Amino Acids as Gustatory Stimuli in the Rat

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ABSTRACT Neural activity in intact chorda tympani nerve of rats was studied with an electronic summator. Neural activity increased when amino acid solutions 0.01 M or above passed over the tongue. Response magnitude, at concentrations close to solubility limits for the amino acids tested, was: DL-methionine < DL-tryptophan < DL-valine < DL-alanine < glycine < 0.1 M NaCl. Maximum response magnitudes to 1 M D-, and 1.2 M DL-alanine, and 1.5 M glycine developed in 1 to 3 minutes. Following such stimulation, a 63 per cent reduction in response to 0.1 M NaCl occurred 60 minutes after the first stimulation (medians). The depression was still present 20 hours later. Responses to glycine and alanine were not depressed. Amino acids *vs.* water preferences were investigated. With ascending concentration sequences, rats selected low concentration DL- and L-alanine and glycine; accepted D-, L-, and DL-tryptophan and low concentration DL-methionine; and rejected high concentration glycine, DL-alanine, and DL-methionine. Descending sequences showed depressed and delayed selection of glycine and DL-alanine, and DL-methionine and D- and L-tryptophan rejection. Both groups rejected DL-valine. It is concluded that glycine and alanine receptor effects differ from those of NaCl, but that all three compounds may affect a common receptor site. Prior exposure to amino acids may modify subsequent neural and/or behavioral responses.

INTRODUCTION

It is of importance to animals to be able to select required metabolites from the many food sources of their environment. Taste probably has an important role in this selection. Many nutrients are long chain, nitrogen-containing, complex organic molecules. Gustatory systems may well have evolved in relation to these complex compounds. Most gustatory investigations have focused primarily on inorganic salts and acids, and some sugars. The amino acids may be a more complex class of stimuli.

Amino acids are recognized as key structural and functional elements in living systems. An extensive literature exists on the relationships between various amino acids and growth, maintenance, or reproduction. Studies at a molecular level are also well represented (*e.g.*, Folkers, 1960). However, only limited attention has been given to amino acids as gustatory stimuli. The crayfish has been reported to initiate feeding activity upon the injection of glycine or DL-glutamic acid into its aqueous environment (Hodgson, 1958), while the blowfly showed no selection of any of a group of 53 amino acids tested (Dethier, 1955). In reference to the rat, it is claimed that glycine is always consumed, whereas tryptophan is always rejected (Aschkenasy-Lelu, 1951).

Electrophysiological recordings from primary receptor structures indicate that glycine is an effective gustatory stimulus in several arthropods, while responses to glutamate are less common, and no responses to alanine have been reported (Hodgson, 1958; Barber, 1961). Alanine, tryptophan, methionine, and glycine, as well as tyrosine, do not produce responses in the catfish (Hideki Tateda, Kyushu University, unpublished data). Nejad (1959) has reported that the responses recorded from the glossopharyngeal nerve of the frog to amino acids decrease with increase in chain length. It appears that the only systematic preference study using amino acids has been done on the blowfly. Electrophysiological studies have primarily used invertebrates, and there seem to be no electrophysiological studies above the class Amphibia. In view of the possible importance of amino acids in the understanding of gustatory systems, correlated examinations of neural and behavioral responses to amino acids are desirable. Systematic studies of this nature on mammals appear to be lacking.

The present study employed both neurophysiological and preference measures in an investigation of gustatory responses to amino acids in the rat. The wealth of information on the rat's gustatory responses to inorganic salts and to certain sugars, as well as data on its nutritional requirements, suggested the choice of this mammal. Amino acids were selected which would not be acidic or basic stimuli, *per se*; would represent several different types of amino acid; and would include those found effective in several other classes of animals. At present, mammalian preference behavior is not predictable from gustatory neural responses. Both classes of data must be obtained so that interrelationships may be searched for. Consequently, the concurrent neurophysiological and preference investigations permitted an evaluation of neural and behavioral interactions.

METHODS AND PROCEDURE

Albino rats of the CFN strain were used throughout the experiments. Rockland rat diet was available *ad libitum*. Distilled water was used at all times. All amino acids

were Nutritional Biochemicals' "standardized" grade. Solutions were stored at 1°C to retard growth of microorganisms. Reagent grade NaCl, KCl, and glycerin; technical grade sucrose octaacetate (SOA); U.S.P. quinine sulfate (QSO_4), and commercial sucrose were used. The pH of the solutions was checked regularly.

Electrophysiology

Sixteen rats were studied (ten female, six male; weights ranged from 220 to 464 gm). Anesthesia was induced with sodium pentobarbital (60 mg/ml; 1 ml per kg, intraperitoneally). The trachea was cannulated, and the chorda tympani nerve exposed, freed from surrounding tissues, and cut close to its entrance into the bulla (Pfaffmann, 1955). Urethane (10 per cent), 1 to 2 ml intraperitoneally, was introduced as an additional anesthetic about 20 hours after initial induction. Silver-silver chloride wick electrodes led to a Grass P5 A.C. preamplifier, a Tektronix cathode ray oscilloscope, and an audiomonitoring circuit. A parallel circuit led the amplified whole nerve activity through a Grass 5P5 amplifier, then into a summator (integrator) circuit, and finally into a Texas recti/riter. The summator rise time (RC) was usually 0.75 second.

The anterior portion of the tongue was placed in a glass flow chamber which permitted test solutions to be passed over the same portion of the tongue consistently. Each stimulus consisted of 25 ml of fluid, at 24°C, which was passed over the tongue in 10 seconds. Each rinse was 80 ml of distilled water which flowed over the tongue in 20 seconds.

Preference Tests

Male rats were used in all experiments. At the beginning of each experiment the rats weighed approximately 110 gm. Prior to the beginning of an experiment the rats had had no preference experience. The rats were housed in individual cages, at the front of which two Richter type, all glass, graduated animal tubes were attached. The amount of fluid taken from each pair of animal tubes was measured once a day to the nearest milliliter. The tubes were then rinsed with tap and distilled water, filled with fresh fluids, and returned to the same cage. The right-left position of the tube containing the test solution was varied once a day according to a restricted random schedule. Every 4 days, the tubes were replaced by a pair of chemically clean tubes, and the concentration of the test solution was changed. Water was always offered in one tube. The water-water control group for which, following the switching schedule, one of the tubes was considered to represent the test solution, provided a baseline against which the effects of chemical and concentration could be compared; both tubes always contained water. Per cent preference was calculated as milliliters of test solution drunk, divided by milliliters of test solution plus milliliters of water drunk, times 100. In all experiments, separate groups of rats were used for each stereoisomer of each amino acid examined. Except for the study of the stereoisomers of alanine (experiment III), each group was subdivided into an ascending concentration sequence subgroup (A), which started with low concentrations first, and a descending concentration sequence subgroup (D), which started with high concentrations first. The number of animals in each group is indicated in the figure legends.

Solutions were prepared within 24 hours of the beginning of each 4 day period. Enough solution for each day was equilibrated to ambient temperature on the day of use.

EXPERIMENT I Rats were pretrained for 7 days on a choice between 0.0002 M QSO_4, or 0.3 M sucrose, and water, following the regular experimental schedule. Half of each experimental group was formed of QSO_4-experienced rats. The amino acids and concentrations used are indicated in Table III and in the text.

The tryptophan groups, after completing their three concentrations, were given a water-water choice for four periods (16 days) and were then rerun. D- , L- , and DL-tryptophan were presented at 0.001 M, 0.005 M, and 0.01 M concentrations.

EXPERIMENT II Animals which met a pre-test criterion of ≥ 70 per cent preference for 0.04 M glycine for 3 out of 4 days became the descending, the water-water, and the 0.0002 M sucrose octaacetate (SOA) *versus* water groups. Rats not pretested for glycine selection became the ascending subgroup. The glycine concentrations listed for experiment I were used, with 0.008 M glycine added for both glycine subgroups, and 0.004 M, 0.002 M, and 0.001 M for the descending subgroups. The SOA and water-water groups were retested on 0.04 M glycine when the descending subgroup reached 0.04 M *during* its *sequence* (days 45 through 48). After this period, the SOA group was also placed on water-water. All four groups were tested on 0.04 M glycine at the *end of* the ascending subgroup's *sequence* (between 0.008 and 0.004 M for the descending subgroup). The ascending subgroup was given two more 0.04 M glycine periods (post-tests) separated by water-water periods.

EXPERIMENT III Thirty-four rats were pretested on either 0.02 M NaCl, or 0.000008 M QSO_4, *versus* water, for one 4 day period. Rats which failed to select the NaCl, or reject the QSO_4 for more than 1 day, either formed the water-water control group or were eliminated from the experiment. Half of each group consisted of quinine-experienced animals. For D- , L- , and DL-alanine, only an ascending sequence was used: 0.005, 0.02, 0.04, 0.08, 0.4, and 0.005 M.

For DL-methionine subgroups, the concentration sequence was as described for experiment I, with 0.03 M and 0.005 M DL-methionine inserted into the sequence for both subgroups, and 0.0005 M, 0.0002 M, 0.0001 M, and 0.00005 M DL-methionine added to the descending sequence. The ascending subgroup was retested on 0.0005 M, 0.001 M, and 0.005 M, in that order, at the end of its sequence. In both cases, the final DL-methionine concentration used was repeated for three successive periods.

RESULTS

Electrophysiology

RESPONSE MAGNITUDE An increase in chorda tympani nerve impulse activity was noted following application to the tongue of various concentrations of glycine, alanine, DL-valine, DL-methionine, and DL-tryptophan (Fig. 1, A and B). No responses were obtained following 0.002 M L-tyrosine (solubility limit is below 0.003 M). Rate of change of magnitudes of summated

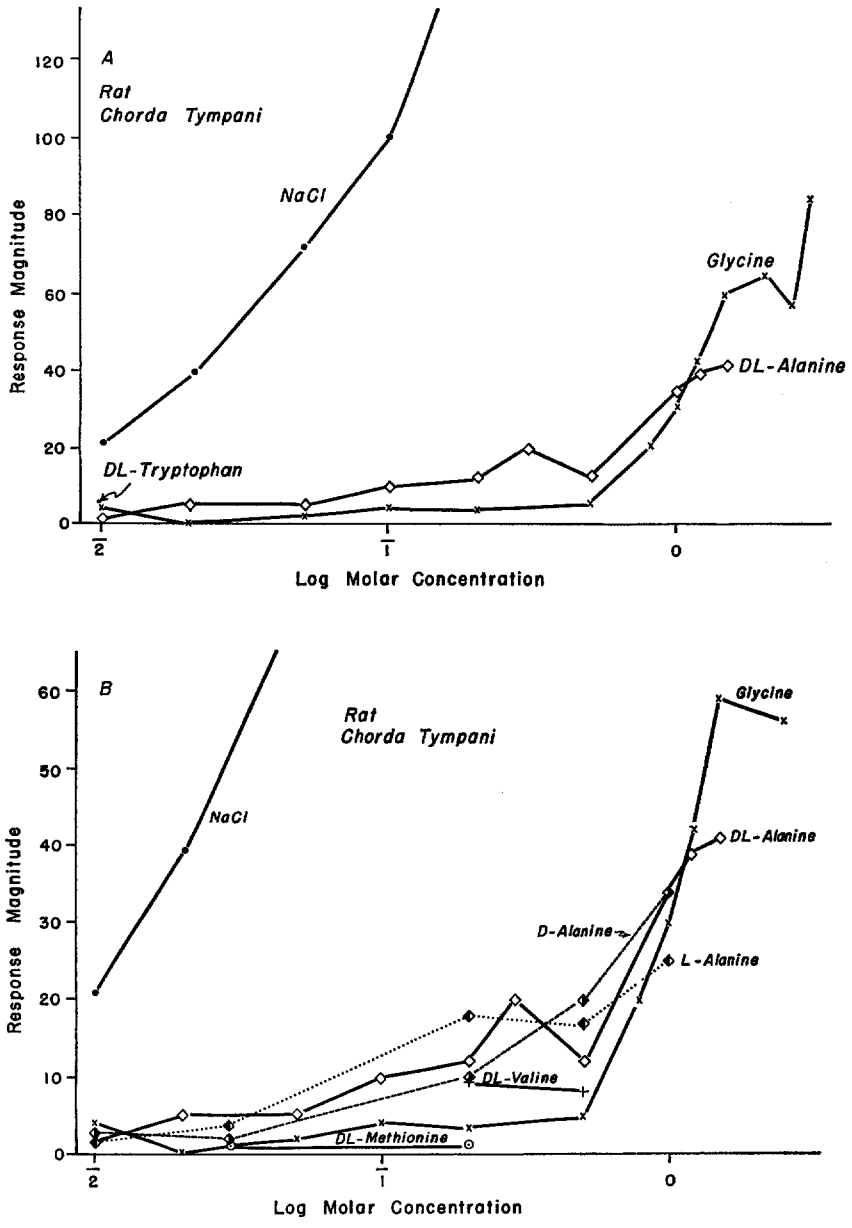

FIGURE 1. Chorda tympani median summated response magnitudes following chemical stimulation of the anterior tongue of the rat (based on sixteen rats). Ordinate represents magnitude of the summated neural response in arbitrary units, adjusted to 100 units for the response to 0.1 M NaCl. A, responses to NaCl, glycine, DL-alanine, and DL-tryptophan. B, responses to NaCl, glycine (except 3.0 M), alanine (D-, L-, and DL-), DL-valine, and DL-methionine. Ordinate expanded (twice).

responses with rising stimulus concentration is given in Table I. Response magnitude increased sharply above 0.5 M, but only slightly between 0.01 and 0.5 M.

For each amino acid studied, the highest concentration used was close to solubility limit. At these maximum concentrations, response magnitude decreased with increasing chain length (Fig. 1). All these magnitudes were less than the response to 0.1 M NaCl; only 3.0 M glycine exceeded 0.05 M NaCl in summated response magnitude.

TEMPORAL CHARACTERISTICS OF THE RESPONSES As the concentration of glycine and alanine solutions increased above 0.5 M, the time interval between

TABLE I

RATE OF CHANGE (DY/DX) OF CHORDA TYMPANI SUMMATED RESPONSE MAGNITUDE (Y) BETWEEN SELECTED STIMULUS-SOLUTION CONCENTRATIONS (X). (BASED ON 15 RATS)

	Concentration range, *moles per liter*			
Amino acid	0.01–0.5	0.5–1.0	1.0–1.5	1.5–3.0
Glycine	0.01	1.1	2.2	1.1
DL-Alanine	0.17	1.0	0.58	—
L-Alanine	0.12	0.41	—	—
D-Alanine	0.13	0.64	—	—

Dashes indicate that no stimuli in that concentration range were used. DL-Methionine and DL-valine showed no clear change in response magnitude between the concentrations used.

stimulus application and peak summated response magnitude became longer (Fig. 2). The stereoisomers of alanine differed in time to peak. The responses to the higher concentrations of alanine and glycine, after rising to peak slowly (Fig. 2), showed little or no consistent falling trend during the remainder of 2 to 4 minute observation periods (Fig. 3, Table II). In contrast, 0.1 M NaCl produced its generally reported pattern (Pfaffmann, 1955; Fishman, 1957) of a rapid rise to peak followed by a negatively accelerated fall in response magnitude (Table II, Fig. 3).

DEPRESSION OF NaCl RESPONSES After two or more stimulations with alanine, 1.0 M or above, responses to NaCl decreased in magnitude, relative to alanine. The median (eight rats) time interval required for NaCl depression to appear was 60 minutes (three stimulations); the shortest interval, 9 minutes. The depression often increased in degree with time. Responses to 1.0 glycerin did not decrease in magnitude, relative to alanine. Using 1.0 M glycerin as a comparison standard, the median (eight rats) maximum reduction in 0.1 M NaCl response magnitude was 63 per cent. Depressions greater than 60 per cent have been noted more than 20 hours after the initial alanine series was

completed (Fig. 4). Potassium chloride response magnitude was similarly depressed.

Glycine, 2.0 M and above, had a similar depressing effect on NaCl and KCl response magnitude, but with less consistency and magnitude of depression. Responses to alanine and glycine were not depressed.

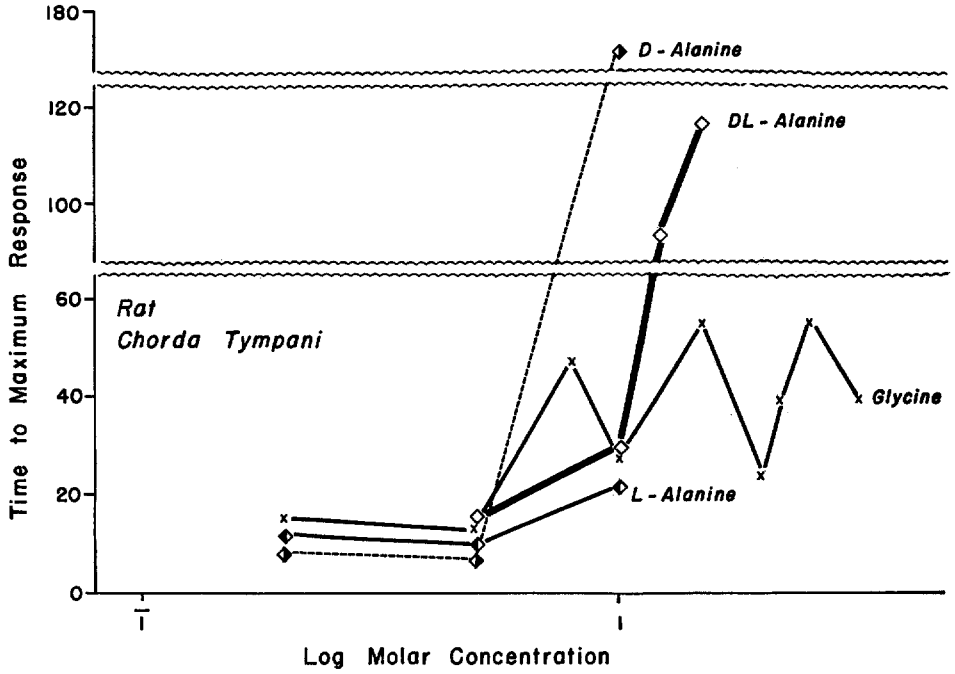

FIGURE 2. Chorda tympani median time to maximum response magnitude following chemical stimulation of the anterior tongue of the rat (based on eleven rats). Ordinate represents time, in seconds, from beginning of fluid movement in flow system (2 seconds required to reach tongue, tongue chamber empty approximately 8 seconds later) to development of maximum (peak) response magnitude. Summator rise time (RC), 0.75 sec.

Preference Tests

EXPERIMENT I. VALINE, METHIONINE, ALANINE, AND GLYCINE The over-all trend was rejection of DL-valine at all concentrations, and rejection of DL-methionine, DL-alanine, and glycine at high concentrations. At lower concentrations, DL-methionine was accepted, while DL-alanine and glycine were selected (Table III). An analysis of variance (Edwards, 1950, p. 284) applied to the data indicated that the type of amino acid and concentrations were significant variables ($P < 0.005$). The over-all effects of direction of testing did not reach significance ($P = 0.07$). The water-water control groups in experiments I, II, and III did not differ significantly from an ideal 50 per

cent group ($P > 0.1$).[1] Preference behavior for each of the above amino acids is illustrated in Fig. 5.

Tryptophan Direction of testing resulted in significant differences in preference for D- and L-tryptophan ($P = 0.01$), but not for DL-tryptophan ($P = 0.07$). The ascending subgroups did not differ significantly from the

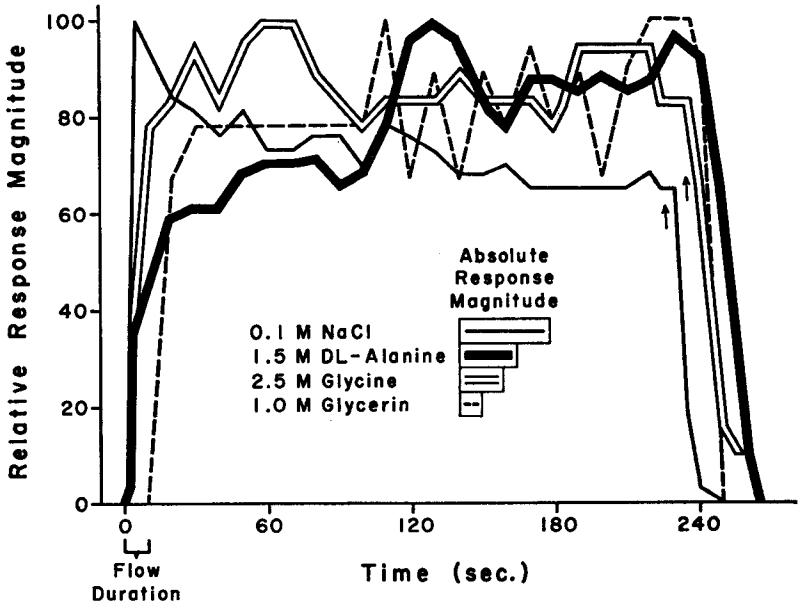

FIGURE 3. Chorda tympani summated response magnitudes following chemical stimulation of the anterior tongue of one rat. Ordinate represents magnitude of the summated neural response to each chemical in arbitrary units, adjusted to 100 units for the maximum (peak) response for each chemical. The bar diagram represents the peak summated neural response magnitudes to the four chemicals, all on the same arbitrary scale (*e.g.*, the response to NaCl was 41 mm pen deflection). The tongue chamber is filled with stimulating fluid for approximately 10 seconds. Summator rise time (RC) = 0.5 sec. Arrows indicate onset of H_2O rinse.

water-water control animals for L- or DL-tryptophan at any of the three concentrations used, or for D-tryptophan at 0.001 M or 0.01 M ($P \geq 0.1$). On the first run, the 0.005 M D-tryptophan per cent preference, 85 per cent, was significantly different from that of the control animals ($P = 0.03$), but this selection tendency did not appear in the second run (per cent preference = 50 per cent, $P = 0.9$).

The descending subgroups on D- or L-tryptophan showed a general trend

[1] This and all subsequent probability values are, unless otherwise noted, based upon the Mann-Whitney U Test (Siegel, 1956). For experiment I, all values are two-tailed. For experiments II and III, all values are, unless otherwise noted, one-tailed.

TABLE II

TEMPORAL CHARACTERISTICS OF THE CHORDA TYMPANI SUMMATED NEURAL RESPONSES TO ONE CONCENTRATION OF NaCl, GLYCERIN, GLYCINE, AND DL-ALANINE

Stimulus solution	Time to peak response, *sec.*	Response magnitude at selected time intervals after first reaching peak, expressed as a percentage of the peak response						
		2 sec.	5 sec.	20 sec.	51 sec.	67 sec.	100 sec.	230 sec.
0.1 M NaCl	13	99	95	86	74	70	—	67
1.0 M glycerin	35	97	90	100	79	72	—	—
2.5 M glycine	56	100	97	86	100	86	—	—
1.5 M DL-alanine	118	97	90	84	85	100	90	—

Tables values are medians based on a total of eleven rats. Dashes indicate that no median data are available for that postpeak interval.

toward rejection of tryptophan. The per cent preference of the descending D-tryptophan subgroup (run 1: 38, 21, 35; run 2: 6, 7, 11) were significantly different from those of the control group at all three concentrations on the second run and at 0.005 M on the first run ($P = 0.028$). More consistent rejec-

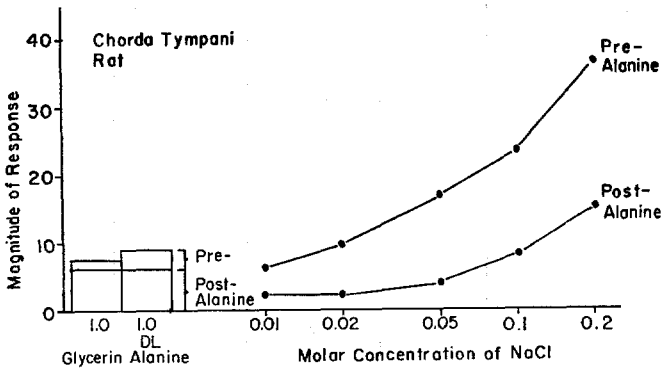

FIGURE 4. Chorda tympani median summated response magnitudes following chemical stimulation of the anterior tongue of one rat. Ordinate represents magnitude of the summated neural response in hundredths of a milliampere. The "pre-alanine" NaCl function was obtained prior to the application to the tongue of DL-alanine above 0.01 M, or of any glycine. The maximum heights of the bars represent the responses to 1.0 M glycerin and DL-alanine, secured within 1 hour after the NaCl series. DL-Alanine, 1.2 M through 1.5 M, and glycine, 2.0 M through 3.0 M, were next presented over a period of 5 hours. The tongue was then removed from the chamber, replaced in the mouth, and left undisturbed for 12 hours. Twenty hours after the initial response magnitudes were determined, the "post-alanine" NaCl function, and glycerin and DL-alanine magnitudes, were determined. Falls in NaCl response magnitude ranged from 60 to 80 per cent. The much smaller decreases in glycerin (20 per cent) and alanine (33 per cent) responses can be attributed to general changes in the preparation during the 20 hour period of no stimulation.

TABLE III

PREFERENCE BEHAVIOR FOR GLYCINE AND DL-VALINE, METHIONINE, AND ALANINE IN THE RAT

Amino acid	Concentration, *moles per liter*																Difference at all concentrations due to direction of testing
	0.001	0.002	0.01	0.02	0.04	0.08	0.1	0.2	0.4	0.6	0.8	1.0	1.5	1.8	2.0	3.0	
DL-Valine																	
Ascending	—	—	⊢	—	—	—	Reject <0.001	—	—	⊣	—	—	—	—	—	—	No ($P = 0.92$)
Descending																	
DL-Methionine																	
Ascending	⊢	Accept (A) 0.37	—	—	—	⊣	Reject 0.028	⊣	—	—	—	—	—	—	—	—	Yes ($P = 0.004$)
Descending	⊢	—	—	Reject <0.001	—	—	—	⊣	—	—	—	—	—	—	—	—	
DL-Alanine			A														
Ascending	—	—	0.16	⊢	—	—	Select 0.002	—	⊣	—	⊢	—	Reject 0.028	⊣	—	—	No ($P > 0.1$)
Descending	—	—	Accept 0.2	0.09	⊢	Select 0.028	⊣⊢	Accept >0.11	⊣	—	⊢	—	Reject 0.028	⊣	—	—	
Glycine									A								
Ascending	—	—	Accept 0.2	0.06	⊢	—	Select 0.028	⊣	1	—	⊢	—	Reject 0.004	—	⊣		No ($P > 0.1$)
Descending	—	—	⊢	—	—	Accept 0.69	—	⊣	—	—	—	Reject <0.001	—	—	⊣		

Tabled values are two-tailed probability figures, based on the Mann-Whitney U Test (Siegel, 1956). Dashes indicate that a concentration was not used. Rejection or selection is deduced when the per cent preference values are significantly different from those of the control group, and the mean per cent preference values are consistently below (rejection) or above (selection) those of the control group. Absence of significant differences is taken as acceptance (A). Experiment I.

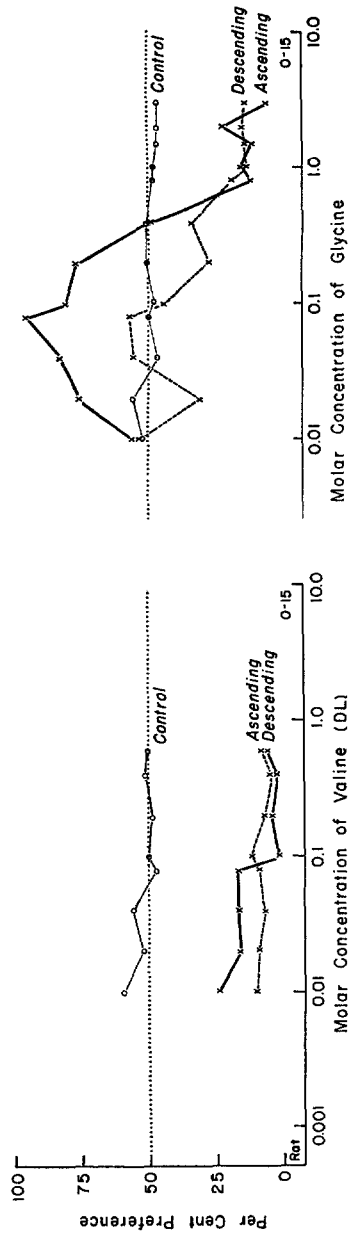

FIGURE 5. Preference functions for DL-valine, DL-methionine, DL-alanine, and glycine. Each point represents the mean per cent preference (milliliters of amino acid ingested divided by milliliters of total fluid consumed, times 100) of four (three for water "control" group. Experiment I.

tion was shown for L-tryptophan. The descending L-tryptophan per cent preferences (run 1: 11, 25, 21; run 2: 2, 11, 21) were all significantly different from the water-water control rat preferences ($P = 0.028$) except the per cent preference for 0.001 M L-tryptophan in the second run ($P = 0.1$). In contrast, the DL-tryptophan descending subgroup was not significantly different from the control group at any concentration in either run ($P > 0.2$). Since two-rat groups were run twice on each of the stereoisomers of tryptophan, the results are less certain than those for the other amino acids studied.

TABLE IV

THE EFFECTS OF EXPERIENCE ON PER CENT PREFERENCE FOR 0.04 M GLYCINE

	Test period					
		During sequence				
Group	Pre-test *Days*, 1–4	17–20	45–48	End of sequence 61–64	Post-test I 69–72	Post-test II 77–80
Descending	87	—	71‡	97§	—	—
H_2O–H_2O	87	—	98‖	99	—	—
SOA–H_2O	79	—	98‖	99	—	—
Ascending	*	89	—	82¶	88**	96‡‡

Dashes indicate that no measurement of preference for 0.04 M glycine was made during that test period. Experiment II.

* On water-water.

‡ Significantly less than "pre-test" preference ($P = 0.03$). This and subsequent P values are, unless otherwise noted, one-tailed, and based on the Wilcoxon Sign Test (Siegel, 1956).

§ , ‖ Significantly different from pre-test preference (‖ $P = 0.03$; § $P = 0.04$, Mann-Whitney U, Siegel, 1956. Two-tailed).

¶ Not significantly different from "During sequence" preference ($P = 1$).

** Not significantly greater than either "End of sequence" preference ($P = 0.1$) or "During sequence" preference ($P = 0.34$).

‡‡ Significantly greater than End of sequence preference ($P = 0.016$). Not significantly greater than During sequence preference ($P = 0.1$).

EXPERIMENT II. GLYCINE The absence of glycine selection by the descending glycine subgroup of experiment I (Table III, Fig. 5) was surprising. More commonly, exposure to high concentrations of a sapid substance which is selected at lower concentrations may tend to depress and delay the selection, rather than eliminate selection completely (*e.g.*, DL-alanine: Table III, Fig. 5). Therefore, it seemed desirable to examine more closely the determinants of glycine preference in the rat. It was possible that high concentrations of glycine were particularly effective repellents. The descending subgroup thus might be rigorously trained to select water during prolonged exposure to the high concentrations. If this were the case, any other strongly rejected substance could be substituted for glycine, and a similar decrease in low concentration

glycine selected produced. The SOA *vs.* water group was introduced to test the effects of rejection training (*i.e.*, water selection training) *per se*. In order to include only known glycine selectors in the critical groups, all the rats except those in the ascending subgroup were given a pre-test period on 0.04 M glycine. Seven out of twenty-four rats tested failed to meet the pre-test criterion and were eliminated. The pre-test procedure also determined the preference levels for 0.04 M glycine prior to any other experience. The effects of the various testing sequences on this preference were measured by retesting the water-water and SOA groups when the descending subgroup reached 0.04 M glycine in its sequence (during sequence test). This held the time interval

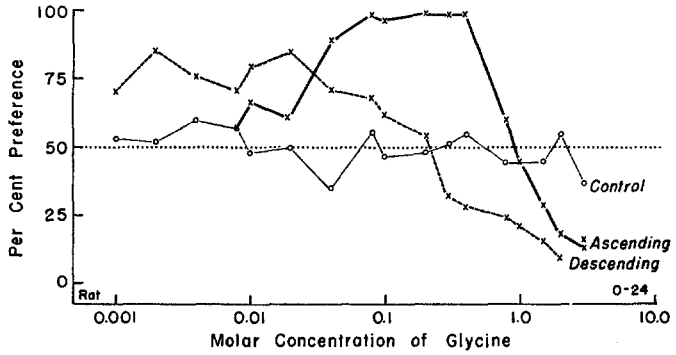

FIGURE 6. Preference functions for glycine. Each ascending point represents the mean per cent preference of six non-selected rats over 4 days; each descending point, that of five selected rats over 4 days. Experiment II.

constant; only the non-water choice differed. Additional retesting of all groups at the completion of the ascending subgroup's concentration sequence (end of sequence test) provided a measure of the stability of the previously determined (during sequence test) treatment effects, and indicated the effects of the ascending sequence on the ascending subgroup's preference for 0.04 M glycine (Table IV).

The results indicated that the per cent preference of the SOA group for 0.04 M glycine in the during sequence test was the same as the preference of the water-water group. Both groups showed a significant increase in preference. The SOA group has strongly rejected the 0.0002 M SOA ($P < 0.001$. Mean preference = 27 per cent). Thus, rejection training, *per se*, did not depress glycine selection (Table IV). In contrast, the pretest preference of the descending subgroup showed a significant decrease in the during sequence test (Table IV). This glycine-related depression of preference for glycine was no longer present 12 days later (Table IV). The descending subgroup did not show the total absence of glycine selection found in experiment I (Fig. 5, Table III) with a descending subgroup of unselected rats. Rather, significant

selection of glycine was found at low concentrations. This selection first appeared at 0.01 M glycine, a concentration one fortieth of the highest concentration selected by the ascending subgroup (Figure 6).[2]

The ascending subgroup did not show a significant decrease from its during sequence preference for 0.04 M glycine when tested at the end of sequence. However, neither did the ascending subgroup show the significant increase in preference found for the water-water and SOA groups (Table IV). After additional testing periods, separated by periods on water, the ascending group did eventually show a significant increase in preference for 0.04 M glycine

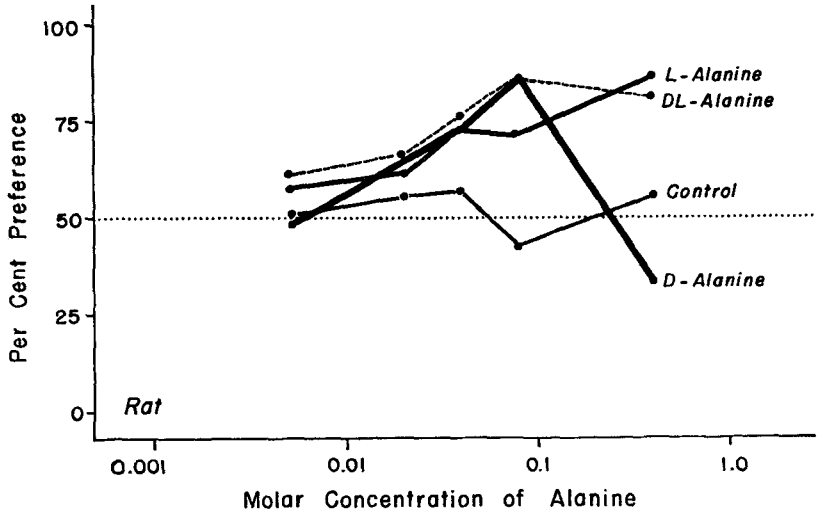

FIGURE 7. Preference functions for the stereoisomers of alanine. Ascending concentration sequence only. Each point represents the mean per cent preference of six rats over 4 days. Broken line, DL-alanine, double line, L-alanine, heavy line, D-alanine. Light line, water-water control group. Experiment III.

(Table IV). The over-all preference pattern of the ascending subgroup (Fig. 6)[3] was similar to the preference pattern found for the ascending glycine subgroup in experiment I (Table III, Fig. 5).

EXPERIMENT III. ALANINE The differences between the stereoisomers of alanine in time to peak response magnitude (Fig. 2) suggested that differences

[2] The descending group showed significant selection of glycine between 0.08 and 0.001 M ($P < 0.001$), with all concentrations except 0.08 M ($P = 0.07$) and 0.004 M ($P = 0.29$) significant ($P < 0.03$). Between 0.4 M (the highest concentration selected by the ascending group) and 0.1 M, none of the concentrations was selected (or rejected, $P \geq 0.1$). The mean per cent preference values showed significant rejection of glycine between 3.0 and 0.8 M ($P = 0.008$).

[3] The mean per cent preference of the ascending group was significantly greater than that of the water-water group between 0.01 and 0.4 M glycine ($P < 0.001$). Significant selection was found at all concentrations within this range ($P < 0.03$) except 0.02 M ($P = 0.13$). The ascending group rejected glycine between 1.0 and 3.0 M ($P = 0.03$).

in preference for the stereoisomers might also be found. This possibility was confirmed (Fig. 7). Both DL- and L-alanine were significantly selected between 0.005 M and 0.4 M ($P = 0.004$). Each concentration in the ascending sequence was significantly selected ($P < 0.03$) except 0.02 M ($P \geq 0.29$). The DL-alanine preference pattern was similar to the preference found for the ascending DL-alanine subgroup in experiment I (Table III, Fig. 5). Preference for DL- and L-alanine differed in the responses to the retest, made after the ascending sequence was completed, of 0.005 M, the lowest concentration used. Although 0.005 M DL-alanine had been selected when presented as the first concentration

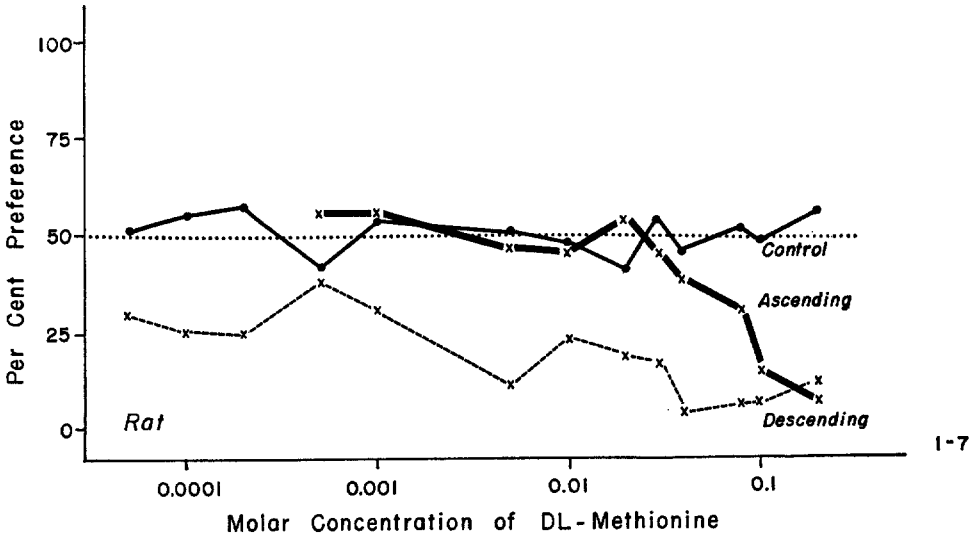

FIGURE 8. Preference functions for DL-methionine. Each point represents the mean per cent preference of four rats over 4 days. Experiment III.

in the ascending sequence (Fig. 7), 0.005 M DL-alanine was not selected ($P = 0.29$) when a retest was made at the completion of the ascending sequence. In contrast, not only was L-alanine selected in the retest period ($P = 0.01$), but also the per cent preference for 0.005 M L-alanine increased significantly, going from 58 per cent when presented at the beginning of the concentration sequence to 78 per cent at the retest ($P = 0.04$, two-tailed).

The D-alanine preference pattern differed from both the DL- and the L-alanine pattern (Fig. 7). No selection occurred at concentrations below 0.04 M ($P > 0.2$). Significant selection of D-alanine was found only at two of the five concentrations used: 0.04 M and 0.08 M ($P < 0.03$). A clear rejection trend appeared at 0.4 M ($P = 0.058$, two-tailed) (Fig. 7). No significant selection of 0.005 M D-alanine was found at retest ($P = 0.2$).

Methionine The profound effect of direction of testing on preference for

DL-methionine found in experiment I (Table III, Fig. 5)[4] suggested that a more detailed examination of preference for DL-methionine was desirable (Fig. 8). Low concentrations of DL-methionine were added to the descending sequence so that the temporal and stimulus concentration aspects of the preference depression could be more thoroughly studied. The ascending subgroup was retested on its lowest concentrations after completing the ascending sequence, thus measuring the effects of the high concentrations on the previously determined preferences (Table V). An indication of the stability of the preference depressions was obtained by repeating for three testing periods the final DL-methionine concentrations used for the ascending and for the descending subgroups.

It was found that the descending subgroup rejected DL-methionine throughout the concentration range tested, from 0.2 through 0.00005 M ($P < 0.001$)

TABLE V

DL-METHIONINE PREFERENCE AFTER ASCENDING SEQUENCE, EXPERIMENT III

Concentration	Mean per cent preference	Probability value (U)
0.0005	30	0.057
0.001	23	0.014
0.005	27	0.029
0.005	39	0.171
0.005	30	0.014

(Fig. 8). Each individual concentration was significantly rejected ($P < 0.03$) except 0.0005 M ($P = 0.1$). The rejection of 0.00005 M DL-methionine was not stable with repeated testing. The per cent preference increased from 30 per cent ($P = 0.03$, rejection) for the first testing period to 46 per cent for the second and third periods ($P = 0.17$, acceptance).

In contrast, the ascending subgroup accepted DL-methionine equally with water between 0.0005 and 0.04 M ($P = 0.28$) (Fig. 8). Within this range no individual concentration was selected or rejected, although there was a trend towards selection of 0.0005 M DL-methionine ($P = 0.058$, two-tailed), and rejection approached significance at 0.005 M ($P = 0.058$). Significant rejection was not found at 0.08 M ($P = 0.29$), but rejection was found at the two highest concentrations in the ascending sequence: 0.1 M and 0.2 M DL-methionine ($P = 0.01$). Thus, 0.1 M DL-methionine, the lowest concentration of DL-methionine at which the ascending subgroup showed rejection during its

[4] In experiment I, the descending DL-methionine subgroup rejected DL-methionine down to 0.001 M, a concentration one-eightieth of the lowest concentration rejected by the ascending subgroup (0.08 M).

sequence, was more than 1000 times more concentrated than the lowest concentration rejected by the descending group (0.00005 M). However, when the ascending subgroup was retested on low concentrations of DL-methionine which it had previously accepted, it now rejected them (Table V). Exposure to high concentrations of DL-methionine, particularly 0.1 and 0.2 M, appears to result in a clear and prolonged conversion of acceptance for lower concentrations into rejection.

DISCUSSION

Neural Responses

Multiunit chorda tympani nerve responses to glycine, alanine (D-, L-, and DL-), and DL-valine, DL-methionine, and DL-tryptophan were studied in the rat. Response magnitudes were low in comparison with NaCl. Stimulation effectiveness changed, often abruptly, with concentration. Consequently, it is difficult to rank these five amino acids with respect to response magnitude. At the highest concentrations used, which approached solubility limits, the order of increasing response magnitude is: DL-methionine < DL-tryptophan < DL-valine < DL-alanine < glycine. The essential (growth and maintenance in the rat (Allison, 1961)) amino acids methionine, tryptophan, and valine occupy the three lowest positions in this sequence.

The time course of alanine and glycine responses differs from that of NaCl. For these two amino acids, maximum response magnitudes were reached slowly. One to 3 minutes were required for the high concentrations to reach peak magnitude, in contrast to 13 seconds for 0.1 M NaCl. Falling trends for alanine and glycine responses were often absent or much delayed during the remainder of 2 to 4 minute observation periods. This suggests that the action of these amino acids on either a common or on discrete receptor sites is quite different from NaCl stimulation. Under some conditions, at least, a common receptor site may be affected by NaCl, glycine, and alanine. The observation that stimulation with high concentrations of alanine and glycine tends to depress subsequent responses to NaCl (*e.g.*, Fig. 4) suggests this. It may be tentatively postulated that some amino acids and NaCl are able to reach and act upon the same receptor site under special circumstances (such as high amino acid concentration). Then, it may be that the results of this joint action on the "NaCl" receptor site can provide information on the composition and organization of the site. Amino acids of various configurations and physicochemical properties would be used, and NaCl response magnitude depression (as well as possible changes in the temporal characteristics of the NaCl response) would be measured and related to the characteristics of the amino acid used.

The specificity of the receptor sites or processes is illustrated in the differ-

ences in time to maximum response (Fig. 2) and in maximum response magnitude itself for D- , L- , and DL-alanine. The "natural" L-form gave the lowest values in both cases. This is in contrast to most processes in metazoans. For example, L-forms of amino acids are actively transported across the small intestine, but the D-isomer is not (Wilson *et al.*, 1960).

Alanine and glycine are reported to taste "sweet" to human beings (Moncrieff, 1951, p. 144, p. 316; Schutz and Pilgrim, 1957) and are selected under certain conditions by the rat (Figs. 5–7). The neural responses to these two amino acids resemble some general characteristics of responses to sugars: (*a*) Relatively slow response build-up (Fig. 2, Table II), (*b*) relatively low magnitude of response (Fig. 1) (Pfaffmann, 1955; Dethier, 1956), and (*c*) sensitivity to configuration (Fig. 2) (Dethier, 1956). The two classes of compounds diverge notably on a fourth characteristic of sugar responses: (*d*) relatively rapid adaptation (Dethier, 1956). Responses to the higher concentrations of alanine and glycine adapt very slowly (Fig. 3, Table II).

The isoelectric points of the amino acids selected for use in this experiment lie between 5.6 and 6.1. The pH of the working solutions ranged between 5.3 and 9.3. The human verbal response threshold for weak organic acids is reported to be pH 3.9 (Pfaffmann, 1959). The hamster neural threshold for acids is pH 5. Sodium hydroxide, pH 9, yields neural responses of only very small magnitude in the hamster while no responses at all are noted at pH 8 (Hagstrom, 1957). The rat yields only very small neural responses to pH 3 inorganic acids (Pfaffmann, 1955) and pH 3.9 acetic acid (Halpern, 1959). In the present experiment, pH 9.5 NH_4OH produced no measurable chorda tympani responses. Consequently, it appears unlikely that the stimulating properties of the amino acids studied are directly related to acidic or basic characteristics.

Neural-Behavioral Interrelations

Preference behavior for glycine and DL-alanine moved from selection to rejection between 0.5 and 1.0 M. In the same concentration range, the chorda tympani response magnitude and time to maximum response increased sharply. This correlation may indicate a peripheral neural factor contributing to the behavioral change. (Preference behavior for D- and L-alanine was not studied above 0.4 M, and chorda tympani responses to the other amino acids did not change appreciably with concentration.)

High concentrations of glycine and alanine produced depression of subsequent responses to NaCl (Fig. 4) and reduced selection behavior to their lower concentrations (Figs. 5 and 6). It may be that the depression of selection results in part from a direct effect of the alanine or glycine on the receptor structures. However, no neural response depressions were noted with trypto-

phan, methionine, or valine. High concentrations of the first two depressed preference and the latter did not (perhaps testing preference for valine at concentrations below 0.01 M would have revealed an effect of exposure to higher concentrations).

Behavioral Responses

For those amino acids which are selected at some concentrations (alanine and glycine), exposure to high concentrations resulted in depression of magnitude of selection, and delay in appearance of selection. In the case of the non-selected amino acids, tryptophan appeared to be rejected only after the rats were exposed to high concentrations, and a broad region of DL-methionine acceptance was converted to rejection. DL-valine rejection was not strongly affected by prior experience within the concentration range studied. However, extension of preference trials to lower concentrations might reveal a pattern for DL-valine similar to that found with DL-methionine.

The concentration range in which human beings describe DL-alanine as sweet is not in accord with the selection functions in the rat. DL-alanine is judged sweet between 0.1 and 1.4 M, while rats begin to select it below 0.01 M and rejection appears below 0.8 M. Similarly, glycine is judged sweet between 0.33 and 2.7 M (Schutz and Pilgrim, 1957), but rats begin to select close to 0.01 M, and switch to rejection by 1.0 M. Also, in both rats and human beings, behavior is affected by the particular configuration of alanine or tryptophan offered. However, correspondence between human judgments (Moncrieff, 1951) and rat preference is poor. For example, L-tryptophan is described as bitter, DL- as sweet, while the D-isomer is said to be tasteless. The rat, after exposure to high concentrations, rejects the D- and L-isomers but accepts the DL-racemate.

All rats were maintained on a diet which supplied adequate amounts of the essential amino acids tested as well as other nutrients. Preference testing under conditions of specific deficiency may result in modified patterns of selection and rejection (*e.g.*, Le Magnen, 1956). It is unlikely that a deficiency state would differentially affect peripheral neural responses to the deficient substance (Pfaffmann and Bare, 1950; Pfaffmann and Hagstrom, 1955).

A broad generalization of selection of glycine and rejection of tryptophan by the rat (Aschkenasy-Lelu, 1951) seems to be an oversimplification of the situation. Nutrition experiments in which amino acids are fed must consider the taste reactions of the animal to the feed or feeds offered. In the case of amino acids, at least, such reactions appear to vary with the particular concentration and configuration of each amino acid used, and with prior experience. They are not predictable from human judgments (the latter point has been found to hold with a wide variety of species and chemicals (Kare, 1961). The

peripheral neural responses upon which the preference behavior depends vary in both temporal characteristics and magnitude with amino acid, concentration, configuration, and prior exposure.

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