-methylphenethylamine (ASME); and monellin. These compounds are all sweet to humans, some very sweet.

The compounds of the third group included the 20 sweeteners the preference ratios of which were significantly higher

![FIG. 1. Results of 2-bottle preference (TBP) tests. Concentration, number of animals tested, and presentation time for each stimulus are shown in Table 1. Error bars are SE. *, significant differences from the preference ratio 0.5; ---, preference ratio level 0.5.]
than 0.5, and therefore these compounds were preferred over water.

Results of CTA tests

Figure 2 shows the consumption of sweeteners by 10 control (●) and 8 conditioned (□) marmosets. It shows the number of licks for each compound expressed in percentage of number of licks for plain water. The sweeteners are arranged in order of increasing consumption by the control group. The level of consumption of sweeteners in the control group can be considered as a measure of liking. The vertical lines on the Fig. 2 separate three groups of compounds that the control animals consumed significantly less than water and therefore did not like, consumed at the same level as water, and consumed at the >100% level and therefore preferred by the control animals.

Spearman correlation coefficient was 0.78 (P < 0.01).

In the conditioned group, sucrose was the least-liked stimulus. Its consumption dropped to 7% of water intake, indicating successful conditioning. Intake of all the compounds consumed more than water by the control group was significantly decreased to 7–30%, indicating that they had a sucrose-like taste quality. Furthermore, in the second group consumption of aceulfame-K, SC45647, NC0035, sucralose, suosan, and CAM was significantly decreased, revealing a sucrlose-like component in their taste. The consumption of the rest of the sweeteners did not significantly differ between the control and conditioned groups.

To see if the concentrations of saccharin, aspartame, and ampame were too low for a preference, we tested several higher concentrations than in Fig. 2. Then the control group consumed these compounds significantly less than water, indicating a nonsweet or aversive taste. In the conditioned group, there was no significant change in consumption (data not shown).

Results of CTA experiments with acid series

Figure 3 shows the results of the experiments with acids and acid/sucrose mixtures in nine control and two conditioned marmosets. The control and conditioned animals gave similar results with acids. At low concentrations, acids were neither preferred nor rejected, but at high concentrations, acids were significantly rejected.

The results of experiments with sucrose/acid mixtures differed between the control and conditioned groups. In the control group, addition of sucrose made acids less aversive and more likeable. The level of consumption of these mixtures
depended on the concentrations used. Although mixtures of high acid/low sucrose were as aversive for the control animals as acid alone, they increased consumption of mixtures with high sucrose/low acid content.

In the conditioned animals, the consumption of all acid/sucrose mixtures was as low as consumption of acids alone. This indicated that marmosets distinguished the taste of sucrose in the mixtures and avoided it.

Results of lickometer tests with salts

Figure 4 shows relationships between concentration and level of consumption for three different salts, NaCl, LiCl, and KCl. The results demonstrated that marmosets tasted all three salts and their consumption significantly decreased with an increase of concentration. The response-concentration curves were also calculated and the data fit a logarithmic curve quite well. The equation for each salt is given in the graphs. The curve fit correlation coefficients for the three graphs in Fig. 4 are 0.86, 0.92, and 0.93, respectively.

As controls we included 0.1 M sucrose, 2.5 mM QHCl, and 20 mM citric acid. The percentage of consumption for sucrose was 144 ± 35.2, for QHCl 16.3 ± 4.4, and for citric acid 12.0 ± 5.1 (means ± SE). This shows that there was no difference between these animals and the CTA controls.

DISCUSSION

With this and our previous studies we continue our comprehensive review of the taste system in marmosets (Danilova et al. 1998a, 2002; Hellekant et al. 1981). In the following, we compare the behavioral findings with the results we previously
recorded in single taste fibers. By combining these methods, we can estimate quite well how a compound may taste to marmosets.

We applied the TBP method in both short (15 min)- and long-term (24 h) tests. Because the behavioral reaction might be influenced by postingestive effects, the data on preference for as many of the sweeteners as possible were obtained in short-term experiments. However, if the compound was not preferred in the 15-min test, it could indicate that either the monkey did not distinguish the taste of the compound (it tasted like water) or the monkey was not attracted to its taste (it was either aversive or neither attractive nor aversive). To force the animal to make a choice, we left the bottles for 24 h. The results were divided into two alternatives: either the animals rejected the compound (it had aversive taste) or they consumed the compound as much as water (it tasted like water or was neither attractive nor aversive). We also used the level of consumption by the CTA control group to add information to our short-term TBP data. In the CTA tests, we made the assumption that if the taste of sucrose is generalized to the compound in question, then it has a sucrose-like taste component.

Table 2 summarizes the results of the electrophysiological, TBP, and CTA experiments. The first column shows the result of our earlier electrophysiological experiments with the same compounds and concentrations as used here (Danilova et al. 2002). We discuss the compounds according to the types of fibers they stimulated or did not stimulate.

Compounds that did not stimulate any CT and NG fibers

Previously we found that seven sweeteners, ASME, brazzein, CAMPA, cyclamate, DMGA, monellin, and NHDHC, did not elicit statistically significant responses in both taste nerves. Here the marmosets showed neither preference nor rejection of these compounds in TBP tests and the consumption of ASME, CAMPA, cyclamate and NHDHC by the control as well as the conditioned animals did not differ from their consumption of water in the CTA tests (brazzein, DMGA, and monellin were not tested in CTA experiments because of lack of compounds). Our TBP results corroborate earlier behavioral experiments in marmosets, which showed no preference for several of the sweeteners used here including ASME, aspartame, CAMPA, cyclamate, and monellin (Glaser et al. 1978, 1995, 1996; Nofre et al. 1996). Taken together the preceding suggests that these compounds in concentrations used here have no taste to marmosets.

This conclusion is interesting because all these seven compounds are sweet to humans. Furthermore, in the Old World primates (rhesus monkeys and chimpanzees) brazzein, cyclamate, monellin, and NHDHC stimulated S fibers and were preferred in behavioral experiments (Hellekant 1997a,b).

Aspartame has to be considered separately. It is commonly used as a sweetener in human consumption. In the rhesus monkey and chimpanzee, 5 mM aspartame stimulated S fibers very effectively and was preferred. In the marmoset, although the average response of 11 S and 15 Q fibers to 5 mM aspartame did not meet the criterion for a response, some fibers (6 S and 6 Q) responded. The finding that some S and Q fibers responded to aspartame suggests a complex taste where its sucrose-like taste is counterbalanced by an aversive taste. The small response of the S fibers suggests that the sensitivity of the New World marmoset’s sweet receptors to aspartame is much lower than that of Old World primates.

It is interesting that CAMPA and DMGA (which did not activate marmoset’s taste fibers and were not preferred) are sweet to humans and in hamster stimulate S fibers and are preferred (Danilova et al. 1998b). Similarly, cyclamate, which gave neither electrophysiological nor behavioral responses in marmosets, is sweet to humans and Old World monkeys and has an attractive taste to mice (Bachmanov et al. 2001). Another example of species differences in taste relates to ω-proline. It is bitter-sweet to humans and not preferred by marmosets, but preferred by some mouse strains (Bachmanov et al. 2001; Haefeli et al. 1998; Schiffman et al. 1979). These examples show that the evolution of the sense of taste may follow paths, which are not always related to the phylogeny.

Compounds that stimulated only Q fibers

We have earlier demonstrated in Old World primates that compounds bitter to humans stimulate Q fibers and are rejected.
This suggests that Q simulated only the Qal. Here in marmosets, all the compounds that stimulate only S fibers provide hedonically negative information in New World monkeys too.

### Compounds that stimulated only S fibers

Of 15 compounds that stimulated only S fibers, 14 were preferred in TBP tests and by the CTA controls. This corroborates earlier findings in marmosets (Glaser et al. 1995; Haefeli and Glaser 1984; Haefeli et al. 1998; Nofre et al. 1996) and observations in other species, including chimpanzee, hamster, pig, and rhesus (Hellekant et al. 1997a,b; Danilova et al. 1998b, 1999). Furthermore, these stimuli were avoided by the conditioned animals in the CTA tests, demonstrating that these compounds share taste characteristics with sucrose. Taken together, the electrophysiological and behavioral results support the hypothesis that activity in the S fibers codes for a taste quality that is intrinsically attractive.

However, if the attractive taste quality of the conditioned stimulus is paired with the negative unconditioned stimulus (LiCl), the same information from the S fibers is processed by the CNS as a cue for rejection. Another example of the plasticity of the CNS in utilization of the peripheral taste input is increased acceptance of bitter taste after “flavor-nutrient conditioning”. Thus after pairing the bitter compound sucrose octaacetate (SOA) with intragastric glucose infusion (positive unconditioned stimulus), the rats demonstrated acceptance and even preference of SOA over a stimulus that was paired with intragastric water infusion (Perez et al. 1998). Although the Q fibers activity was unchanged and signaled the aversive taste of...
SOA, other nontaste factors played a role in the decision-making.

CAM was the only compound in the group that was not preferred. It is possible that the concentration of CAM was too low to produce a significant preference. However, the input from the S fibers was revealed in the CTA tests because the consumption of CAM was suppressed in the conditioned animals.

**Compounds that stimulated S, Q, and H fibers**

This group comprised compounds of different taste qualities: acids, acid/sucrose mixtures, and some sweeteners.

**ACIDS.** Both the control and conditioned animals demonstrated stronger aversion with increased acid concentration. In the electrophysiological experiments with acids, the H and Q fibers responded with activity sustained during stimulation and 2–3 s after the end of stimulation (Danilova et al. 2002). Many S fibers showed small transient activity at the beginning of stimulation and then an off response during rinsing after stimulation. We think that although there was some response in the S fibers, it was insufficient to cause intake and balance the effect of Q fibers.

**ACID/SUCROSE MIXTURES.** Reactions to acid/sucrose mixtures depended on concentrations used. The Q fiber activity induced aversion and the S fiber activity consumption. Increasing sucrose concentration in the mixtures increased S fiber activity (signaling an intrinsically attractive taste) which then changes the behavior from aversion to preference. In contrast, the conditioned animals avoided the mixtures because the S fibers signaled the taste quality that they learned to avoid. As mentioned in the preceding text, the conditioning occurs in the CNS. The peripheral taste fiber activity remains the same whether the animal is conditioned or not, but how CNS utilizes the activity is changed with conditioning.

**SWEETENERS.** Ampame, acesulfame-K, NC00174, NC00351, D-phenylalanine, saccharin, stevioside, and xylitol are all sweet to humans but with an additional bitter taste component (Schiffman et al. 1995; unpublished observations). As shown in Table 2 they elicited various reactions in the TBP and CTA tests. We suggest that the balance between the Q and S fiber activity determined the intake of these compounds.

In some cases S fibers exert stronger influence than Q fibers: acesulfame-K, NC00174, and xylitol were preferred and NC00351 was not rejected. The input from the S fibers was revealed by the fact that the intake of these four sweeteners was suppressed in the conditioned animals. However, it is likely that these stimuli also have another non-attractive taste component, because as mentioned they also stimulated Q and in some cases H fibers. The Q fiber input can explain why the control animals in the CTA tests consumed acesulfame-K and NC00351 just as much as water, in spite of the fact that these compounds stimulated S fibers.

In other cases the behavioral results indicated that the Q fiber component exerted stronger influence: ampane, D-phenylalanine and stevioside were rejected in the TBP tests and consumed less than water by the control animals in the CTA test. Similar result with ampane has been reported in marmosets (Glaser et al. 1996). Indeed, the nerve responses to these three compounds showed a large Q fiber component. The influence of the S fibers is difficult to estimate because of a low level of consumption of these stimuli by the control animals in the CTA tests.

Of course there are limitations with regard to this. These limitations might include the impact of other fiber types, or the internal status (hunger, thirst, etc.).

**Relationship between behavioral and electrophysiological results**

Based on the preceding results and data from our previous studies, especially in primates, we suggest that the S fiber input gives a hedonically positive effect and the Q fibers to a negative effect. These inputs are then processed and combined in CNS with other factors, such as satiety, experiences, homeostasis, etc. It is implicit that this peripheral input is only one of the factors that influence intake.

Provided that our model is valid, one should be able to predict the intake of a compound in naïve animals using electrophysiological recordings. As a measure of intake we used the preference ratios in the TBP tests here and as a measure of the neuronal activity we suggested to use Net responses from the S and Q fibers in the CT and NG: Net response = (S_CT + S_NG) – (Q_CT + Q_NG). The S and Q fibers’ responses to all bitter or/sweet compounds used here were taken from our previous study (Danilova et al. 2002).

We found a strong linear relationship between the net response and the preference ratio. The Pearson correlation coefficient for these two parameters was 0.85 (P < 0.01). Thus the analysis generally supports our hypothesis that intake reflects balance between S and Q fiber inputs in which S fibers serve as a hedonically positive and Q fibers as a hedonically negative input.

**Responses to salts**

In many species, fibers responding predominantly to salts, N fibers, have been described and a number of studies suggest that these fibers code for salty taste. This conclusion is supported by data in rats in which treatment with the sodium channel blocker, amiloride, abolished the N-fiber activity and disrupted the discrimination of salt from other taste qualities (Scott and Giza 1990).

As shown in Fig. 4 the intake of NaCl, LiCl, and KCl changed with concentration. This shows that marmosets can taste these salts. On the other hand, we have earlier identified only one N fiber in the CT and none in the NG. This undercuts the idea that salty taste is coded by N fibers in this species (Danilova et al. 2002). However, we recorded in 35% of the CT fibers that these salts first inhibited the activity and then gave an off response. This suggests that the temporal pattern may also be used for the coding of salty taste in marmosets. The possibility of a temporal pattern being used to discriminate qualities has been suggested based on data from rat nucleus tractus solitarius (Di Lorenzo and Victor 2003).

Other mechanisms may be at play in taste receptor cells (TRC) and add gustatory information to the Q and S fibers. Considering the fact that there are several cell types in the taste buds and that some of these cells have no evident exposure to the content in the taste pore and several neuropeptides have been found in the taste buds, it is quite likely that some kind of processing of the chemical information occurs in the taste bud (cf. Ewald and Roper 1994; Herness et al. 2002a,b).
Relationship between S/Q fibers and T1R/ T2R receptors

As mentioned in the introduction, two families of taste receptors, T1Rs and T2Rs, have been identified. The finding that the human T1R2/T3 responded to compounds sweet to man, whereas the mouse T1R2/T1R3 responded only to compounds attractive to mice suggests that differences in S fiber responsiveness are caused by differences between the receptors (Li et al. 2002; Zhao et al. 2003).

Furthermore, our finding that some sweeteners were not preferred by marmosets, although they are liked by Old World monkeys, suggests dissimilarities between T1Rs also within the primate order. In this context, it should be mentioned that Nofre et al. inferred that species differences in sweet taste are caused by amino acid differences in their sweet receptor (Nofre et al. 1996). This is supported by recent data from Li et al., who found sequence variations in T1R2/T1R3 among 18 primate species and suggested that this could account for differences in the taste response to aspartame (Li et al. 2003).

Electrophysiological data in rhesus monkeys and chimpanzees show a better response to sweet in the CT than the NG, whereas the murine T1R receptors are located mostly on the back of the tongue, which is supplied by the NG nerve (Hoon et al. 1999). Consequently one would expect that the response to sweet in the NG would be better than in the CT. This seemingly contradiction between molecular and electrophysiological data was recently resolved by Liao and Schultz, who demonstrated that T1R genes are expressed selectively in the fungiform papillae of humans and therefore most likely also in other Old World primates (Liao and Schultz 2003). On the other hand, our data in marmosets show little difference between the sweet responses in CT and NG nerves and may indicate that New World primates have a more even distribution of T1Rs on their tongues.

This study, as well as all our previous primate studies, strongly favors the theory of labeled line coding. We would prefer to replace labeled with dedicated. Several studies supporting this theory have recently been published. First, T1Rs and T2Rs never are found in the same TRCs but are located in different TRCs (Nelson et al. 2001). This means that bitter and sweet tastes have to be processed by different TRCs. It does not necessarily mean that sweet and bitter are conveyed in separate nerve fibers because as is well known a taste fiber can innervate more than one TRC. However, there is no reason to assume that the separation between bitter and sweet is not maintained in the taste fibers. Second, in a recent study, Zhang et al. (2003) demonstrated that active transduction in bitter receptor-expressing cells, but not in sweet receptor-expressing cells, is sufficient for aversive responses to bitter. This provides a foundation for our idea that dedicated Q fibers are linked to aversion. Third, Zhao et al. (2003) demonstrated that inactivation of both T1R2 and T1R3 receptors abolished the preference for sweeteners but not aversion to bitter. Fourth, they showed that mice, which previously did not discriminate between water and spiradoline, an opioid ligand, strongly preferred it after the opioid receptor was expressed specifically in T1R2+ expressing TRCs (Zhao et al. 2003). They also wrote: “together, these results establish that dedicated taste pathways mediate attractive and aversive behavior and strongly support the concept of taste coding using labeled lines,” which corroborates our results in a number of studies.

In summary, this and previous studies characterize the gustatory system of a New World monkey, *C. jacchus jacchus*, with behavioral and electrophysiological techniques. By combining nerve recordings and behavioral techniques, we have presented a further link between S fiber activity and preference and Q fiber activity and rejection. This strengthens our hypothesis that intake is influenced by S and Q fibers, where S fibers serve as a hedonically positive input and Q fibers as a hedonically negative input.

ACKNOWLEDGMENTS

We thank especially Dr. D. Abbott for access to the animals and Dr. Jean-Marie Tinti and Prof. Claude Nofre, Laboratoire de Biochimie Structurale, Université Claude Bernard, Lyon, France, for compounds.

GRANTS

This research was supported in part by Division of Research Resources Grant RR-00167 to the National Primate Research Center, University of Wisconsin-Madison.

REFERENCES


