Preference conditioning alters taste responses in the nucleus of the solitary tract of the rat

BARBARA K. GIZA, KAREN ACKROFF, STUART A. MCCAUHEY, ANTHONY SCLAFANI, and THOMAS R. SCOTT

Department of Psychology, University of Delaware, Newark, Delaware 19716; and Department of Psychology, Brooklyn College of City University of New York, Brooklyn, New York 11210


The visceral consequences of feeding have a marked effect on a subject's subsequent response to the taste of the ingested substance. This relationship is studied experimentally by pairing the taste of a chemical with either toxins or nutrients, and so creating conditioned aversions or preferences, respectively.

Conditioned aversions are generated reliably by a single pairing of a novel taste (the conditioned stimulus (CS)) with a toxin (the unconditioned stimulus (US)) that causes malaise (14). The subsequent avoidance of the conditioned taste is assiduous and lasting. This profound change in behavior has been associated with alterations in the activity evoked by the CS at various gustatory relays, among them the first central synapse in the nucleus of the solitary tract (NTS) (6). Electrophysiological recordings at this site revealed a change in the response profile evoked by a saccharin CS in conditioned rats, such that it more closely resembled the profile elicited by quinine, the prototypical aversive stimulus. They also disclosed a sharp, phasic response to saccharin that was not present in unconditioned animals. Thus the distribution of evoked activity to the saccharin CS, both across neurons and over time, was modified to be more like that of an aversive stimulus.

The establishment of conditioned taste preferences (CTPs) has proven more difficult (24), which suggests that it may be more urgent for an animal to avoid a specific toxin than to seek a specific nutrient. However, experimental protocols have been developed through which a reliable CTP may be created by associating a taste with the administration of nutrients (3, 5, 10, 25, 26).

Conditioning alters taste responses in the nucleus of the solitary tract. Responsiveness of acid-oriented neurons to MgCl2 in MG rats was lower than in Controls, and its profile was more distinct from those of acidic and bitter stimuli. Total activity to citric acid was unchanged in CI rats. However, its temporal profile showed a decreased phasic component, making citric acid temporally distinct from nonsugars. Therefore, the responses to both CS+ were modified, each in its own manner, to be more distinct from those of aversive stimuli. The effects of preference conditioning, however, were weaker than those of aversive conditioning.

conditioned flavor preferences; electrophysiology; intragastric infusions

THE VISCERAL CONSEQUENCES OF FEEDING HAVE A MARKED EFFECT ON A SUBJECT'S SUBSEQUENT RESPONSE TO THE TASTE OF THE INGESTED SUBSTANCE. THIS RELATIONSHIP IS STUDIED EXPERIMENTALLY BY PAIRING THE TASTE OF A CHEMICAL WITH EITHER TOXINS OR NUTRIENTS, AND SO CREATING CONDITIONED AVERSIONS OR PREFERENCES, RESPECTIVELY.

Conditioned aversions are generated reliably by a single pairing of a novel taste (the conditioned stimulus (CS)) with a toxin (the unconditioned stimulus (US)) that causes malaise (14). The subsequent avoidance of the conditioned taste is assiduous and lasting. This profound change in behavior has been associated with alterations in the activity evoked by the CS at various gustatory relays, among them the first central synapse in the nucleus of the solitary tract (NTS) (6). Electrophysiological recordings at this site revealed a change in the response profile evoked by a saccharin CS in conditioned rats, such that it more closely resembled the profile elicited by quinine, the prototypical aversive stimulus. They also disclosed a sharp, phasic response to saccharin that was not present in unconditioned animals. Thus the distribution of evoked activity to the saccharin CS, both across neurons and over time, was modified to be more like that of an aversive stimulus.

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METHODS

Behavioral Methods

Subjects. The subjects were 61 female Sprague-Dawley-derived rats born in the laboratory from Charles River CD stock (Wilmington, MA). The animals, which were 13–16 wk old, were individually housed in standard wire-mesh cages in a vivarium maintained at 21°C under a 12:12-h light-dark cycle. They were divided into control (Control; n = 14), MgCl2 (MG, n = 23), and citric acid (CI, n = 24) groups.

Surgery. The rats of the MG and CI groups were surgically implanted with gastric cannulas according to a method described elsewhere (10). Briefly, a stainless steel cannula was implanted into the fundus of the stomach and fixed to the stomach wall by means of a purse-string suture and polypropylene mesh. The shaft of the cannula passed through an opening in the abdominal wall and the skin. When not in use, the cannula was sealed with a stainless steel screw.
Intra gastric infusions were accomplished using an electronic esophagus apparatus described by Elizalde and Sfalfani (10). Rats were placed in test cages similar to their home cages but modified so that powdered chow was available from a food cup accessible through a 64-mm hole in the back wall. Also, a slot in the cage floor permitted two catheters attached to the gastric cannula to be connected to a dual-channel infusion swivel located below the cage. Plastic tubing connected the swivel to two peristaltic infusion pumps. The infusion pumps were operated automatically by drinkometer circuits and a microcomputer whenever the rat drank from one of two stainless steel sipper tubes located at the front of the cage. The rate of infusion was 1.3 ml/min, and ±1 ml of fluid was infused intragastrically for each milliliter of fluid consumed. An important feature of this system is that the rats controlled the amount and pattern of intra gastric infusions by their voluntary drinking behavior.

Test solutions. The CS were 0.10 M MgCl₂ or 0.01 M citric acid, prepared with tap water. The intragastric infusates were tap water and a 32% (wt/vol) solution of Polycose (hydrolyzed corn starch, Ross Laboratories). For the CI group, citric acid was the CS—paired with intragastric Polycose, and MgCl₂ was the CS—paired with intragastric water; the CS flavors were reversed for the MG group. The Control group drank the MgCl₂ and citric acid solutions without intra gastric infusions.

Procedure. The MG and CI rats were allowed 10 days to recover from surgery. They were then transferred to the test cages and attached to the infusion system. For several days, the rats were infused with water as they drank water from two spouts. Powdered Purina Chow and plain or flavored water (as described below) were available ad libitum 23 h/day throughout the experiment; the equipment was serviced during the remaining 1 h/day. Daily oral fluid intakes and infused water or Polycose solutions were measured to the nearest 0.1 g.

On training days 1 and 3, the rats were given access to the CS—solution paired with intra gastric Polycose infusion. On days 2 and 4, the rats were given access to the CS—paired with intra gastric water infusion. On test days 5 and 6, the rats underwent two-bottle preference testing with the CS+ and the CS—solutions paired with their respective infusions. This 6-day cycle was then repeated with the CS—presented on days 7 and 9 and the CS+ on days 8 and 10. After these 12 days of training, the rats were given sequential two-bottle preference tests with the CS+ vs. water and the CS—vs. water for 2 days each. The MgCl₂ solution was presented first to all rats so that one-half the animals received their CS+ first and the others their CS—. CS remained paired with their original infusions, and water intake was paired with water infusion. Throughout the training and testing, the left-right positions of the CS+ and CS—solutions were alternated to control for side preferences.

The Control rats were housed in the vivarium throughout the study with ad libitum access to chow. They received the same number of days and sequence of access to MgCl₂ and citric acid solutions and two-bottle preference testing as the MG and CI groups.

Apparatus. The MG and CI rats were allowed 10 days to recover from surgery. They were then transferred to the test cages and attached to the infusion system. For several days, the rats were infused with water as they drank water from two spouts. Powdered Purina Chow and plain or flavored water (as described below) were available ad libitum 23 h/day throughout the experiment; the equipment was serviced during the remaining 1 h/day. Daily oral fluid intakes and infused water or Polycose solutions were measured to the nearest 0.1 g.

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Following training at Brooklyn College, the rats were transferred to the University of Delaware. They were housed under similar conditions in the vivarium there, and a subset of the rats was given 24-h two-bottle preference tests (without intra gastric infusions) to reassess their responses to the CS tastes before electrophysiological testing.

Electrophysiological Methods

Subjects and selection criteria. All 14 rats of the Control group served as subjects for electrophysiology. Rats of the MG and CI groups that demonstrated a final test preference of >75% for the CS+ over the CS— and for the CS+ over water during testing in Brooklyn were accepted as potential subjects for electrophysiological recording. Of the 47 conditioned rats, all met the CS+ vs. CS— criterion but only 33 met the more stringent CS+ vs. water criterion. Seventeen rats were selected from this group for retesting in Delaware, all of whom still met the 75% criterion. The final five of these animals were not used in neural recording because they had received unreinforced exposure to the CS+. However, when it became clear how resistant a conditioned preference is to extinction, the remaining rats were used. Of the 28 rats that underwent electrophysiological recording, 27 contributed neural data: 15 from the MG group and 12 from the CI group.

Surgical procedure. Surgical levels of anesthesia were induced by an intramuscular injection of 100 mg/kg Ketaset followed in 10 min by 48 mg chloral hydrate, which was subsequently administered as needed. A tracheotomy was performed to permit tracheal clearing and artificial respiration. An esophageal fistula was implanted to avoid chemical and mechanical postigestive effects during gustatory stimulation of the oral cavity, pharynx, and esophagus. The rat was mounted in a nontraumatic head holder, and a slender length of polyvinyl tubing through which a fine stimulus spray could be administered to the entire oral cavity was placed in its mouth and secured. The rat’s heart rate was monitored and the interbeat interval maintained at ±180 ms through adjustments in respiration rate and depth of anesthesia. Rectal temperature was maintained between 35 and 37°C. Portions of the occipital and parietal bones were removed, and the cerebellum was aspirated to expose the surface of the medulla.

Recording. Activity from single neurons was isolated by micropipettes (1.0 μm ID; Z = 5–10 MΩ at 1,000 Hz) filled with 1.6 M potassium citrate. Initial coordinates used to locate the NTSs were 2.7 mm anterior to the obex, 1.7 mm lateral to the midline, and 1.0 mm ventral to the surface of the medulla. The electrode was advanced in 50-μm steps, and neural activity was tested until robust gustatory responses were encountered. Action potentials were identified by consistency of waveform, spike amplitude, and the requirement that each spike occur within an interval of <1.5 ms. Single-unit potentials were amplified, filtered, and displayed on an oscilloscope using conventional recording techniques. A direct-coupled four-channel tape recorder was used to preserve unit activity, the onset marker pulse, and a voice commentary for later analysis off-line.

Stimuli and stimulus delivery. Sixteen stimuli representing the basic taste qualities as well as more complex stimuli (MSG, sodium-saccharin, and Polycose) were employed (see legend to Fig. 3). Twelve solutions were prepared in distilled water. Five percent tap water was included in the sucrose and glucose solutions to ensure adequate conductivity to activate a stimulus onset marker; citric acid and MgCl₂ (the 2 CS) were mixed in tap water transported from Brooklyn to ensure that the tastes of the two CS did not differ from those on which the rats were trained.

Five milliliters of solution at room temperature was sprayed over the tongue at a rate of 2 ml/s. Gustatory-evoked activity was recorded for 3 s before (spontaneous) and for 5 s following (evoked) stimulus onset. The moment of stimulus contact with the tongue was marked by a transistor-transistor logic device that passed an 11-nA current through the rat, an amount two orders of magnitude below the threshold for electric taste (4). Each taste was followed by a 20-ml distilled water rinse and by a minimum rest period of 60 s. Stimuli were presented at intervals of no less than 90 s to
prevent adaptation effects. Additional rinses and rest periods were occasionally required for baseline activity levels to be reestablished. Stimuli were presented in a quasi-random order with the stipulation that chemicals representing similar taste qualities not be applied consecutively. If the neuron remained well isolated at the end of the series, stimuli were reapplied to obtain a measure of response reliability.

Data analysis. Independent analyses of the electrophysiological data were performed off-line. Spontaneous activity was subtracted from the evoked response to yield net spikes per second for the 5-s poststimulus period. These counts were used to calculate response magnitude, breadth of tuning, and average spontaneous rate across all cells in each group. Derived analyses, including calculation of interstimulus and interneuronal correlation matrices, multidimensional scaling, planned comparisons, analysis of variance (ANOVA), and post hoc tests, were all performed with the Systat package. Hierarchical cluster analyses, used to identify subgroups of cells with similar response profiles, were conducted using the Clustan routine. Summary statistics, including calculation of mean evoked rates, were obtained for each neuronal subgroup as they had been for the entire population of cells. Where significant group × stimulus interactions were obtained, response rates were compared across the Control and each CS+ group. Net spikes for each of 50 consecutive 100-ms bins were averaged across neurons to give poststimulus time histograms (PSTHs). Finally, individual correlations, which were used to generate the multidimensional spaces, were compared between groups using a Z test for independent correlation coefficients.

RESULTS

Summary of Findings

The neural effects of a CTP were significant and consistent with the expectation that the CS+ would be represented by a neural profile that implied a less aversive taste quality. There was a decrement in the evoked response magnitude to MgCl2 in MG rats relative to those from Controls, a difference that arose mainly from the subset of cells that responded well to acids and quinine. When the relative similarity of response profiles evoked by the taste stimuli was represented in a multidimensional space, MgCl2 took a position at the margin of the bitter stimuli, significantly farther from the others than in the space from Control rats. In contrast, the effect of conditioning on the response to citric acid among CI rats was expressed through the temporal aspects of the response. Specifically, the phasic activity normally associated with acidic stimuli was attenuated and delayed in rats with conditioned preferences for citric acid, such that the time course of the evoked response was significantly less similar to those of all bitter chemicals and HCl. As a consequence, citric acid joined sugars in a temporally based multidimensional space.

Behavior

The analyzed behavioral data are from the 27 experimental and 14 Control rats that contributed neural data. Oral intakes during the one-bottle training and two-bottle test days are presented in Fig. 1. Intakes were expressed as 2-day means and analyzed with repeated-measures ANOVA, followed by t-tests or Newmann-Keuls tests of group differences. To simplify data presentation, only results from the second cycle of training and testing and subsequent solution-vs.-water tests are discussed.

During one-bottle training (Fig. 1A), the conditioned rats drank more CS+ than CS− overall [F(1,25) = 39.9, P < 0.001]; there was no group effect, but the group × CS interaction was significant [F(1,25) = 10.8, P < 0.005]. The MG rats drank more CS+ [F(1,25) = 4.4, P < 0.05] and less CS− [F(1,25) = 8.3, P < 0.01] than the CI rats. In the two-bottle test (Fig. 1B), the conditioned rats drank substantially more CS+ than CS− [F(1,25) = 293.8, P < 0.001]; the overall CS+ preference was 93%. The CS+ preferences of the MG and CI groups were very similar. The conditioned rats also drank more CS+ than plain water [F(1,25) = 366.6, P < 0.001]; the overall CS+ preference was 91% (Fig. 2). The same rats, in contrast, drank much more water than CS− [F(1,25) = 193.6, P < 0.001]; the overall CS− preference was 10% (Fig. 2). There were no reliable differences between the CS vs. water preferences of the MG and CI groups.

The Control rats consumed similar amounts of citric acid and MgCl2 during one-bottle days (Fig. 1A), but slightly more citric acid than MgCl2 during two-bottle days (Fig. 1B). When water was available, they drank substantially more water than citric acid or MgCl2 (t13 = 11.70 and 12.35, respectively, P < 0.001, Fig. 2). Relative preferences for
Electrophysiology

Spontaneous rate and response criterion. The mean ($\pm$ SE) spontaneous rate among neurons from Control rats was 11.6 ± 1.3 spikes/s. Spontaneous rates from neurons in MG animals had a mean of 10.1 ± 0.9 spikes/s vs. 16.0 ± 1.6 spikes/s among cells from CI rats. The spontaneous activity in cells from MG rats did not differ from Controls, whereas that from CI cells was significantly elevated [$F(1,123) = 4.25, P < 0.05$]. Although the spontaneous rate obtained in CI cells is rather high, it is not unprecedented (19), nor is there an obvious relationship either to conditioning or to exposure because rates were lower in both the MG and Control groups.

The criterion for classification as a taste cell was an evoked response to at least three stimuli that exceeded the spontaneous rate by 1.65 SD ($P < 0.05$, one-tailed), sustained for 5 s. The numbers of taste cells recorded from the Control, MG, and CI groups were 57, 61, and 68, respectively.

Evoked activity. Reliability. A quantitative measure of the reliability of the evoked responses was derived by reapplying the stimulus series while recording from a given cell. Each unit's responses to the first series of applications were then organized in one column and paired with its responses to the second application in another. The Pearson correlation coefficient between the two columns generated across neurons served as a measure of reliability. With no variability, the reapplication of a stimulus should evoke the identical response to that elicited by its initial application, and the resulting correlation across many reapplications would be +1.00. We performed this analysis on the basis of 1,175 stimulus reapplications (409 in CI, 408 in MG, and 358 in Control groups). The resulting correlation coefficients were +0.96, 0.95, and 0.97, respectively, indicating a high degree of stability throughout the system during the 45–60 min during which recording typically proceeded.

Breadth of responsiveness. NTS cells showed typically broad responsiveness across the stimulus array. The accepted measure of breadth is the entropy coefficient introduced by Smith and Travers (29). The relative responsiveness of a neuron to each of four basic taste stimuli [$0.1 \text{ M NaCl}, 1.0 \text{ M glucose}, 0.01 \text{ M HCl}, \text{ and } 0.01 \text{ M quinine-HCl (QHCl)}$] is used to calculate a cell's coefficient of entropy, a value that may range from 0.0 (total specificity to one chemical) to 1.0 (equal responsiveness to all four). The absolute value of the few inhibitory responses was used in calculating the breadth-of-tuning coefficient, which, by virtue of its logarithmic form, cannot manage negative numbers. By this measure, the mean ($\pm$ SE) coefficient of entropy among the neurons from MG and CI rats was 0.80 ± 0.01 for both groups and did not differ from the value derived from the Control rats, 0.80 ± 0.02. Therefore, by the criterion of responsiveness across the four basic taste stimuli, cells became neither more selective nor more broadly tuned as a result of the conditioning procedure.

1 The breadth-of-tuning metric provides an index of the distribution of a cell's response across the four prototypical stimuli. It is calculated according to the formula $H = -k \sum_{i=1}^{n} p_i \log_2 p_i$, where $H$ is breadth-of-tuning coefficient; $k$ is a scaling constant, which is 1.661 for four stimuli; and $p_i$ is the proportion of the neuron's response devoted to any one stimulus. The value of $H$ ranges from 0.00 for a neuron that responds to only one of the prototypical stimuli to 1.00 when the response is equal to all four.
RESPONSE RATES. Response rates across all NTS cells obtained from MG and CI rats were compared with those recorded from Controls (Fig. 3). Planned comparisons conducted on the responses to MgCl2, QHCl, and Polyclose were significantly lower in MG rats \((P < 0.05\) for each comparison), whereas those from CI rats were lower only to QHCl \((P < 0.05\).

DIVISION INTO NEURON TYPES. We categorized neurons according to the similarity of their response profiles. The net discharges evoked by the same four basic stimuli in 5 s constituted the response profile for each of the 186 neurons. Correlation coefficients were then calculated between all pairs of profiles in each group, providing a matrix of relative functional similarity among the cells. Each matrix was subjected to cluster analysis, the results of which identified three neural subtypes. Of 57 neurons recorded from Control rats, 55 could be included in one of these three discrete neural subtypes, identified by their most effective stimuli as sugar-oriented (S-cells, \(n = 11\)), sodium-oriented (N-cells, \(n = 21\)), and acid-oriented (H-cells, \(n = 23\)). In the MG group of rats, 59 of the 61 cells could be included in corresponding groups of S- \((n = 13)\), N- \((n = 16)\), and H-cells \((n = 30)\). Of 68 neurons recorded from CI rats, 67 were either S- \((n = 24)\), N- \((n = 28)\), or H-cells \((n = 15)\). The distribution of neurons into the three subtypes was significantly different between the CI and Control groups \((\chi^2 = 6.46; P < 0.05)\), but did not differ between the Controls and the MG group.

Within each of the three groups of rats, response rates obtained from cells within each subtype were subjected to ANOVA and post hoc comparisons. For the ANOVAs, the least significant of the three subtype \(\times\) stimulus interactions was \(F(30,840) = 30.49; P < 0.001\). Post hoc tests confirmed that the responses of neurons within a subtype differed significantly from those of other cells to the stimuli that defined that subtype, e.g., in the response of S- vs. H-cells to glucose. For these comparisons, the least significant value was \(F(1,52) = 4.21; P < 0.05\). The mean profiles from each subtype in Control, MG, and CI rats are shown in Fig. 4.

There is evidence that these neuronal subtypes represent functionally discrete coding channels within the taste system \((6, 13, 27)\). Therefore, we analyzed the responses of corresponding subtypes of neurons in each group of rats. In both MG and CI rats, S- and N-cells showed little difference from the corresponding cells in unconditioned Controls. However, in the H-cell subtype of MG rats, responses were lower for all nonsugars, and significantly so for MgCl2 \([F(1,51) = 5.55; P < 0.025]\) and QHCl \([F(1,51) = 6.02; P < 0.025]\). Thus neurons that bear the primary responsibility for coding aversive chemicals were rendered less responsive to several of those stimuli, and significantly so to the CS+, through

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**Fig. 3.** Evoked spike rates across all NTS neurons to each stimulus in three groups of rats. N, 0.1 M NaCl; L, 0.1 M LiCl; M, 0.1 M MSG; K, 0.1 M KCl; CA, 0.1 M CaCl2; NH, 0.1 M NH4Cl; MG, 0.1 M MgCl2; Q, 0.01 M quinineHCl; AC, 0.01 M acetic acid; Cl, 0.01 M citric acid; H, 0.01 M HCl; G, 1.0 M glucose; SU, 0.5 M sucrose; F, 1.0 M fructose; SA, 0.03 M sodium-saccharin; P, 0.2 M Polyclose. There were no differences in mean evoked activity (X), although responsiveness to quinine was reduced in both conditioned groups and to MgCl2 and Polyclose in the MG group. \(^* P < 0.05\) in MG; \(^* P < 0.05\) in CI rats. CRL, Control.

**Fig. 4.** Mean evoked response to each stimulus (as in Fig. 3) now divided into the contribution from each of the three neuron types. Sugar-oriented cells (S-cells; A) and sodium-oriented cells (N-cells; B) were largely unaffected by the conditioning procedure. However, the acid-oriented cells (H-cells; C) in MG rats were significantly less responsive to two bitter chemicals, including the CS+. \(^* P < 0.05\) in MG; \(^* P < 0.05\) in CI rats.
conditioning. There were no significant differences between the response magnitudes of H-cells in Control and Cl rats.

Taste quality. ACROSS-NEURON PROFILES. If the effect of creating a preference for the CS+ is to alter its taste quality, that change should be evident in the relationship of its response profile to those of other chemicals in the stimulus array. The fact that the CS+ was conditioned to be more appetitive implies that its response profile should be modified to resemble those of appetitive stimuli more and of aversive stimuli less. Only the latter was the case. The correlation between the response profiles generated by MgCl2 and quinine was +0.85 in Control rats, but just +0.66 in the MG group (Z = 2.40; P < 0.01). Indeed, MgCl2 evoked a profile that was significantly less like those of all bitter stimuli [KCl, CaCl2, and NH4Cl (Z > 1.87; P < 0.05 for all comparisons)] in MG rats.

The effect of conditioning on the across-neuron profile for citric acid was less pronounced. Only the correlation between profiles representing citric acid and quinine was lower in CI (r = +0.61) than in Control rats (r = +0.77; Z = 1.75; P < 0.05), as predicted.

There was no increase in similarity between the profiles of the CS+ and appetitive stimuli (sugars), however. The correlation between the profiles of MgCl2 and the three sugars in Control rats were all in the range +0.02 to +0.14. In MG rats, they ranged from 0.00 to +0.11 and in CI rats from −0.08 to +0.03 (all nonsignificant).

A spatial representation of the relationship among all stimuli, based on these correlations, is shown in Fig. 5 for all three groups. The general organization of these multidimensional spaces was typical of that seen in other studies. There was a cluster of Na+/Li+ salts, another cluster of sugars, and a broad area of acids, bitter salts, and quinine. Sodium-saccharin was appropriately positioned between the sugar and Na+/Li+ clusters; Polycose was not closely tied with any group, but was nearest the Na+/Li+ salts. Neither the MG nor the CI space was drastically reconfigured by conditioning. In the MG group, the loss of similarity between the profiles of MgCl2 and the other bitter stimuli caused MgCl2 to be placed at the edge of the acid-bitter cluster, but not clear of it (Fig. 5B). There were no apparent movements in the CI versus the Control spaces (Fig. 5C).

TEMPORAL PATTERNS. In the analysis above, we considered only the net discharges from each stimulus-neuron interaction during 5 s of evoked activity. Yet the time course of this accumulation not only carries reliable information regarding taste quality (9), but also may be sufficient to activate appropriate reflexive responses to chemicals in behaving rats (7). Therefore, we generated a temporal profile in which the evoked

Fig. 5. Taste spaces based on similarity of response profiles to the stimulus array in each group of rats. MgCl2 was significantly farther from other bitter and sour stimuli in the MG group (B) than in Controls (A), yet still remained part of that cluster. Effect of conditioning on citric acid (C) was not apparent in this profile.
response to each stimulus was divided into 50 100-ms bins, which were then collapsed across neurons. PSTHs for the prototypical stimuli are shown in Fig. 6 to illustrate the characteristics of each. Quinine elicits a sharp peak of phasic activity, with a rapid decline to a low tonic level; thus the phasic response dominates its PSTH. NaCl also evokes a pronounced phasic response, but then maintains a plateau of tonic activity at about one-half the phasic rate. HCl elicits a smaller phasic peak relative to its tonic response, which continues to decline over seconds. The most disparate PSTH is evoked by glucose; the phasic portion is less evident, and the response continues to build throughout the first second after stimulus onset.

The first 2 s of the PSTH capture the character of each response. Therefore we proceeded with correlation matrixes and multidimensional representations as before, with data limited to that time period.

The temporal profile (PSTH) representing citric acid was altered by the conditioning procedure in the CI group. The correlations between the PSTHs of citric acid and those evoked by LiCl, KCl, CaCl₂, NH₄Cl, MgCl₂, HCl, QHCl, and Polycose were significantly lower in CI rats than in Controls (Z > 1.89; P < 0.05; one-tailed, for all comparisons). Differences in those between citric acid and NaCl, MSG, and sodium-saccharin approached significance (Z > 1.36; P < 0.10). Thus the temporal representation of citric acid shifted decisively away from those of all stimuli except the sugars. The basis for this change in temporal character of the response to citric acid in CI rats was a reduction in the phasic peak of the PSTH, and its delay into the 2nd second of evoked activity (Fig. 7). This brought the temporal profile into sharper contrast with those of the nonsugar stimuli and into closer alignment with

Fig. 6. Poststimulus time histograms for the 4 prototypical taste [0.01 M quinine-HCl (A), 0.1 M NaCl (B), 0.01 M HCl (C), and 1.0 M glucose (D)] stimuli in the Control group. Quinine generated the highest ratio of phasic-to-tonic activity, and glucose, the lowest.

Fig. 7. Poststimulus time histograms to citric acid (0.01 M; A) and MgCl₂ (0.1 M; B) in Control and their respective CS⁺ groups. Phasic response to citric acid was selectively reduced, resulting in a temporal profile significantly more distinct from those generated by nonsugars. Discharge rate to MgCl₂ was reduced modestly and almost uniformly in MG rats, with no major distortion of the temporal profile.
the response to glucose, which is characterized in the rat by a slow onset and domination by tonic activity. To quantify this shift, we took the spikes during the first second of evoked activity as the phasic response and the mean spikes/s over the next 4 s as the tonic response. We then calculated the phasic-to-tonic ratio as a measure of the domination of the phasic peak of activity in the temporal profile. This ratio ranges from <1 for sugars to >5 for sharply bitter chemicals (28). The phasic-to-tonic ratio for citric acid was 1.7 in Control rats; in the CI group, it was 1.2, the same as the phasic-to-tonic ratio for glucose.

In contrast, the reduced overall response to MgCl₂ among MG rats noted in the section above resulted from only minor changes in both the phasic and tonic portions of the PSTH, and so no alteration in the temporal profile (Fig. 7B). The phasic-to-tonic ratio for this bitter stimulus was 4.0 in Control rats versus 4.4 in the MG group. Consequently, the correlations between MgCl₂ and other stimuli in the MG group did not differ from the corresponding values in Controls. The PSTH of neither citric acid nor MgCl₂ was altered when these stimuli were used as CS₂.

Correlation coefficients were calculated between each pair of PSTHs (n = 120) within a group and were collected into matrices. These served as the basis for generating a multidimensional space representing the temporal similarity among the responses to these 16 stimuli. These spaces are shown in Fig. 8 for each group. With the notable exception of citric and acetic acids in the CI group, stimuli assumed typical positions in each space. Bitter stimuli were at one extreme and sugars at the other, with salty salts and acids between them. Polycose at 0.2 M and 0.03 M sodium-saccharin both elicited PSTHs similar to those of salty salts or HCl. In the space derived from MG rats, MgCl₂ maintained its location in the midst of the bitter salts and quinine (Fig. 8B), reflecting the fact that the shape of its PSTH was unmodified by conditioning. However, in the CI space (Fig. 8C), citric acid assumed a location among the sugars. There was no generalization to the other stimuli, which held to the same relative positions as in the Control space (Fig. 8A).

DISCUSSION

The behavioral effects of taste-nutrient conditioning were pronounced: there was a striking increase in preference for the CS₁, relative both to the CS₂ and to water. This replicates prior results obtained with other flavor cues (1, 10).

The electrophysiological effects of the taste-nutrient conditioning were much smaller, yet significant. Afferent signals are carried in a spatiotemporal code, both components of which have been shown to be informa-

Fig. 8. Taste spaces representing similarity of the stimuli based on time courses of the activity they evoke. In the space derived from the CI rats, citric acid shifted significantly away from all nonsugars to join the sugar cluster. A: Control. B: MG. C: CI.
tive in identifying a stimulus. The effect of preference conditioning on the signal for MgCl₂ was manifested in the spatial distribution of activity (i.e., in the discharge rate across neurons); the effect on the code for citric acid was primarily in the temporal distribution.

Increased behavioral preference implies that there should be an underlying improvement in the hedonic profile of the CS+, i.e., the neural profile should become less similar to those of aversive stimuli and more like those that are appetitive. Evidence for the former is strong; for the latter, less so.

It is assumed by both major theories of gustatory neural coding (11, 13) that the acid-oriented subtype of neurons (H-cells) have the primary responsibility for signaling aversive taste qualities. The response of these cells to MgCl₂ was significantly lower in the MG group of rats. Moreover, this difference was sufficient to alter the across-neuron profile for MgCl₂ such that it was significantly less similar to the profiles of stimuli that rats avoid and humans describe as aversive (sour and bitter). Indeed, the correlation between the profiles evoked by MgCl₂ and quinine (the prototypical bitter stimulus) in MG rats was just +0.66, significantly lower than in the Control group of this study (+0.85). However, this difference was not sufficiently pronounced to move the profile for MgCl₂ beyond the fringe of the group of aversive stimuli. Thus the basic character of MgCl₂ was retained despite its behavioral preference. It did not become more closely affiliated with the profiles for innately appetitive sugars.

The neural effects of conditioning on citric acid were not manifested in overall spike rates or in the magnitude of response within any neural subtype. Moreover, there was no significant effect on the profile evoked by citric acid across neurons; its position in the multidimensional space was not different in the CI group from in Controls. Rather, the effects were apparent in the time course of the response. The phasic peak of activity that has been associated with the response to aversive stimuli (28) was blunted and delayed in the CI group, making the PSTH significantly less similar to the temporal profiles of salts, HCl, and quinine, and so driving it toward the sugars in the multidimensional space. In no other group of rats, in this study or in eight others in which we have examined the arrangement of PSTHs in the NTS, has citric acid appeared in the midst of sugars.

This result is in agreement with the findings of Scott and Mark (28) that the same set of neurons responds to both benign organic acids and toxic alkaloids, but that a clear discrimination can be made on the basis of the time course of those responses. If a slower response onset is what makes a marginally acceptable organic acid distinct from a highly aversive alkaloid, then the enhancement of that distinction, seen here, could underlie its increased appetitiveness following conditioning.

Still, the neural effects we report here do not appear of sufficient magnitude to account for the behavioral preference for the tastes of the CS+. Rather, higher-order relays must be involved. Associative processes governing CTAs are reported to be more invested in the parabrachial nuclei (PBN) than the NTS (12), although the same may not be true for conditioned preferences (22). The increased release of dopamine in nucleus accumbens to the CS+ implies that the activity of ventral forebrain nuclei involved in reward may be modified by preference conditioning (18). Our data suggest that the role of the NTS may be relegated to decreasing the intensity of the rejection reflexes that are coordinated in the hindbrain when an innately aversive CS+ is encountered. That would reduce interference with the instructions for acceptance perhaps originating in PBN or ventral forebrain.

CTAs and CTPs are behavioral complements of one another. By means of a CTA, an innately preferred stimulus can be made aversive; through a CTP, a neutral or aversive stimulus can come to be preferred. Both are highly resistant to extinction. There are neural complements to these processes as well. With development of a CTA, the neural response to the CS increases and that increase is contained primarily in a phasic peak of activity. Creation of a CTP is shown here to be associated with a reduced response to the CS+ or with a reduction and delay in the phasic peak of activity that is normally associated with an aversive stimulus.

However, the extent to which CTPs mirror conditioned aversions is limited. Whereas lactose-induced aversions may be mild (20), the standard regimen of intraperitoneal LiCl causes assiduous avoidance of the CS and orofacial reactions to imply disgust and evokes somatic and autonomic reactions such as lying prone, piloerection, and diarrhea. In contrast, preference conditioning does not necessarily increase CS intake (10, 21). In fact, there even remains the question of whether a CTP reflects a true increase in the hedonic value of the CS+. It may be that taste preference conditioning is a form of instrumental learning in which the animal consumes the CS to obtain nutrients. On the other hand, acceptance of the CS+ is more highly resistant to extinction than is a typical operant response (10). Also, Mark et al. (18) have reported that, following preference conditioning, the taste of the CS+ elicits an increased dopamine release in the nucleus accumbens, a neurochemical marker for reward (31).

The pronounced behavioral changes observed in taste aversion learning are associated with significant neural effects. In the NTS, as noted above, both the spatial and temporal components of the sensory code for the CS are altered by conditioning (6). The increase in NTS activity following conditioning has also been confirmed in c-Fos studies (30). At the level of the PBN, both electrophysiological (8) and c-Fos (33) results have demonstrated that neural activity to the CS increases and that the nature of the response is altered to reflect its aversive quality. Electrophysiological changes have also been demonstrated in the hypothalamus following aversive conditioning (2), whereas neurochemical changes associated with aversion have been reported in the nucleus accumbens (17). The neural effects of a conditioned preference, reported here at the level of the NTS, are less striking.
In the short-term, at least, it is more important for an animal to reject a toxin than to select a nutrient. The taste system has mechanisms to reject a chemical 1) if that chemical has a toxic evolutionary history that selected for those that rejected it (such that it is innately aversive) and 2) if the protective evolutionary bias is in error, and the effect of a chemical is toxic to the individual (a conditioned aversion). Thus, when an unfamiliar chemical is swallowed, the animal takes an experiment, and there must be a mechanism, the CTA, for assessing its outcome. Although biased in the animal’s favor by its evolutionary endowment, it is an experiment nonetheless. The readiness of a rat to make the connection between taste and malaise with one pairing and the pervasive effects seen in the central nervous system bear testimony to the effectiveness of this mechanism.

Consumption of a novel, innately aversive chemical is a less likely experiment for a rat to perform in the wild. An aversive stimulus is unlikely to be accepted in large enough quantities to have a positive visceral impact. The decision to reject is made at the first stage, and the experiment is foregone. Initially appetitive tastes may be made even more preferred by postigestive nutritive consequences, although the effect of such preference training on the neural code for the CS+ is yet to be determined.

The taste system is primarily a goad to feeding. Its activity offers the intense reward that encourages consumption. As a system that is normally set to promote feeding, there are several factors that can decrease its driving force, CTAs among them, but few that can make it even more effective. Satiety, for example, has major consequences on taste activity in the NTS (15, 16) and elsewhere in the nervous system (23); in contrast, the impact of food deprivation on taste activity is less pronounced (19). The modest neural consequences of a CTP reinforce this limitation.

This research was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (DK-31135 and DK-30964) and a National Institute of Mental Health Research Scientist Award (MH-00983) to A. Sclafani.

Address for reprint requests: T. R. Scott, 220 Wolf Hall, Univ. of Delaware, Newark, DE 19716.

Received 11 October 1996; accepted in final form 11 J une 1997.

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