# A Neurobehavioral Analysis of l-Amino Acids as Taste Stimuli

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Sensory physiologists have approached the study of complex perceptual phenomena only after investigating more simple stimulus-response interactions. Researchers in vision have used spots, bars and angles as basic stimuli (29, 30, 39); auditory researchers have used pure tones (20, 36). In gustation most research has emphasized the use of sugars and inorganic salts and acids (61). Although our understanding of each sensory system has advanced by utilizing these simple stimuli, dramatic and often unexpected results have been obtained when physiologists advanced to the level of using more complex, biologically relevant stimuli. In vision the discovery that some frog retinal ganglion cells are specifically tuned to respond to moving spots or edges (40) greatly accelerated research on feature detection and introduced the concept of biological significance to sensory physiologists. The discovery of pheromones at about the same time by Karlson and Liischer (35) served to emphasize the importance of biologically adaptive stimuli and the efficacy with which a sensory system could process them. In the auditory system biologically significant stimuli have been discovered in a variety of animals including the frog (8), barn owl (52), moth (64) and monkey (87). Researchers in gustation have not yet used complex organic stimuli other than sugars to any great extent in neurophysiological studies. We undertook to initiate this process by defining the neural and behavioral responses to 12 l-amino acids. These were chosen to represent both essential and nonessential classes and to cover a wide range of molecular weight and pH.

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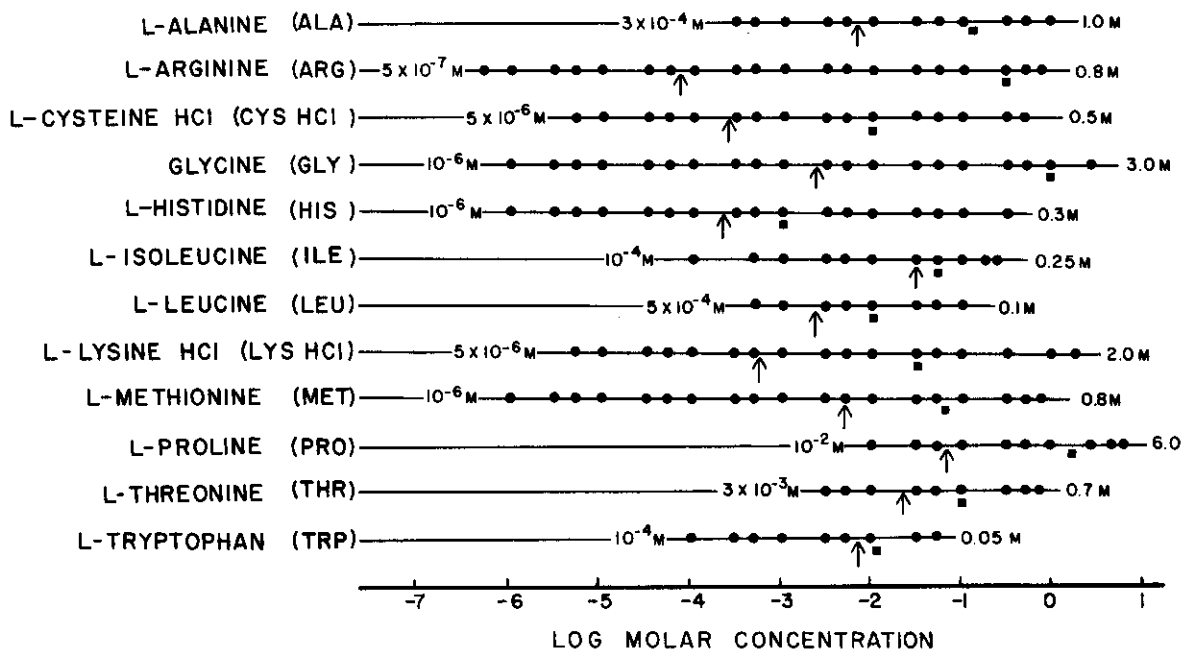



**Figure 1.** Summary of the name, abbreviation, concentration range, neural threshold (arrow) and half-maximum concentration (square) for each amino acid.

*Self Regulation of Amino Acids.* An adequate supply of amino acids is necessary for normal protein anabolism as well as construction of other important nitrogenous compounds. Although most of these amino acids are obtained through catabolism of ingested protein sources, others freely occur in plant and animal tissue (80). The ingestion of some amino acids is considered "nonessential" in the sense that these molecules can be synthesized from other sources within the body; other amino acids which must be derived from the diet are referred to as "essential." However, the terms "essential" and "nonessential" refer only to the ability of the body to synthesize particular amino acids; both types are critical for normal development. Failure to ingest the essential amino acids in sufficient quantity (and in proper balance) can result in retardation of growth and depression of food intake (66) as well as in metabolic disorders, histopathology and death (27).

Evidence that individual amino acids can be effective taste stimuli for the rat comes from studies which have imposed dietary deficiencies, presumably resulting in the pathologies mentioned above. When subsequently offered various diets with specific amino acids available, rats made a nutritionally balanced selection over a period of several days (26, 41). By doing so they resumed normal growth and restored the sensitive balance of plasma, muscle and brain amino acid levels which were disrupted during the period of deficiency (42, 53). Moreover rats exposed to an amino acid imbalanced diet developed a conditioned aversion to it (41) which encouraged them to sample other, possibly replete food sources. Upon encountering the corrected diet, they developed a conditioned preference which resulted in the restoration of their physiological balance even though the diets were not labeled with any flavors other than those of the amino acids themselves (65).

Since so little is known of the neurobehavioral correlates of amino acid perception, our studies had to begin with basic issues: 1) What are the effective neural and behavioral concentration ranges for these stimuli? 2) What preferences do rats show for this important class of nutrients and how do preferences change with concentration? 3) What neural coding characteristics do amino acids demonstrate in the peripheral nervous system? 4) In what ways does the rat's qualitative perception of these amino acids differ from the human perception? Accordingly, to what extent can neural processes in the rat be generalized to those of humans?

### 1. Chorda Tympani Multiunit Responses to Amino Acids

Schiffman and colleagues (69, 71) have shown in a series of psychophysical experiments that amino acids, when used as gustatory stimuli, vary widely in threshold as well as in perceived intensity at equimolar, suprathreshold concentrations, In a study by Halpern et al. (24),

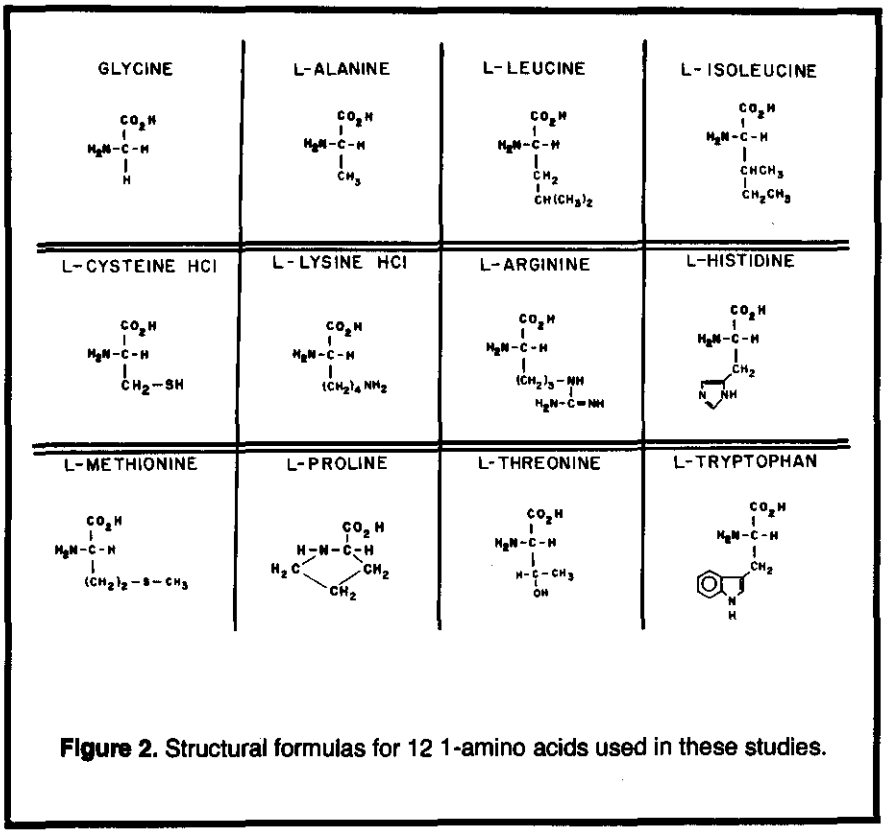



**Figure 2.** Structural formulas for 12 1-amino acids used in these studies.

which included both electrophysiological and behavioral measures, similar results were obtained for the rat. However, since the stimulus battery used by Halpern et al. was small and consisted mostly of nonphysiological d and dl isomers, comprehensive data are still lacking for the rat. Thus, the effective concentration range for most amino acids is unknown. For investigators wishing to expand their battery of gustatory stimuli to include amino acids, these data are prerequisite. In our first two experiments we defined the effective concentration range of 12 l-amino acids electrophysiologically and behaviorally.

The 12 amino acid stimuli were all l-isomers except glycine which has no optical rotation. Two were monohydrochloride derivatives: cysteine hydrochloride and lysine hydrochloride. The range of concentrations used in this experiment extended from below the neural threshold for chorda tympani

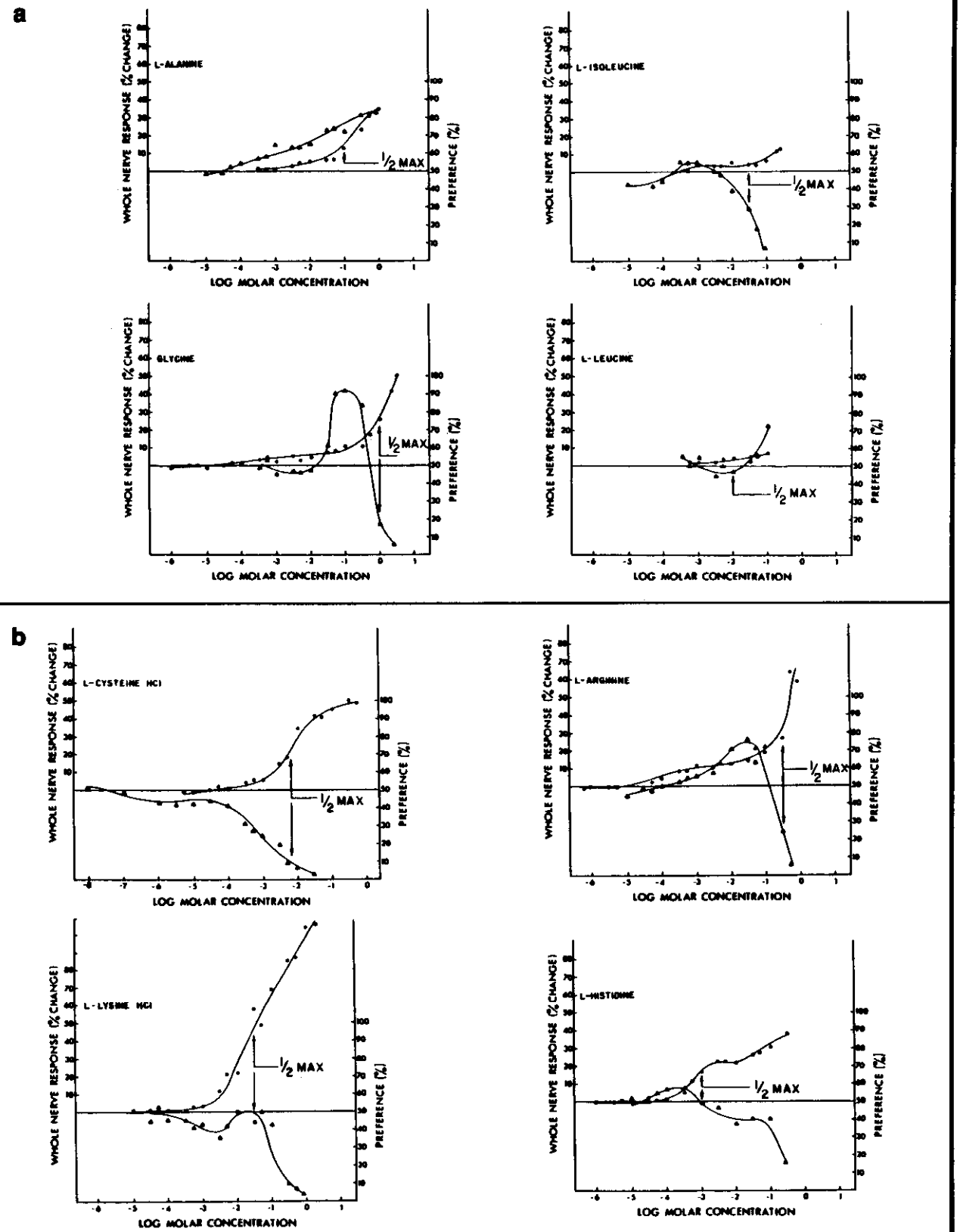
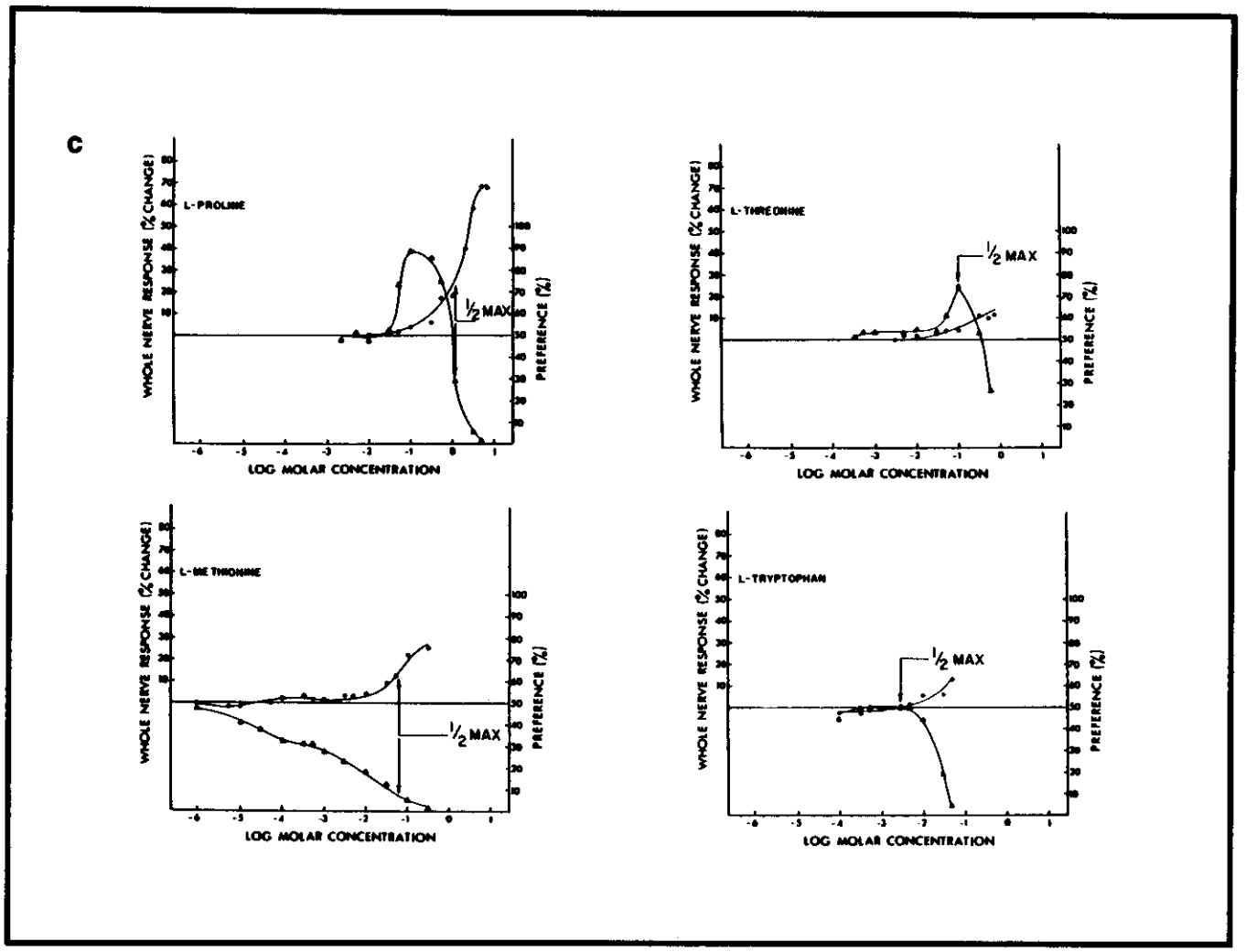



**Figure 3.** Multiunit response magnitude (left ordinate) and percent preference (right ordinate) of each amino acid plotted as a function of stimulus concentration. The neural response magnitude was plotted as a percent increase above the resting level of multiunit activity. Neural: o—o Behavorial: △—△ Figure 3a at top, 3b at bottom, 3c on page 6.




(CT) activation up to the saturation limit for each solution. Each stimulus with its abbreviation and range of concentrations is shown in Figure 1. Chemical and structural formulas appear in Figure 2.

We anesthetized each of 80 adult, male Sprague-Dawley rats, placed it in a headholder and drew its tongue into a glass flow chamber for stimulation. We then exposed the CT nerve, cut it near its entrance to the bulla and draped it over an uninsulated tungsten electrode.

We amplified, filtered and displayed the neural response to each stimulus using standard electrophysiological techniques. We also stored the neural and stimulus onset marker signals as well as voice commentary on a four channel AC tape recorder for later analysis. The neural detection threshold was designated as a one standard deviation increase in neural activity above the average spontaneous activity maintained over five sec.

Of the 12 1-amino acids we examined, the lowest thresholds were for those stimuli having either an HCl radical (Cys HCl, Lys HCl) or basic side chain (Arg, His). This relationship was not a direct pH effect since both His (pH = 7.65) and Cys HCl (pH = 1.80) had essentially the same threshold. Similarly, Schiffman et al. (71) have also found acidic or basic side chains or an HCl radical to be characteristics of low threshold amino acids for humans. They also reported that the highest thresholds were for amino acids typically described by human subjects as sweet: Gly, Ala, Thr and Pro. Those same sweet amino acids in this experiment had thresholds approximately two log units above the low group. Other non-sweet amino acids also had high thresholds, however, thus eliminating any simple axiom describing threshold in terms of either pH or quality.

*Intensity–Response Functions.* Figures 3a-c show the magnitude of the neural response elicited by each stimulus plotted as a function of stimulus intensity. Except for Figure 3c, the stimuli are grouped into each graph according to structural or psychophysical criteria. Figure 3a shows the neural response functions for Gly, Ala, Ile and Leu. All four amino acids have aliphatic side chains but it is noteworthy that their stimulating ability decreases with an increase in carbon chain length: Gly > Ala > Leu = Ile. Studies by Halpern et al. (24) and Yoshii

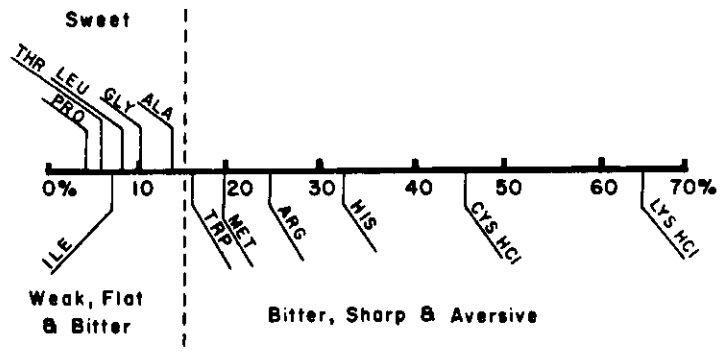



**Figure 4.** Response magnitude of each amino acid at 0.1 M plotted as a percent increase over the resting activity of the CT. Qualitative descriptions are derived from the human psychophysical literature. Trp was only soluble to 0.05 M. Its projected effectiveness at 0.1 M is shown here.

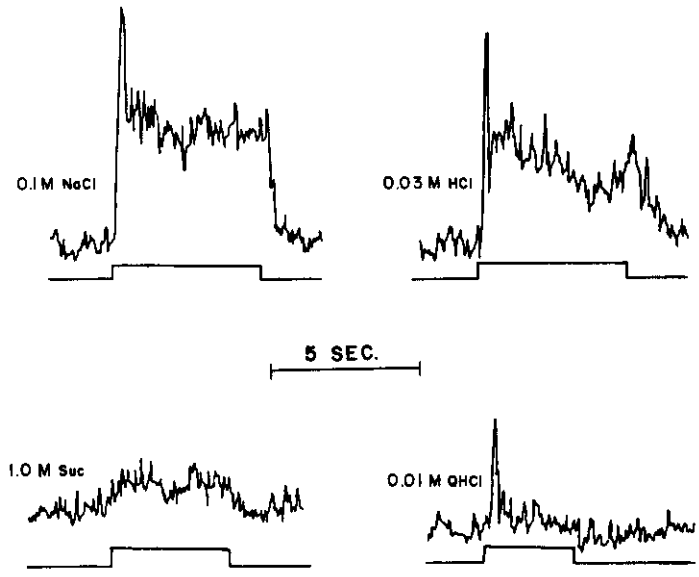



**Figure 5.** Integrated, multiunit CT responses to the four primary taste stimuli. Clockwise from the upper left: 0.1 M NaCl, 0.03 M HCl, 0.01 M QHCl, 1.0 M sucrose.

et al. (90) have also reported a loss of stimulating effectiveness for amino acids during ascension in a homologous chemical series.

The stimuli included in Figure 3b have either a basic side group (His, Arg), an HCl radical (Cys HCl) or both (Lys HCl). These pH-related stimuli as a group not only had the lowest thresholds but ranked 1st, 3rd, 4th and 6th in terms of response magnitude at their maximum concentrations. The stimuli which ranked 2nd and 5th, Pro and Gly respectively, achieved that status largely through their free solubility which allowed testing into the 5-6 molar range. This effect of concentration upon the maximum response is evident only for amino acids which are not pH-related. The Pearson product-moment correlation between maximum response and solubility (g/l at 23°C) was non-significant for the pH-related stimuli in Figure 3b ($r = +.08$, $N = 4$) but was highly significant for the remaining stimuli ($r = +.83$, $t = 4.39$, $p < .01$, $N = 8$).

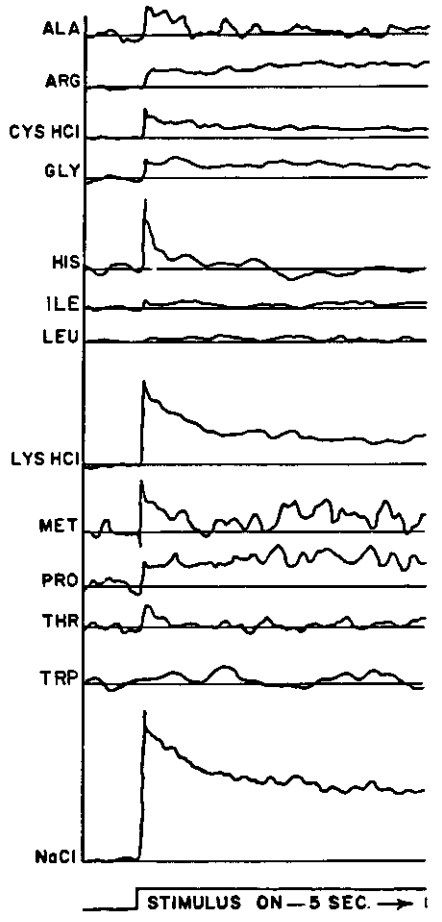
The neural response functions for the four remaining amino acids, Thr, Trp, Pro and Met, which have few chemical or psychophysical attributes in common, are shown in Figure 3c. Neither Trp, which is a heterocyclic amino acid, nor Thr, which contains a hydroxyl group, was an effective stimulus at CT. Met resembles Cys HCl in that they both contain a sulfur atom, but Met has a pH close to 7 and perhaps for this reason is a much less potent stimulus. Pro, the only amino acid included in this study, was an effective stimulus, but only at concentrations exceeding 1 M.

The analysis so far has been drawn from the data contained in Figures 3a-c. These intensity—response curves not only address questions concerning thresholds and maximum responsiveness, but also the issue of stimulus effectiveness. For example, the aliphatic amino acids exhibited their most dynamic increases in the neural response at concentrations approaching their solubility limit. This contrasts with Lys HCl, Cys HCl and His which were more effective stimuli because they had a wider dynamic response range not totally dependent upon the solubility limit of the chemical itself. An appropriate measure of stimulus effectiveness would be a comparison of the response magnitude evoked by an equimolar concentration of each chemical. This analysis is summarized in Figure 4 for a 0.1 M concentration of each amino acid. The most ef-

fective stimuli were those with acidic properties, Lys HCl and Cys HCl, followed by the two basic amino acids, His and Arg. All four of these chemicals impart a bitter, sharp and aversive taste in human subjects. This contrasts with the amino acids at the ineffective end of the spectrum which humans report as either sweet or weak, flat and bitter. The aliphatic amino acids are in this group. Although several organizing principles are no doubt responsible for this continuum of neural effectiveness, the most obvious one is molecular weight. The lighter amino acids were generally ineffective while the heavier ones evoked moderate or even robust responses. The Pearson product-moment correlation between molecular weight and neural effectiveness was significant ($r = +.71$, $t = 3.19$, $p < .01$, $N = 11$).

*The Half-Maximum Response Concentrations.* Further investigations whether at the behavioral level or at the level of single neurons, will often require the use of a single concentration for each stimulus. The convention with other stimuli has been to use the concentration which evokes one-half of the maximum CT whole nerve response (½ max) (13, 15, 54). This ensures that the concentration selected will be in the nervous system's responsive midrange and therefore within acceptable physiological limits. The concentration which produced the ½ max response for each amino acid is marked by a square in Figure 1.

*Time Course Analysis.* Sensory neurons in almost every modality exhibit at stimulus onset a rapid, phasic burst of activity followed by a prolonged, but less vigorous, tonic response. This pattern of responding is evident in the gustatory system but the balance between phasic and tonic periods varies as a function of the stimulus. The time courses of individual applications of NaCl, HCl, sucrose and QHCl to the rat shown in Figure 5 illustrate some of the different response patterns commonly observed in the gustatory system. The neural response to HCl and NaCl is typically rapid but HCl, as with most acids, maintains a lower tonic response level. QHCl shows an even greater relative reduction than HCl in the tonic response. Sucrose and many other sugars evoke responses which build over the first few seconds. Each time course is consistent for each stimulus and often generalizes to other stimuli of the same quality (e.g., sucrose→fructose and



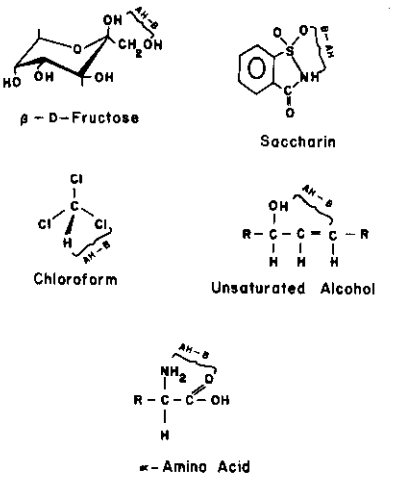




**Figure 7.** Molecular structures of five sweet chemicals with the AH-B complex designated for each. From Shallenberger and Acree (77).

**Figure 6.** Integrated, multiunit CT activity evoked by each of the stimuli of Experiment 1. All records represent the neural activity of the 1/2 max stimulus concentration. Each record represents a computer average of from 2-10 stimulus applications, adjusted in size relative to the 0.1 M NaCl comparison stimulus.

$HCl \rightarrow HNO_3$). Among amino acids there was excellent consistency in the time courses among the individual applications of each stimulus. Figure 6 shows the average time course for each amino acid at its ½ max concentration. Both Lys HCl and Cys HCl evoked a strong transient response which decayed slowly until a moderate tonic level of firing was achieved. This contrasts with Ala, His, Met and Thr which produced only a minimal tonic response after a rapidly decaying phasic period. Perhaps the most unusual time courses were produced by Pro, Arg and to some extent by Gly. The neural response to Gly did not decay appreciably beyond the phasic period and for Arg and Pro, the response actually increased. A similar effect has been reported for Gly by Halpern et al. (24) but not by Tateda (85). These time courses reflect single neuron latencies and will be an important control for sampling bias during discussion of the single unit analysis of the same amino acid stimuli.

From the whole nerve response it is clear that amino acids represent a diverse class of gustatory stimuli. Some amino acids had an effective concentration range as narrow as .74 log molar units (Trp) while others were neurally effective across almost four log molar steps (Arg). Neural thresholds ranged from $9 \times 10^{-5}$ M for Arg to $8 \times 10^{-2}$ for Pro. This variability is as extreme as that observed in rats and humans when simpler acids, salts, sugars and alkaloids are used as gustatory stimuli.

### 2. Taste Preferences for Amino Acids

*The Chemistry of Taste Quality.* Although the molecular bases of taste quality is still a poorly understood area, there are certain organizing principles of chemical structure which do have some predictive value. The relationship between salty and sour tastes and ionized salts and acids has been recognized for over one hundred years (45, 67, 86). More recently Shallenberger and Acree (74-77) have provided a description of sweet taste based upon an AH-B triad where A and B represent electronegative atoms and AH is a covalent bond to a hydrogen atom. The critical feature of this complex is the distance between the AH and B components which must be close to 3 nm in order to support a hydrogen bond. Figure 7 shows the position of the AH-B system in several sweet molecules. Moncrieff (45) has approached the physical substrate of taste quality at a molar level by considering the interaction of specific groups within the molecule. Each of these approaches can contribute to an understanding of amino acid taste quality in humans and presum-

ably in rats.

As with all gustatory stimuli each amino acid achieves its taste quality not only by its constituent parts, but also by their spatial configuration. This is best illustrated by the structural relationship between acetic acid and Gly (see Figure 8a). Acetic acid is a very sour stimulus due mostly to its acidic carboxyl group. The addition of an amino group to acetic acid in Gly offsets the action of the carboxyl group and results in a sweet taste. The same effect is also noted for propionic acid (with Ala the resultant amino acid; see Figure 8b) as well as many hydrazides, amides and ureas. The change in taste quality from acetic acid to Gly (and other $\alpha$-amino acids) is due mostly to the proximity of the $NH_2$ and COOH groups which form an AH-B system. In amino acids where $NH_2$ is attached to either the $\beta$ or $\gamma$ carbon, proximity is lost, the hydrogen bond is not formed and a sweet taste seldom results (see $\beta$-Ala in Figure 8c). A second factor responsible for the sweet taste of Ala and Gly is their low molecular weight. The R groups for both amino acids are very small and hence cannot overpower the sweet taste of the $-CNH_2COOH$ core. In larger amino acids the sweet core is only a minimal part of the molecule and thus does not dominate the overall taste quality. It is commonly observed among amino acids, glycols and other gustatory stimuli that as the carbon chain length or molecular weight increases in a homologous series, an initially sweet stimulus gradually becomes either bitter or tasteless. Carbon chain branching can have a similar effect upon taste quality (45).

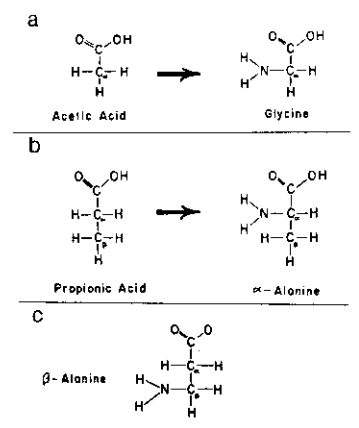



**Figure 8.** The relationships between acetic and propionic acids and their amino acid derivatives. The molecular configurations of Gly and $\alpha$-Ala are illustrated in Figures 8a and 8b. The impact of the $-NH_2$ group upon the taste of each amino acid is directly related to its proximity within the molecule to the acidic -COOH pole. $\beta$-Ala, shown in Figure 8c, is less sweet than $\alpha$-Ala because these groups are more widely separated.

The taste quality of the aliphatic amino acids is consistent with the generalizations for molecular weight and carbon chain branching already outlined. The decreasing order of sweetness for Gly, Ala and Leu corresponds to their increasing molecular weight with Leu, the largest of these stimuli only occasionally being reported as sweet (33, 89). Ile, the branched isomer of Leu, is always reported as tasting bitter (38, 69).

The sweetening ability of the basic amino group in the amino acid core is largely due to the resultant neutrality of the chemicals. Since the addition of a second or third amino group to an amino acid would shift the pH into the basic range where most stimuli are bitter, His and Arg are probably bitter. Although pH is related to the molecular species, it is also dependent on concentration. On this basis it could be predicted that at low concentrations where pH is close to 7.0, both His and Arg may be sweet. There are very few comprehensive studies relating taste quality of amino acids to concentration, but some of them do suggest this sweet/bitter continuum for Arg (38, 83). The impact of the second amino group upon the taste of Lys HCl is much more difficult to predict because of the buffering capability of the HCl radical. Humans report that Lys HCl elicits a complex taste with both salty and bitter components.

The hydroxyl group is a constituent part of many sweet tasting compounds including glycols, glycerines and sugars. Accordingly, the hydroxylic amino acids Serine and Thr are sweet (69, 71).

The presence of a sulfur atom in an aliphatic chain is usually sufficient to cause both an obnoxious odor and a bitter taste. This is a general observation among mercaptans, thiophenols, thioethers and disulfides. However, if the sulfur atom is fully oxidized or stabilized in a heterocyclic ring, the odor and taste may be very agreeable as in the case of sweet tasting saccharin. In Met and Cys HCl the sulfur atom is fully expressed and both stimuli are bitter and very aversive, although only Met acts as a strong odorant (69).

Pro and hydroxyproline are both extremely sweet stimuli but since they are the only imino acids, it is difficult to speculate about how their components interact to induce sweetness.

It has not been possible to link the chemical composition of aromatic stimuli to taste quality beyond the fact that most are either sweet or bitter. Trp is reported by human subjects to be flat and bitter (69).

A summary of the taste quality of these amino acids in humans and their extrapolation to the rat is included in Table 1. Generalization of taste quality from the human psychophysical literature to the rat is risky but may be justified on several grounds. The first is that rats treat the four classes of prototypical stimuli in much the same way as humans do. Sugars and salts are preferred at low concentrations and rejected at high concentrations; acids and alkaloids are avoided. Secondly, here is evidence at both neural and behavioral levels derived from rat, hamster, cat and monkey, suggesting that the organization of gustatory information into four discrete quality classes, sweet, sour, salty and bitter, is not unique to humans (17-19, 51, 56).

The purpose of our second ex-

periment was to determine the preference behavior of the rat for this array of amino acids and to compare these data with the predictions of taste quality based upon the human psychophysical literature.

The preference test was a 24-hour two-bottle choice in which one bottle contained the test stimulus and the other distilled water ($DH_2O$) (62, 63). Subjects were 30 male albino rats. For those in the experimental group the test stimuli were the same 12 amino acids as were included in the whole nerve study. Control subjects were offered $DH_2O$ in both bottles. Each stimulus concentration was tested for 36-108 rat-days during which the positions of the amino acid test solution and the $DH_2O$ tube were counterbalanced to offset possible side preferences. This experiment spanned 401 days of which 378 were actual test days.

*Behavioral Thresholds.* The criterion for designation of either a preference or aversion threshold was based upon the variability of control subjects (one standard deviation or 7.5%) around a 50% chance level. The control subjects actually consumed 50.35% of their total fluid intake from the test stimulus bottle ($DH_2O$) (N = 930 rat-days).

The pH-related amino acids were predominant in the group of amino acids evoking low threshold behavioral responses (see Table 2). This is consistent with the electrophysiological data presented earlier and the human psychophysical literature (38, 71, 89). The amino acids which evoked high behavioral thresholds in this experiment were Leu, Thr, Gly and Pro. Each of these amino acids was found by Schiffman et al. (1979) and Kirimura et al. (38) to be a high threshold, sweet stimulus for human subjects except for Leu which was described as bitter. Most of the other human literature also describes Leu as bitter (16, 33, 38, 83, 89), but the preference data in this experiment do not support this finding (see Figure 3). Leu is never rejected by rats as a bitter stimulus would be, and in fact, is preferred at high concentrations. The Pearson product-moment correlation between the behavioral thresholds of the rat and the human psychophysical thresholds reported by Kirimura et al. (38), Yoshida and Saito (89) and Schiffman et al. (71), was +.57 ($t = 2.19$, $p < .025$, N = 12). As Table 2 shows, the behavioral thresholds of the rat for Met and Ala were much lower than the corresponding thresholds of human subjects. However, for the remaining 10 amino acids the human psychophysical thresholds were only an average of 4.1 times higher than those for the rat. This difference is smaller and a bit more consistent than that observed between the neural and behavioral threshold data we previously obtained for the rat. There was no consistent ordering of neural and behavioral thresholds for the rat and differences larger than 1 log unit in molar concentration occurred for Cys HCl as well as for Met and Ala.

The pH-sweetness continuum of threshold reported here for the rat is only a general scheme representing the extremes of threshold sensitivity. In humans, on the other hand, the entire pH-sweetness continuum can be translated into a molecular weight effect. As the molecular weight increases, threshold generally decreases ($r = -.73$, $t = 4.94$, $p < .001$, N = 12) while the taste quality changes from sweet to bitter or tasteless. The low correlation between molecular weight and threshold found in this experiment ($r = -.30$, nonsig) may be an indication that the two-bottle preference test provides a rather imprecise measure of threshold. It is unlikely that the two-bottle preference test has the same power as the human verbal report especially at low concentrations where some stimuli may be hedonically neutral.

**Table I**

| Amino Acid | Predicted Quality for the rat | Basis for Predicted Quality | |
|---|---|---|---|
| | | Molecular | Human Psychophysical |
| Ala | sweet | low molecular wt./AH-B complex. | Schiffman, 1975 |
| Arg | sweet @ low conc. bitter @ high conc. | effect of $-NH_2$ at neutral pH. high pH stimuli are bitter. | Kirimura et al., 1969 |
| Cys HCl | bitter or sour | sulphur atom/ HCl radical. | Schiffman, 1975 |
| Gly | sweet | low molecular wt./ AH-B complex. | Schiffman, 1975 |
| His | sweet @ low conc. bitter @ high conc. | effect of $-NH_2$ at neutral pH. high pH stimuli are bitter. | ------- |
| Ile | bitter | branching of aliphatic chain. | Schiffman, 1975 |
| Leu | weak sweet | relatively low molecular wt./ AH-B complex | Kaneko, 1938 |
| Lys HCl | salty or bitter | ------- | Schiffman, 1975 |
| Met | bitter | sulphur atom | Schiffman, 1975 |
| Pro | sweet | ------- | Schiffman, 1975 |
| Thr | sweet | -OH group | Schiffman, 1975 |
| Trp | bitter | ------- | Schiffman, 1975 |

*Hedonic Characteristics.* The data for low suprathreshold concentrations (< 0.05 M) clearly support the predictions of taste quality for the rat listed in Table 1. The aliphatic amino acids, Gly, Ala and Leu, were each preferred by the rat in the same order that human subjects rank them according to sweetness (89). Ile, the only bitter aliphatic amino acid, was rejected by the rat at all concentrations. Thr and Pro, both sweet tasting stimuli to humans, were also preferred. The sulfurous amino acids, Met and Cys HCl, which were predicted to taste either bitter or sour, were avoided. It was hypothesized that Arg and His may taste sweet at low concentrations where pH = 7.0. Although both stimuli were preferred at low suprathreshold concentrations, there was no apparent relationship to pH. His became aversive at a concentration of 0.001 M (pH = 7.52) whereas Arg was preferred up to 0.16 M (pH = 11.0). Trp, a bitter tasting stimulus to humans, was rejected at all suprathreshold concentrations. No amino acid once rejected was ever accepted at a higher concentration, though Lys HCl did assume a hedonically neutral taste at a concentration both above and below which it was avoided.

So far we have found an apparent similarity between the human psychophysical reports and the data derived from the rat at both neural and behavioral levels. In light of this it may seem initially perplexing that the similarity between the rat neural and behavioral thresholds themselves is less striking. However, Pfaffmann (59), who first reported the same disparity of neural and behavioral thresholds, has shown one rather obvious reason for this occurrence (60). The CT does not carry all of the afferent gustatory activity of the oral cavity. Approximately ⅓ of the tongue and the entire perilingual area are innervated by fibers of the glossopharyngeal and vagus nerves. For the glossopharyngeal nerve the neural thresholds for QHCl and HCl are almost one log unit lower than those observed during neural recording from the CT for the same stimuli. When the behavioral thresholds are compared with both the CT and glossopharyngeal neural thresholds, much better agreement is found (60). Since the neural activity of CT is largely gustatory and the behaving subject is utilizing the broader perception of flavor, we should expect less than complete correspondence of neural and behavioral data. Flavor is an amalgam of gustatory, olfactory, tactile, temperature and even visual information. To the extent that nongustatory processes contribute to the flavor of an amino acid, the neural and behavioral data will appear disparate. For Met in particular the strong, sulfurous odor may have been responsible for the very low behavioral threshold observed in our preference study.

Most early reports of amino acid taste quality in humans expressed the generalization that d-isomers taste sweet and l-isomers bitter (4, 33, 34). This view has been tempered somewhat by more recent studies which have shown that l-isomers often have a sweet or sour taste (69, 83). The lack of a consensus may be due to differences in concentration which in the present experiment exerted a profound effect upon preference behavior. Of the seven amino acids that were initially preferred at low suprathreshold concentrations, five were subsequently avoided at higher concentrations (see Figure 3a-c). This change in preference behavior occurs on the average at only a 16% activation of CT, well below the average ½ maximum activation for these stimuli, 22.5%. For this reason it is unlikely that the reversal of preference observed for these five stimuli was due to overstimulation with unreasonably high concentrations. A more likely explanation would be either mediation by postingestional factors, a concentration dependent shift in quality, or both.

Research by McCleary (44), Pfaffmann (58) and Oakley and Pfaffmann (50) shows that the decline in taste preference observed in rats for fructose, glucose, sucrose and sodium chloride begins as these stimuli become hypertonic. Furthermore, this is a cumulative effect of fluid consumption directly related to the osmotic pressure of the stomach contents (44, 78, 82).

**Table II**

| | AMINO ACID | MOLAR CONCENTRATION AT THRESHOLD ($10^{-3}$ M) | | NEURAL THRESHOLD ($10^{-3}$ M) | HUMAN TASTE THRESHOLD / RAT BEHAVIORAL THRESHOLD |
|---|---|---|---|---|---|
| | | EXPERIMENT 2 | HUMAN PSYCHOPHYSICS* | EXPERIMENT 1 | |
| AMINO ACIDS with a BASIC SIDE CHAIN and HCl RADICAL | Cys HCl | 0.005 | 0.02 | 0.3 | 4.0 |
| | His | 0.2 | 2.6 | 2.6 | 13.0 |
| | Lys HCl | 0.4 | 3.0 | 0.8 | 7.5 |
| | Arg | 1.3 | 4.6 | 0.1 | 3.5 |
| AMINO ACIDS with an ALIPHATIC SIDE CHAIN | Ala | 0.2 | 19.6 | 8.0 | 98.0 |
| | Ile | 4.0 | 15.4 | 30.0 | 3.8 |
| | Gly | 25.0 | 46.0 | 3.5 | 1.8 |
| | Leu | 56.0 | 24.0 | 2.5 | 0.4 |
| OTHER AMINO ACIDS | Met | 0.002 | 5.4 | 7.0 | 2700.0 |
| | Trp | 9.0 | 7.7 | 9.0 | 0.9 |
| | Pro | 10.0 | 47.0 | 80.0 | 4.7 |
| | Thr | 30.0 | 50.0 | 20.0 | 1.7 |

*Geometric means of human threshold values reported by Yoshida and Saito (89), Kirimura et al. (38) and Schiffman et al. (71).

As such, the postingestional effects of hypertonic taste stimuli are most clearly seen in long-term preference experiments, such as the 24 hour two-bottle test. In the present experiment Gly, Pro, Arg and Thr, which underwent a reversal of acceptance, were most preferred at approximately 0.1 M which is nearly isotonic; only one amino acid, Ala, was preferred at clearly hypertonic concentrations. Shuford's data suggest that the reversal of preference for sucrose and glucose due to osmotic pressure is not mediated by a change in taste quality (78). Nevertheless, with these amino acids about which much less is known, this conclusion may be premature. Stone (83) has already shown that minor taste components of several amino acids may become more fully expressed as the concentration is increased. The nonmonotonic preference curves of Lys HCl, Arg and His may be the result of a quality change due perhaps to their widely fluctuating pH values. For Gly, Pro and Thr the issue is less clear. We will examine the relative taste qualities of these amino acids in our final study and from those data some insight is possible into the stability of these qualities as concentration changes.

### 3. The Neural Coding of Amino Acid Taste Quality

The whole nerve activity we recorded earlier is suitable for determination of neural thresholds, time courses and effective concentration ranges of gustatory stimuli. It is also directly related to perception of stimulus intensity (5, 81). However, the whole nerve technique, by recording averaged signals, misses the activity of individual neurons, the basic units for transmission of information by the nervous system. As a result, it is unable to address many sophisticated questions and areas of inquiry. Single unit recording techniques, on the other hand, tap the information carried by individual neurons and enable study of such neural coding issues as the basis for sensory quality.

The earliest single unit investigations of quality coding in gustation by Zotterman (91) and Pfaffmann (56) established that neural coding of sensory quality is not organized as a labeled-line system of the type envisioned by Mueller and later by von Frey (6). When stimuli representative of the four putative basic taste qualities (salty, sour, sweet and bitter) were applied to the tongue, single axons of the glossopharyngeal and chorda tympani nerves were more often responsive to several stimuli than to one. This broad tuning of single neurons has subsequently been verified in many other species and at several levels of the nervous system (Benjamin (3), rat ventrobasal thalamus; Nagaki et al. (48), cat chorda tympani; Norgren and Pfaffmann (49), rat parabrachial nucleus; Smith et al. (79), hamster nucleus of the solitary tract). As a result of this discovery, Pfaffmann (56, 57) suggested that stimulus quality is not represented by the activity of any single neuron but rather by the pattern of activity among many neurons. This hypothesis has since been elaborated upon by Erickson (13, 14, 88) and is now called the across-fiber pattern theory (AFP). According to the AFP theory, stimuli which taste alike should evoke a similar pattern of activity in the neural population. The index of similarity used by Erickson is the Pearson product-moment correlation coefficient (10, 21, 73). A pair of stimuli evoking quite dissimilar patterns of neural activity would have a low or negative correlation and thus would not be expected to taste similar. Support for the AFP theory has been obtained by Ganchrow and Erickson (21) who found that at both CT and bulbar levels of the rat gustatory system, stimuli judged to be similar tasting by humans (45) and rats (46) evoked highly correlated profiles of neural activity. This effect was independent of stimulus intensity for those stimuli that maintain a stable taste quality over a wide concentration range. For stimuli such as KCl and sodium saccharin (NaSac) for which a concentration-dependent shift in quality has been reported for both humans (12) and rats (46), the neural profiles show a corresponding shift. For example, NaSac is sweet at low concentrations but becomes increasingly bitter at higher concentrations. The neural analog to this perceptual change is a shift in similarity of the NaSac pattern from sucrose to QHCl with increasing stimulus intensity.

The success of the AFP theory in gustation is due largely to its quantitative base which gives it good descriptive and predictive powers. Its descriptive capabilities are fully realized by using the coefficients of profile similarity to construct Euclidean coordinate systems. Within these Euclidean spaces, or taste spaces, gustatory stimuli (or even neurons) can be arranged according to their degree of similarity. Contiguous data points within the space represent stimuli (or neurons) which have similar profiles. Although the dimensions of this space are not specified by the computer program which constructs it, Schiffman and Erickson (70) have hypothesized that hedonic value, molecular weight and pH may represent three of the most important variables. The same program may be used for analysis of behavioral data (23, 43, 68, 69, 72).

The AFP theory is also very attractive from a theoretical point of view. It is an efficient mode of information transfer yet can provide the redundancy necessary for reliable operation (55). In addition, the AFP theory has the capability of encoding many diverse qualities, an important characteristic for the gustatory system which, in the rat, operates with only several hundred peripheral neurons.

In the following two experiments we investigated the specific taste qualities of amino

acids, first neurally, then behaviorally, as analyzed by the AFP theory.

We isolated 40 single units from the CTs of 32 adult, male, Sprague-Dawley rats by carefully dissecting small nerve fascicles free from the larger nerve trunk. The stimuli were 0.1 M NaCl plus the same 12 amino acids used in the first experiment, each at its ½ max concentration (See Figure 1).

*Stimulus Effectiveness.* The 12 amino acids we applied to the tongue ranged widely in effectiveness. Arg, Pro, Lys HCl, Cys HCl and Gly each evoked responses in more than 70% of the units tested, while His, Met, Trp, Ile and Leu produced responses in fewer than 20%. This index of stimulus effectiveness correlated +.90 with that obtained by ranking each amino acid according to its magnitude of multiunit response at the ½ max concentration (Figure 3).

*Time Course Analysis.* The time courses of single unit responses were complex and varied enormously across amino acids. Some responses (Lys HCl) were purely phasic while others (Pro, Gly) were often long-latency tonic (Figure 9). These time courses have been observed by other investigators but only as isolated events. In the present experiment 59.1% (123 of 208) of the responses obtained were of these types; an additional 7.7% (N = 16) were inhibitory responses. Thus, the majority of responses evoked by these amino acids were not of the short latency, phasic/tonic genre commonly observed in the gustatory system when other simple salts, acids and sugars are used as stimuli.

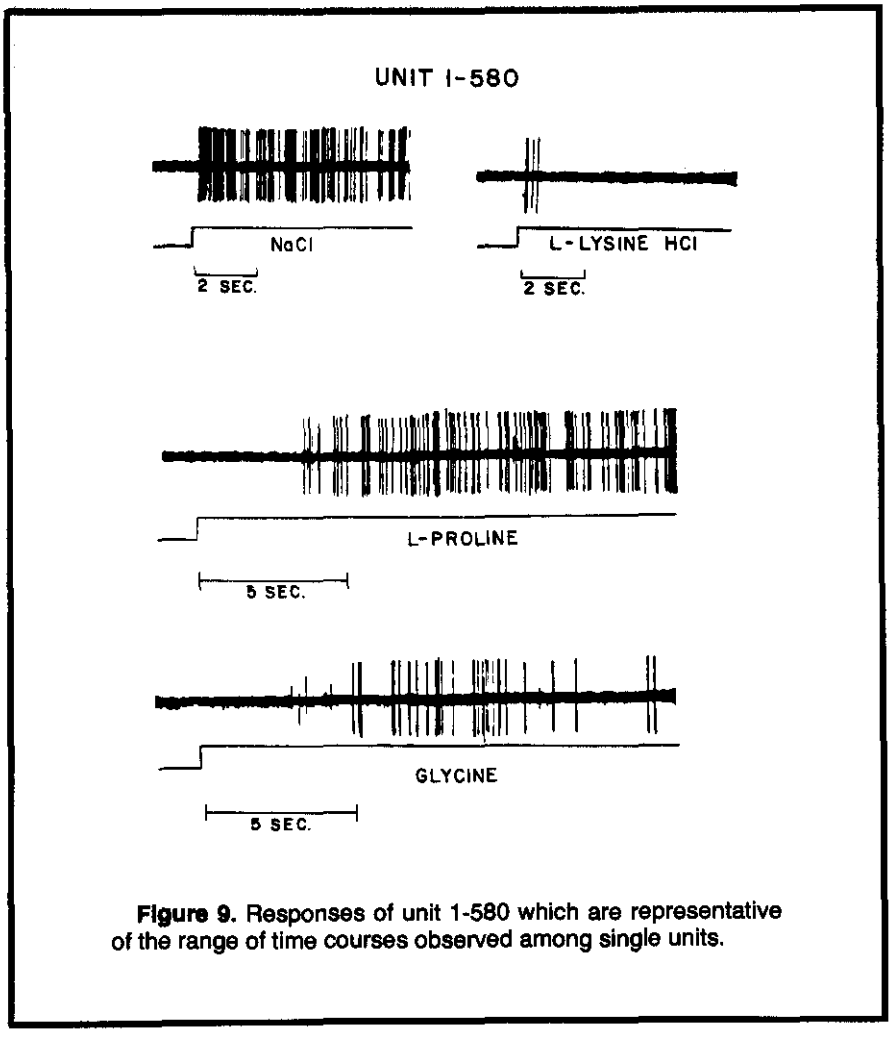



**Figure 9.** Responses of unit 1-580 which are representative of the range of time courses observed among single units.

*Latency of Response.* Response latency at the CT may be as short as 25-30 msec when the tongue is stimulated with various electrolyte solutions; sugars produce longer latencies but even these rarely exceed 500 msec. Thus it is extraordinary that 32.7% (N = 67) of the response latencies in this experiment exceeded 1 sec and 25% (N = 52) exceeded 2 sec. Long latency responses (> 1 sec) were characteristic of particular stimuli rather than individual neurons. Sixty-one percent (41 of 67 cases) of the long latency responses reported here were associated with either Gly or Pro. More than one-half of the cells which responded to either Gly or Pro (51.1%) showed latencies greater than 1 sec and these ran to as long as 25 sec. While it is possible that such extreme latencies could result from stimulation of free nerve endings (2, 37) several lines of evidence make this unlikely. First, in spite of their high concentrations, neither Gly (1.0 M) nor Pro (1.15 M) are irritants which could theoretically permeate the tongue's surface to reach the free nerve endings. Both Arg (pH = 11.2) and Cys HCl (pH = 2.0), on the other hand, are caustic yet produced very few latent responses. Second, the fibers which showed the long latency responses had normal chemical sensitivity and in every case responded to at least one other chemical with a short latency, phasic/tonic response.

*Inhibition.* Reports of response inhibition at the level of the CT are somewhat scarce in part because of the low spontaneous rate of these fibers. In our recordings the mean spontaneous rate of the 40 single units was 5.45 spikes/5 sec. There were 13 fibers with a spontaneous rate greater than 4 spikes/5 sec which

could be analyzed for possible inhibitory responses. Of these 13 fibers, eight produced a total of 14 inhibitory responses, four of which are illustrated in Figure 10. There was no tendency for either specific stimuli or fibers to be associated strictly with response inhibition.

*PST Analysis.* The temporal pattern of evoked discharge, whether phasic or tonic, was primarily a function of the stimulus rather than the fiber. Cys HCl, His and Met evoked sharp phasic onsets from most cells while Lys HCl, Pro, Gly and Ala aroused almost no phasic bursts. These temporal characteristics generally agree with psychophysical reports of sharpness from humans. These features are being treated with increasing importance as researchers continue to discover that the time course of a response is a critical variable in coding taste quality and intensity (9, 25).

*Taste Space Analysis.* The three dimensional taste space based on these single unit data is shown in Figure 11. The three dimensions have been labeled A, B, C because no physico-chemical properties clearly define the axes.

In an ideal preparation all three applications of NaCl should be positioned in the same locus. In this space the existence of a relatively tight cluster indicates stability of the single unit preparation during the recording session.

Cys HCl and Lys HCl are grouped together in this space probably because of the HCl radical each has. However, this is not a pH effect because Cys HCl has a pH of 2.0 while the pH of Lys HCl is 5.8. Neither is their proximity due to their actual perception by rats. Cys HCl was strongly avoided by the rats in the preference study presented earlier; Lys HCl was almost neutral at the ½ max concentration.

On the basis of their behavioral and neural similarities, it was expected that Gly and Pro would form a tight pair in the taste space. The data from our final experiment will also support this view. The modest spread of Gly and Pro was most likely due to the fact that their long latency responses could not be included in this analysis.

Other psychophysical, behavioral and neural determinants of this taste space will be discussed later when the behavioral taste space is presented.

### 4. Taste Quality and Behavioral Responses

The measure of amino acid hedonics derived from our two-bottle preference tests, though consistent with predictions

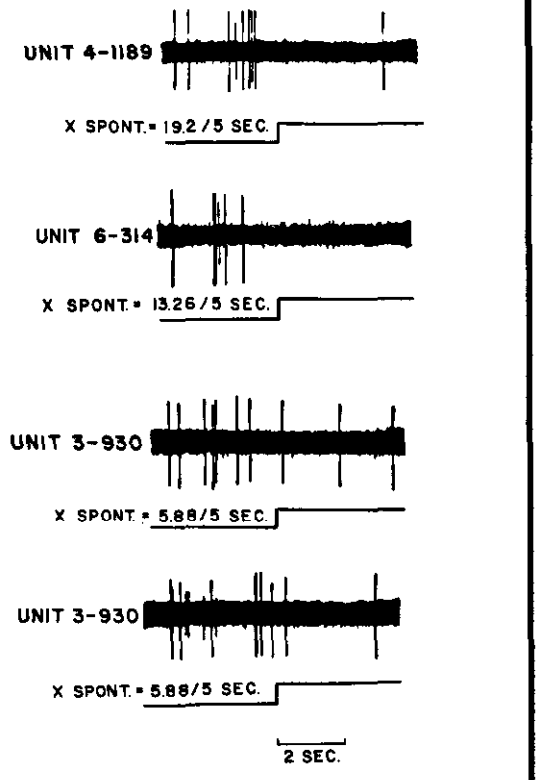



**Figure 10.** Inhibitory responses observed among single CT fibers.

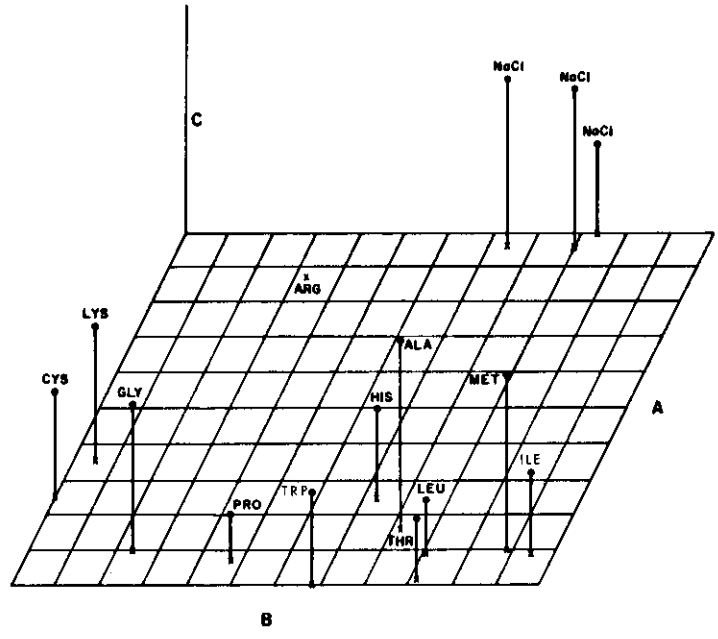
based upon human psychophysical reports, do not specifically describe taste quality. On the basis of a preference experiment alone a qualitative distinction cannot be made between two appetitive stimuli (e.g. NaCl and sucrose) or between two aversive stimuli (e.g. QHCl and HCl). It is possible to address this problem through an examination of the "baitshyness" effect which rats and many other animals demonstrate under both natural and laboratory conditions.

Baitshyness or a conditioned taste aversion (CTA) is formed when a novel food or liquid is paired with gastrointestinal malaise. The animal will subsequently avoid the conditioned substance even if it otherwise would be considered palatable. The conditioned aversion is very persistent but will eventually extinguish especially if the subject has access to the original taste cue when it is not paired with illness (7).

The ability of a conditioned aversion to generalize to other stimuli on the basis of their taste qualities has been demonstrated in several studies. This tendency serves as the basis for using a CTA paradigm to make a qualitative assessment of the rat's gustatory experience. If, for example, a CTA to amino acid "X" generalizes very strongly to NaCl, we infer that the rat perceives both NaCl and amino acid "X" similarly. Another amino acid "Y" may generalize equally to NaCl and sucrose. Therefore, to the rat amino acid "Y" does not taste exactly like either NaCl or sucrose but rather has a complex taste which is somewhat salt-like and sucrose-like. An analysis of the conditioned aversion generalization gradients for the stimuli in this experiment provides a measure of relative amino acid taste quality for the rat.

The subjects were 192 adult, male rats who were divided into 12 groups of 16 and assigned to either experimental (N = 160) or control groups (N = 32). The drinking schedule for each subject was manipulated by restricting its daily access to water to a 15 min session at 1100 hrs and a 1 hr session at 1600 hrs. Acclimation to this drinking schedule was complete in about seven days. The 1 hr drinking session was continued to ensure adequate hydration of the subjects and a stable level of fluid deprivation on a day-to-day basis.

After this shaping period each of the 16 subjects in an experimental group was given access to one of 16 CSs (the 12 amino acids plus NaCl, HCl, sucrose and quinine) during the 15 min drinking session. Each subject then received an intraperitoneal injection of LiCl (127 mg/kg), the UCS, to induce gastrointestinal malaise. Control subjects drank $DH_2O$ before the LiCl injection. Following a 48 hr recovery period each of the 16 test stimuli, including the CS, was presented in random order to each subject on a daily basis during the 15 min drinking session. Each chemical was used as both a CS and test stimulus in order to maximize the number of taste quality comparisons (both primary and complex) and thereby expand the descriptive power of the experimental paradigm. The amount of each test stimulus consumed was recorded to the nearest ½ ml. The same CS-UCS pairing was repeated for each subject after the fifth and tenth stimuli were presented in order to maintain a robust taste aversion.




TASTE SPACE GENERATED FROM CHORDA TYMPANI SINGLE FIBER ANALYSIS

**Figure 11.** Spatial representation of gustatory similarity for the stimuli included in the single fiber experiment. This taste space, constructed in three dimensions, accounted for 93% of the data variance; one and two dimension solutions accounted for 66% and 87% respectively.

*Data Analysis.* The strength of the CTA to a particular test stimulus was expressed in terms of percent suppression according to the following formula:

$$\% \text{ suppression} = \left(1 - \frac{^{ml}\text{CTA}}{^{ml}\text{Control}}\right) 100$$

High suppression scores represent strong generalization from the CS to the test stimulus thus

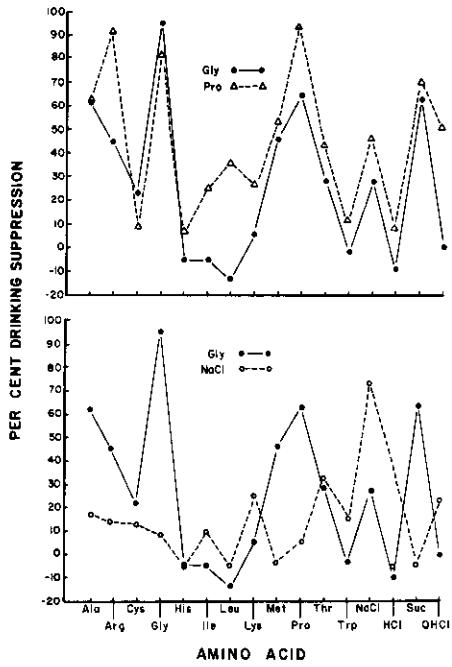



**Figure 12.** Spectrum of drinking suppression scores for two highly correlated conditioned stimuli (Gly and Pro) and two uncorrelated conditioned stimuli (Gly and NaC1). High correlations imply high qualitative similarity; low or negative correlations imply dissimilarity.

indicating a similar taste quality for each. However, a more comprehensive index of taste similarity for two stimuli would be a comparison of their suppression scores across an array of CSs. This is illustrated in Figure 12 for Gly, Pro and NaCl from data of the present experiment. Gly and Pro show a highly correlated pattern of suppression across the array of 16 test stimuli ($r = +.81$) suggesting a very similar taste quality for them. Gly and NaCl, on the other hand, show an uncorrelated pattern of suppression ($r = +.02$) characteristic of stimuli having unrelated taste qualities. Pearson product-moment correlations were calculated for each pair of test stimuli based upon their suppression scores across the array of 16 chemicals employed as CSs. These correlation coefficients were used to position each amino acid within a taste space.

A stronger aversion (indicated by percent suppression) was formed to the CS than to any other stimulus by the experimental subjects. This was expected because of the dominant role taste cues play in the formation and generalization of a CTA. However, the range of drinking suppression scores for the CSs (17.7 – 98.0%; $\bar{x} = 68.1\%$) clearly shows that each CS was not an equally effective cue. Previous investigators, also noting this fact, have described the most effective CSs as being very salient (32). Several studies have attempted to specify the relevant stimulus characteristic which makes one CS more salient than another during CTA training. CS intensity, duration and quantity have all been studied and found to be directly related to the degree of aversion produced, but it is unclear what common factor these variables may share (1, 11). It has been suggested that these quantitative aspects of the CS relate to palatability (22, 84) or the degree of neophobia rats readily demonstrate to novel stimuli (47). Other research by Kalat suggests that intense or prolonged CSs merely distinguish the cue from the background of daily experience (31).

In our experiment the stimulus characteristic which related most strongly to cue salience was hedonic intensity. Hedonic intensity was defined as the absolute difference in taste preference from hedonic neutrality (50%) based upon the two-bottle preference data presented earlier. The Pearson product-moment correlation between degree of CTA suppression during the CS retest and hedonic intensity was +.87 ($t = 5.58$, $p < .001$; see Figure 21). This does not necessarily counter or supersede results of previous studies which have identified other stimulus characteristics as the basis for CTA conditioning. It is possible that all of these factors (hedonic intensity, novelty, CS intensity, etc.) contribute to a stimulus ensemble from which cue salience emerges. As such, no single factor would be responsible for either making a tastant salient or determining the effectiveness of aversive conditioning. This view represents a step away from the current interpretation that salience is merely an abstraction and

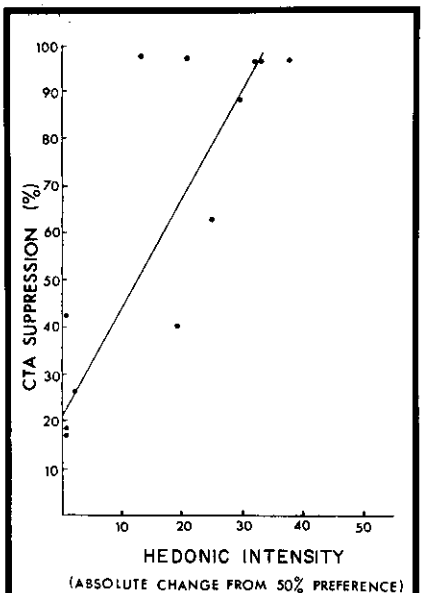



**Figure 13.** Percent suppression of drinking as a function of hedonic intensity. In general, subjects showed poor suppression when tested with CSs which were of low hedonic intensity (i.e. $30\% < x < 70\%$).

thus cannot represent a stimulus attribute. However, by recognizing cue salience as a relevant characteristic of the CS, the role of the CS in food aversion learning may be better understood.

*Taste Space Analysis.* Since multidimensional scaling techniques organize a data set in such a way that proximity represents similarity, the taste space in Figure 14 should receive corroborative support either from our electrophysiological or behavioral data presented previously or from the human psychophysical literature. The following taste space description proceeds from this assumption.

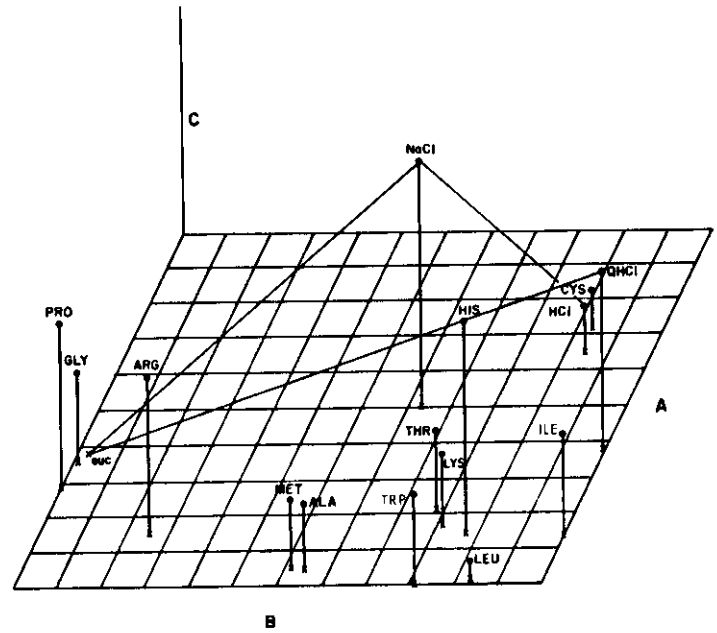
In the human psychophysical literature Gly and Pro are most often described as sweet (38, 69, 77, 89) and in this taste space both stimuli are within the same cluster as sucrose. The gustatory similarity of Gly and Pro is further emphasized by the electrophysiological and behavioral data presented earlier. Both stimuli showed very high neural and behavioral thresholds. At intermediate concentrations Gly and Pro were the two most preferred stimuli of the dozen tested but each became progressively more aversive at concentrations above .6 M. The integrated, multiunit CT responses of Gly and Pro both showed a moderate transient portion with a very high or even building sustained response. This has been verified at the single unit level where a significant number of CT fibers showed long latency building responses.

According to the psychophysical data collected by Kirimura et al. (38), Arg undergoes a qualitative change as concentration increases. The two-bottle preference data and the CTA taste space support the sucrose-like character of Arg at concentrations up to .1 M. At higher concentrations a saltiness or bitterness is reported (69). In the CT single unit taste space Arg shifted away from sucrose in the direction of NaCl because a higher concentration (.3 M) was used (rats would not consume .3 M Arg in the CTA study, requiring us to reduce the concentration in order to include Arg in the experiment). This concentration change is qualitatively significant because the rat's preference for Arg drops from 63% at .1 M to 24% at .3 M.

In human psychophysical experiments Cys HCl is most often described as sulfurous and complex rather than sour, salty or sweet (69). The contiguity of Cys HCl and HCl in this multidimensional space suggests that for the rat Cys HCl evokes a rather simple, acidic taste, almost primary in nature.

The neurally ineffective amino acids all cluster in the lower right hand corner of the CTA taste space indicating that they are behaviorally nondescript as well. Other amino acids which evoked moderate multiunit CT responses were positioned in this "neutral" corner if in the two-bottle preference test they were determined to be of low salience (i.e. $30\% < x < 70\%$). For example, Lys HCl which evoked the largest multiunit CT response of any amino acid tested, was hedonically neutral at its ½ max concentration, did not show good self-suppression in the CTA experiment, and thus moved away from Cys HCl, its neural neighbor, into the ineffective corner of the taste space. Histidine, another neurally effective stimulus, suffered a similar fate. In both cases a significant neural response and behavioral preference had been established at a lower concentration. Thus, a clear distinction seems to exist between the recognition of a taste and the salience of that taste in the subject's memory.

Ala and Met are contiguous in the CTA taste space just as they were in two dimensions of the CT single unit taste space. However, there are no other data which would support such a finding. Ala is preferred in the two-bottle preference test (75%); Met is rejected (9% acceptance). The human psychophysical liter-




TASTE SPACE GENERATED FROM CTA STIMULUS SIMILARITY GRADIENTS

**Figure 14.** Spatial representation of gustatory similarity for the stimuli included in the conditioned taste aversion study. This taste space, constructed in three dimensions, accounted for 88% of the data variance; one and two dimension solutions accounted for 69% and 83% respectively.

ature describes Ala as predominantly sweet whereas Met is described as sulfurous, obnoxious and bitter (69). In addition, Met has a distinct olfactory component which Ala lacks. Perhaps further studies will clarify this apparent paradox.

*Taste Constancy.* The qualitative information contained in the CTA taste space enables us to return to a question which arose before concerning taste constancy. Does the reversal of taste preference observed for Arg, Gly, Pro and other stimuli necessarily reflect a concentration-dependent shift in taste quality? As discussed above, the CT single unit and CTA taste spaces do indicate that Arg undergoes a shift in quality from sucrose-like to salt-like with increasing concentration. Gly and Pro, on the other hand, maintain strong sucrose-like qualities even though at these concentrations their preferences had dropped to 17% and 30% respectively. These data support Shuford's (78) findings that highly palatable but concentrated stimuli may be rejected during long-term preference experiments. However, further research is necessary to determine if the same mechanism, osmotic pressure, was responsible in this case.

*Taste Primaries and Multidimensional Scaling.* Perhaps the most protracted debate within gustation has centered around that of primary taste qualities. In the nineteenth century the debate was focused mainly on the number and type of primaries; today some investigators question their very existence (see Woolston and Erickson (88) for a comprehensive review). Nevertheless, most investigators today support the idea of taste primaries as Henning (28) described it in his taste tetrahedron. The corners of the tetrahedron are occupied by the primary taste qualities salty, sweet, sour and bitter. In 1916 Henning predicted that transitional or complex tastes would reside along the continuum between two primary tastes or on one face of the tetrahedron bordered by three primary tastes. The gustatory stimuli considered to be the prototypes for the four primary qualities are NaCl, sucrose, HCl and QHCl.

Each of the recognized primary taste stimuli was included in the CTA study and the multidimensional analysis summarized in Figure 14. As primary stimuli, NaCl, sucrose, HCl and QHCl should form a perimeter within the taste space which encloses the remaining gustatory stimuli. Clearly this was not the case. There were many amino acids that showed poor generalization to all four of the primary stimuli and thus were positioned away from them in a corner of the taste space. Such a configuration is incompatible with Henning's model of four primary taste qualities.

There are several possible interpretations for the failure of the Henning model in this experiment. The first is that certain nongustatory characteristics of these complex tasting stimuli influenced construction of the taste space. The placement of malodorous Met and several pH-related stimuli at the edge of the taste space supports this view. However, other amino acids placed in the same corner of the space have no apparent olfactory or trigeminal components (e.g. Ala, Leu, Thr). Nevertheless, the possibility remains that some uncontrolled, extragustatory characteristics of the stimuli were present and exerted some influence upon the space.

A second, but less likely possibility, is that the segregation of amino acids at the lower edge of the space represents the influence of an additional taste primary. Japanese taste researchers often include a fifth primary, umami (meat-like), in their discussions of taste quality (38). The stimulus prototype for the umami taste is an amino acid, monosodium l-glutamate, but Kirimura et al. (38) have shown that among the amino acids tested here, only Met and Ala impart an umami taste.

The last possibility to be discussed here is Erickson's view that gustation is not based upon a system of primary qualities. According to Erickson, taste quality varies continuously along one or more stimulus dimensions. As such, in a taste space analysis, stimuli such as NaCl, sucrose, HCl and QHCl would be expected to diverge from one another (since they are qualitatively dissimilar); however, there would be no requirement that they form a perimeter to confine the other stimuli. In this regard, when a "primary" stimulus is located along the edge of a taste space, it simply means that the stimulus in question represents an extreme position along one or more stimulus dimensions. Unfortunately, the configuration of stimuli in the CTA taste space provides little insight into what these relevant stimulus dimensions may be. Until these underlying dimensions are identified, multidimensional analyses such as the present one should be described as consistent with Erickson's model, but not a true test of it.

*Implications of Amino Acid Taste Quality for Dietary Self-Selection.* The normal preference animals show for sweet and salty stimuli reflects the close association that exists between quality and nutrition. Most sweet substances are nutritious and NaCl, the most common of salts, is a vital electrolyte. However, the results of our preference study illustrate that quality is not the sole determinant of nutritive value. Most of the essential amino acids (5 of 9) were avoided in the preference test even at low suprathreshold concentrations while the other nonessential amino acids (Gly, Pro, Ala) were preferred. A similar qualitative paradox exists for saccharin, LiCl, and Pb-acetate. All are preferred by rats but saccha-

rin has no nutritive value; the latter two are poisonous.

These apparent inconsistencies are bothersome to the experimenter but may provide clues to solve the riddles of food ingestive behavior. Consider that amino acids, unlike sweet carbohydrates, do not represent an energy supply which should be ingested in voluminous quantities. Ingestion of disproportionate amounts of amino acids produces many deleterious effects (27). Thus, there may be no selective pressure for an appetitive taste among the range of amino acids, vitamins and other micronutrients. In fact, for successful amino acid self-regulation it may be advantageous for the chemicals to be bitter or sour because taste thresholds are lowest for these qualities. This may be an important point because amino acids usually comprise only a small part of any foodstuff.

We have described several aspects of amino acid gustatory stimulation in this report. Most convey the same message: despite some peculiarities in response time courses, amino acids bear striking similarities to the salts, acids and sugars which the majority of investigators use as taste stimuli. As such, the study of amino acids here has not discovered a gustatory feature detector or defined the relationship between taste and nutrition. However, by determining the gustatory properties of amino acids, future research into nutrition and food selection may proceed in a logical fashion through several levels of nutrient complexity and at successive levels of the nervous system.

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