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THE TASTE REACTIVITY TEST. I. MIMETIC RESPONSES TO GUSTATORY STIMULI IN NEUROLOGICALLY NORMAL RATS

HARVEY J. GRILL* and RALPH NORGREN

Rockefeller University, New York, N.Y. 10021 (U.S.A.)

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SUMMARY

One or two bottle preference tests, i.e., relative fluid consumption, constitute the primary methodology for determining acceptance or rejection of tastes in animals other than humans. These tests require organisms to initiate and maintain drinking behavior and, therefore, can not be applied to preparations which do not eat or drink spontaneously. The taste reactivity test, a new method for assessing responses to gustatory stimuli, circumvents this shortcoming. A 50 μl taste stimulus is injected directly into the oral cavity of a freely moving rat and the immediate response videotaped for frame by frame analysis. Each of the sapid stimuli used (4 concentrations of sucrose, NaCl, HCl, and quinine HCl) generated a stereotyped response derived from a lexicon of 4 mimetic (movements of lingual, masticatory, and facial musculature) and 5 body response components. Responses to taste stimuli were highly consistent within and between rats. For example, sapid sucrose, NaCl and HCl stimuli elicited a response sequence beginning with low amplitude, rhythmic mouth movements, followed by rhythmic tongue protrusions, and then lateral tongue movements. No body movements accompanied these mimetic responses. In contrast, quinine in concentrations at and above 3×10^{-5} *M* (1/2 log step above the absolute behavioral threshold for quinine) elicited a response pattern beginning with gaping and proceeding through as many as 5 body responses. These normative data for the intact rat can be directly compared to the taste reactivity of neurally ablated preparations which do not spontaneously feed or drink. Such comparisons can be utilized in determining the neural substrates necessary for the execution and regulation of ingestive behavior.

INTRODUCTION

Ingestion and rejection constitute the final sequences of feeding behavior. Al-

* Present address: University of Pennsylvania, Department of Psychology, Philadelphia, Pa. 19104, U.S.A.

though these responses can be modified (i.e. the concentration of QHCl consumed is a function of hours of deprivation), they are usually unencumbered by the complex constraints from learning and environment that obscure other aspects of food seeking and manipulation (i.e. meal patterns, amount consumed, satiation, diet selection). Ingestion and rejection of tastes have been traditionally measured with fluid consumption tests. The two-bottle preference test directly contrasts ingestion of water with a taste solution. Rejection is inferred from the lack of ingestion. The one-bottle acceptance test infers acceptability from relative fluid consumption.

The use of fluid consumption tests is dependent upon organisms which are capable of initiating and maintaining the appetitive and consummatory phases of drinking behavior. Therefore, ingestion and rejection responses of neurologically aphagic and adipsic animals have not been assessed. In order to compare the gustatory reactivity of normal rats and neurologically deficient ones, which do not consume food and water, a non-appetitive test is required.

The fluid consumption test provides the organism with two options, either to drink or not. While this binary decision offers a high contrast picture of responsivity to tastes, the underlying decision process remains unexplored. For example, two-bottle preference test data directly implies that rats will reject a solution of either 0.01 *M* HCl or 0.001 *M* QHCl. Is the rejection of the quinine equivalent either quantitatively or qualitatively to the rejection of the acid? Is the rejection based on a single sampling or is it based upon repeated sampling, implying possible post-ingestive effects? A test reflecting the range of afferent gustatory events more accurately (as verbal reports from humans do) may be a more sensitive index of the organism's interpretation of the stimulus.

The limitations of the fluid consumption test have led to the development of the taste reactivity test. This method requires applying a small calibrated quantity of taste solution directly into the oral cavity of a freely moving rat and videotaping the resultant response for frame by frame analysis. Response dynamics can then be timed and categorized to yield normative data. These data, i.e. the sequencing and duration of response components, can be directly compared to the taste reactivity of neurologically impaired animals. Such comparisons are required to establish anatomical boundary conditions for the elicitation of ingestion and rejection responses by gustatory stimuli.

METHODS

Fistulae

Under pentobarbital anesthesia (35 mg/kg i.p.) 12 male Sprague–Dawley, Charles River rats, weighing 250–375 g, were implanted with 2 intraoral fistulae[9,15]. Each fistula was placed just anterolateral to the first maxillary molar, brought out subcutaneously and anchored to the skull with cranioplastic. Obturators of PE 10 tubing (polyethylene tubing, Clay–Adams) heat sealed to PE 100 tubing were inserted to keep the fistulae clean and patent during the course of the experiment. Rats were individually housed and maintained under a normal 12 h light/12 h dark cycle. All testing was performed during the light phase.

Apparatus

Tests were carried out in a Plexiglas cylinder 10 in. in diameter and height. A ball bearing swivel containing two, 3 in. pieces of 17G stainless steel tubing was mounted in the center of a removable lid. Eleven inch lengths of PE 160 tubing were connected from the swivel to the rats' fistulae. The observation cylinder was placed on a stand that accomodated a removable clear plastic floor. Elevation pegs on the plastic floor facilitated air circulation between floor and cylinder. A mirror was mounted between the legs of the stand enabling observation of the rat's mouth during testing. A foot switch activated seconds timer was hung directly below the mirror.

Calibrated 50 μl injections of taste stimuli and distilled water were dispensed from 1.0 ml hypodermic syringes. Either a 23G 3/4 in needle or a teflon hub (acid containing syringes) was fit on each syringe. Calibrated fluid injections were delivered to the oral cavity via a 3 ft. length of PE 50 tubing ending in a 25 mm nozzle consisting of PE 10 tubing. The PE 50 passed freely through the swivel and attached PE 160 tubing but only the PE 10 could pass into the rats' fistulae. This insured that a fixed length of PE 10, 1–2 mm, would protrude into the oral cavity, so that fluid was squirted directly into the mouth and not back up the lumen of the fistula. Animals did not respond to this short length protruding into the oral cavity, although they did to 5–8 mm of the same tubing.

This apparatus enabled rapid changing of the taste stimuli without restraining or otherwise interrupting the animal's movements. The process of changing gustatory stimuli, removing one length of PE 50 and replacing it with another, took approximately 10 sec.

Experimental analysis of taste reactivity was done exclusively with the videorecorded response. Even though rats were free to move, they settle down within minutes of being placed in the testing cylinder. The only stimulus to evoke movement during the response was sapid quinine. The rat's head was made to fill the entire screen when the focal length of the videocamera (Sony AVC 3260, 75 mm zoom lens with 5 mm teleextender) was approximately 8 inches from the surface of the mirror. The resolution of ongoing oral behavior afforded by this system compensated for the difficulty of tracking a moving animal. The responses to stimuli were recorded at 60 frames/sec (Sony AV 3650) for subsequent frame by frame analysis. Aside from identifying the stimulus, no verbal description accompanied a trial to reduce biasing the subsequent visual analysis.

Stimulus selection

Four concentrations of each of the 4 standard taste stimuli were selected from within the range of existing fluid consumption data by the following criteria. Each concentration step should yield observable differences in reactivity from its adjacents. The weakest concentration should be detectably different from distilled water while the strongest should not produce physiological damage to the oral cavity. Pilot testing yielded the following selection. The ascending series in molar or normal solutions for each taste was as follows: sucrose, 0.01, 0.03,0.3, 1.0; quinine hydrochloride (QHCl), 0.000003, 0.00003, 0.0003, 0.003; sodium chloride (NaCl) 0.03, 0.3, 1.0, 1.3; hydro-

chloric acid (HCl), 0.003, 0.03, 0.1, 0.3. The strongest solutions may seem quite concentrated, however, the quantities delivered were minute (50 μl) and pilot tests indicated that there were no cumulative ill effects (i.e., there was no change in the threshold or duration of responses over repeated trials). All chemicals were reagent grade; the solvent was distilled water. Sucrose solutions were prepared weekly.

Stimulus size

Halpern[5] has estimated the rat's usual volume per lick to be 5 μl. This figure can vary as a function of spout diameter. Whereas a 5 μl stimulus might be limiting in terms of threshold effects related to stimulus size and not stimulus concentration, a 50 μl stimulus should not. Pilot data using either a 50 or 100 μl stimulus volume did not yield dramatic differences in response to a fixed concentration. The 50 μl stimulus size was selected to reduce fluid intake-during a test session. The total volume of fluid injected per test day including water was 1.4 ml.

Testing

Rats were handled daily during their 1 week recovery from surgery. On 4 subsequent days rats were familiarized with the apparatus and received 2 midrange concentrations of each of the 4 taste stimuli. Formal testing followed. A test day consisted of an ascending series of sucrose concentrations followed by an ascending series of QHCl concentrations, or similarly, an ascending series of NaCl followed by HCl. Formal testing included 4 such days, two ascending series of sucrose, QHCl, NaCl and HCl in total. The stimulus order was chosen because QHCl and HCl may have longer lasting residual effects than either sucrose or NaCl.

A standard test day occurred as follows. Rats were adapted to the test cylinder for 10 min. The first ascending series was either sucrose or NaCl. Two water rinses always preceeded and followed each taste stimulus. Time between oral injections was between 30 and 45 sec post termination of the previous response and cessation of all activity. The 2 rinses following the most concentrated stimulus of the first series was followed by flushing of the rat's fistula with 5 cc of room air and a 10 min intertest interval. The second ascending series was either QHCl or HCl and proceeded in the same manner as the first series.

Initially some animals were tested under either ad lib food and water or one of the following deprivation conditions: 0.5 h post tube fed 12 ml meal (2 Kcal/ml)), 3 meals daily, sweetened condensed milk/water diet[9], 1 h post tube fed, 3–4 h post tube fed, 5 h post tube fed. Tube fed animals had no other access to food and water. In deprivation conditions of 3 h and beyond, the animal's activity was so great as to make it impossible to track its mouth with the videocamera. The duration of taste reactivity was also greatly reduced. Conversely, results from ad lib to 1 h food deprived yielded no systematic differences in taste reactivity (i.e. response duration, response components, component concentration thresholds). Therefore, the data analyzed in this report have been combined from ad lib, 0.5 h and 1 h food deprived rats.

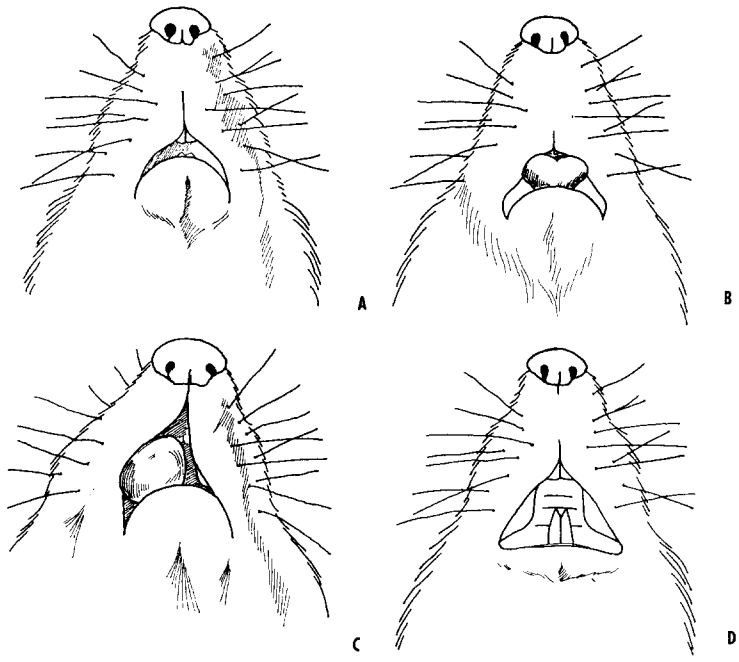

Fig. 1. The lexicon of 4 mimetic response components comprising taste reactivity: rhythmic mouth movements, A; rhythmic tongue protrusions, B; lateral tongue movements, C, and gapes, D. Of the 4 mimetic components only mouth movements and gapes have been observed to initiate response sequences.

Data analysis

For each animal, two responses at each stimulus concentration were examined frame by frame (16.6 msec per frame). The components of each response were sequenced, timed and categorized. These components were then photographed and/or traced from the videoscreen for comparison. Taste reactivity was quantified as follows: total response time, sequence of response components, and duration, frequency and magnitude of the individual response components.

RESULTS

Responses to taste stimuli were highly consistent within and between rats. The response observed after a 50 μl intraoral injection of a taste stimulus could be conveniently divided into mimetic components (movements of lingual, facial and masticatory musculature which are displayed in Fig. 1) and body components. Each of the sapid solutions tested generated a stereotyped response derived from a lexicon of 4 mimetic and 5 body response components. The sequence of response components was not independent across stimulus categories (only two of the 9 components initiated sequences) and the form of some components varied between stimuli. Each component will be described separately first, and the total sequences resulting from sapid stimulation subsequently.

Mouth movements consist of low amplitude, bilaterally symmetric movements of

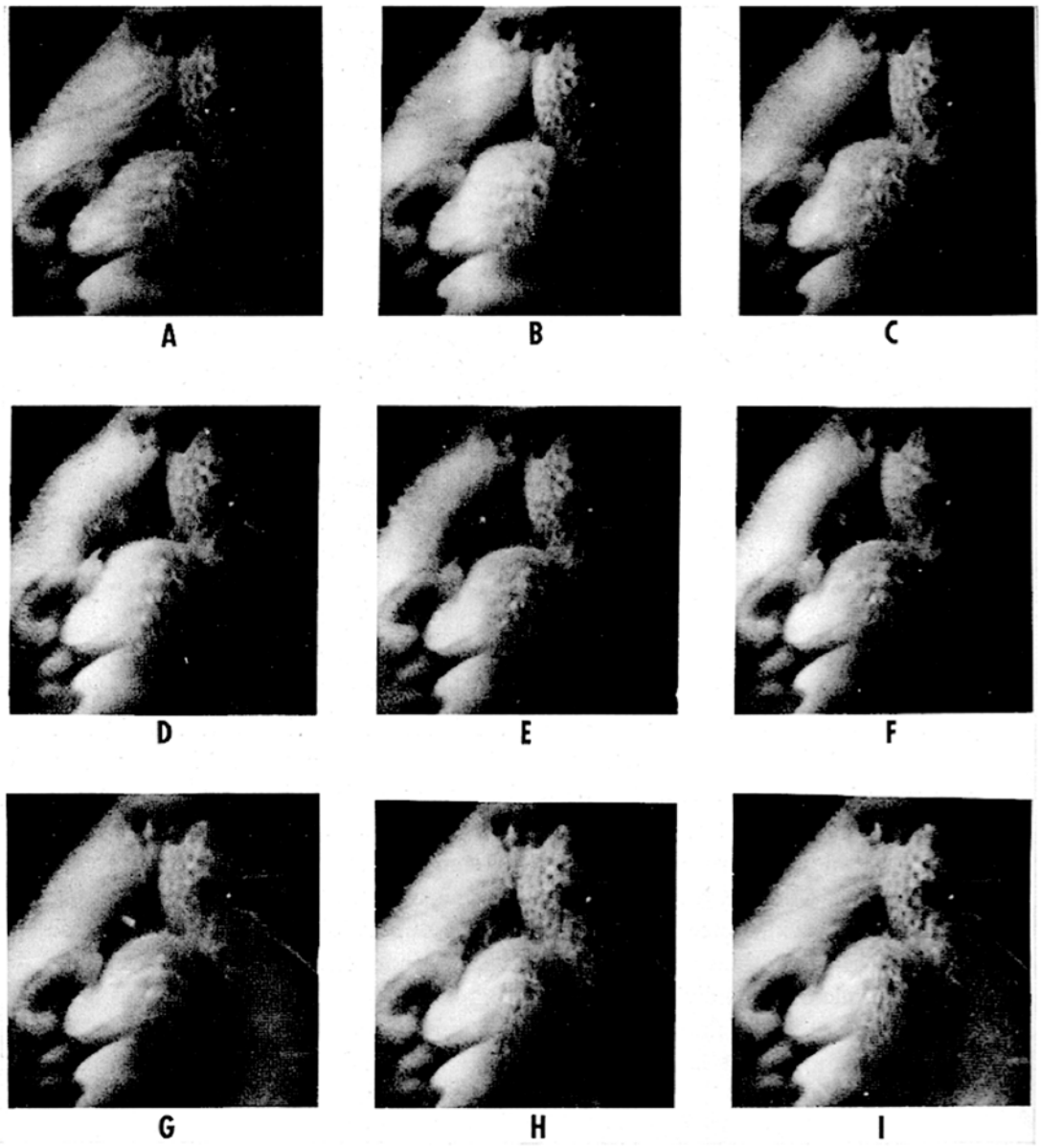

Fig. 2. Consecutive videoframes (16.6 msec separating each frame) of a lateral tongue movement elicited by 50 μl of a 0.3 *M* sucrose stimulus. The tongue emerges in either corner of the mouth, pushing the upper lip laterally (B–E) as the tongue moves forward. The maximal extension of the tongue is shown in E. Tongue retraction (F–I) is morphologically and chronologically identical to tongue extension (B–E).

the mandible that occur rhythmically (6.6 cycles/sec). The mandible opens to its maximal extent rapidly, within a 16.6 msec (single frame) (see Fig. 1A). The mandible stays open for an additional frame; jaw closure is maintained for 116.2 msec before reopening.

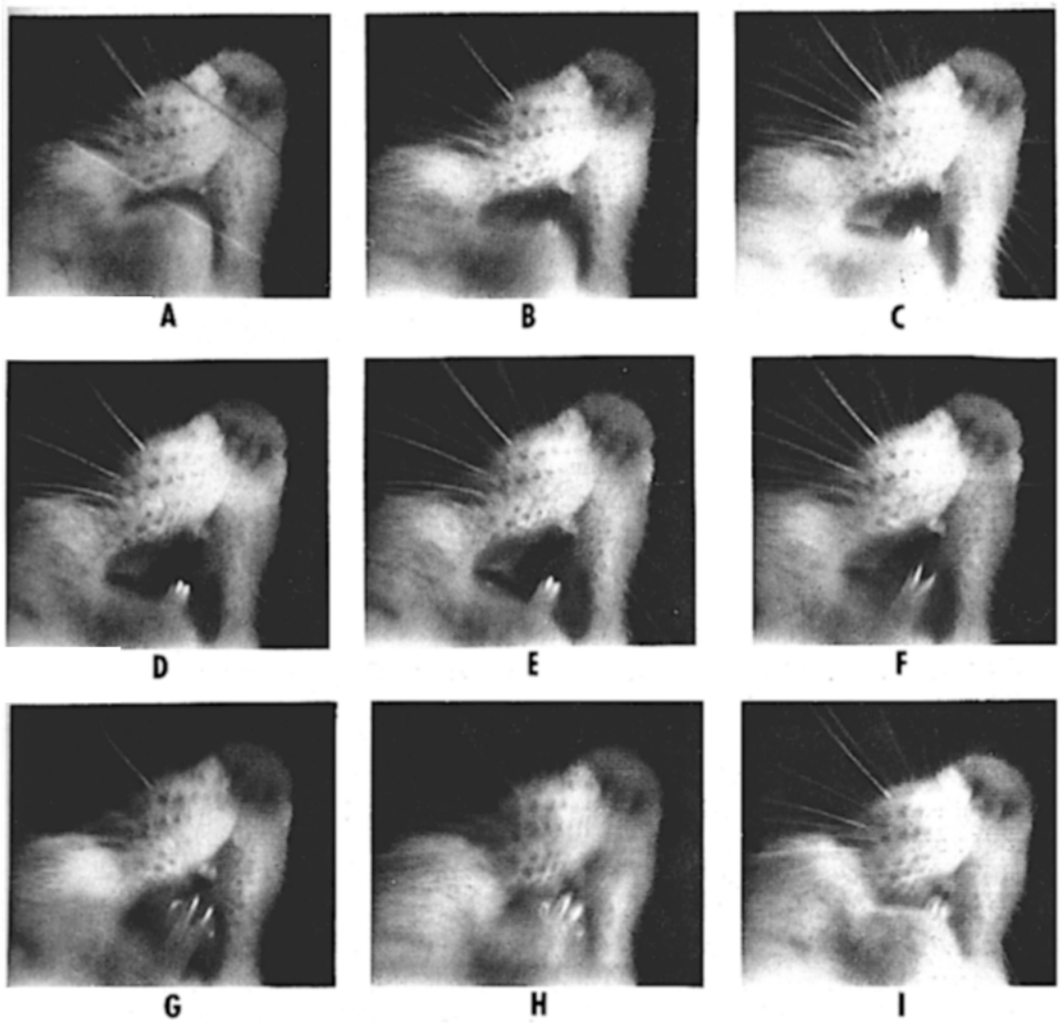

Fig. 3. Consecutive videoframes (16.6 msec separating each frame) of an individual gape elicited by a 3×10^{-4} *M* QHCl stimulus. The corners of the mouth retract (B–F) to a maximal mouth opening (F). The lower lip retracts to reveal the incisors (F and G). The tongue is initially retracted from its extended resting position (B–F) and then extends at jaw closure (G–I).

Tongue protrusions. When mouth movements initiate a response they are stereotypically followed by rhythmic protrusions of the tongue (8.8 cycles/sec). The anterior tip of the tongue visibly emerges directly on the midline, to cover the upper incisors, during the mandible opening of a mouth movement (Fig. 1B). Protrusion and retraction of the tongue take place in approximately 49.8 msec; protrusion accounting for one half of the total time. The jaw closed position is maintained for approximately 66.4 msec before reopening for the next protrusion. The tongue protrusion is not added on to the mouth movement cycle. The duration of the mouth movement that contains a tongue protrusion is shorter (116.2 msec) than a mouth movement that occurs alone (149.4 msec).

Lateral tongue movements. A response that begins with mouth movements and tongue protrusions is generally followed by lateral tongue movements (Fig. 1C). As shown in Fig. 2, the tongue emerges on the side of the mouth, extending the upper lip laterally as the tongue moves forward. Lateral movements occur singly with a variable duration (85–215 msec) or in pairs of equal duration. Duration was timed from the opening of the midline cleft of the upper lip (Fig. 2B) to its closing (Fig. 2I). Although single lateral movements occurred irregularly on either side of the mouth, the second protrusion of a pair always occurred on the side opposite the first after an 80–90 msec delay.

Gaping. The response to certain taste stimuli begins with gaping (Fig. 1D). In Fig. 3 it can be seen that the mandible rapidly lowers and concomitantly the corners of the mouth retract posteriorly and dorsally revealing the internal oral labia. Retraction of the corners of the mouth (Fig. 3B–G) forms a triangular shaped mouth opening that is held for approximately 83 msec. During the maximal mandible opening the lower lip retracts to expose the incisors (Fig. 3F and G). At rest the rat's tongue extends over the lower incisors to touch the dorsal portion of the upper incisors[7]. In gaping the tongue retracts from this resting position during the opening of the mandible and extends at closing (Fig. 3). The tongue moves forward to push against the lower incisors during jaw closure (Fig. 3G and H).

Gapes generally occurred in rhythmic bursts (2–6) with inter-gape intervals of 85–115 msec. Gaping differs from yawning (the only other large opening of the mandible) in duration and appearance. The mean duration of a gape was 166 msec, while a yawn lasted 1000 msec. The corners of the mouth are not retracted in yawning, so the mouth assumes an eliptical shape approximating the passive stretching of the mandible of an anesthetized rat.

All mimetic responses, except gaping, proceeded in the absence of body movement. That is, freely moving rats did not alter their position during movement of the facial, lingual and masticatory musculature. In response to specific stimuli, however, rats performed a sequence of 5 body movements during and after gaping. *Chin rubbing* was defined as lowering the head which brings the mouth in direct contact with a substrate (i.e. floor, wall) and projecting the body forward by flexion of the dorsal neck, pectoral and forelimb musculature. The lower lip was passively retracted as the head moved forward. Neck extension was in the forward direction only; there were no lateral movements of the head. Chin rubbing occurred as a single extension (500 msec $\pm$ 90 msec) or as a sequence of extensions and relaxations (persisting for several seconds) terminating with relaxation of the musculature and elevation of the head to its normal resting posture approximately 1 cm above the substrate.

Head shaking consisted of rapid side to side movements of the head. The alternation of these movements was faster than 60 cycles/sec since these movements blurred in individual videotaped frames. 'Wet dog shaking' seen in response to fluid on the dorsum of many animals is analogous except that head shaking is restricted to the head and neck musculature. *Face washing* is a face-forelimb grooming strategy of rats consisting of active contact between forelimbs, oral cavity and face while the rat rears on its hindlimbs and can last several seconds. A rapid (faster than 60 cycles/sec)

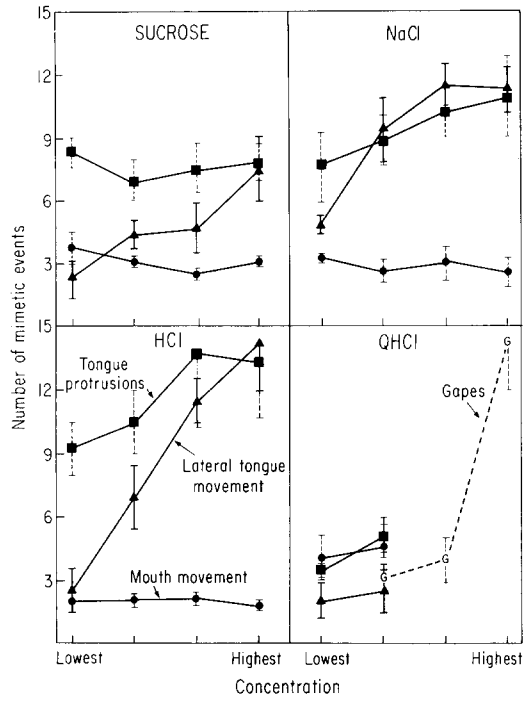

Fig. 4. The response sequence elicited by sucrose, NaCl and HCl proceeds from mouth movements, to tongue protrusions, to lateral tongue movements. Circles represent the number of initial mouth movements before the appearance of the first tongue protrusion. Squares signify the number of tongue protrusions before the first lateral tongue movement. The total number of lateral tongue movements (▲) and gapes (G) occurring within a response are also plotted as a function of stimulus concentration. Mouth movements and tongue protrusions appear to be less concentration dependent than lateral tongue movements and gapes. Brackets represent standard deviations.

alternating or *flailing of the forelimbs* follows face washing in response to certain taste stimuli. *Paw pushing* — in quadrupedal posture, the rat simultaneously extends one forelimb forward against the substrate and retracts the other back, actively rubbing the forepaws on the substrate. Intervals between extensions of each forelimb ranged from 166–250 msec. Paw pushing can persist for seconds as a continuous behavior. Face washing is elicited by specific gustatory stimuli but is also seen without apparent antecedents in normal rats as part of grooming behavior. Unlike face washing, chin rubbing, head shaking, forelimb flailing and paw pushing are not seen in intact rats without antecedent gustatory stimulation.

A 50 μl intraoral injection of sucrose (0.1, 0.3 or 1.0 *M*) always elicited a stereotyped sequence of mouth movements, tongue protrusions and lateral tongue movements (Fig. 4). The first lateral tongue movements appeared about 2.5 sec ($\pm$ 0.4 sec) after the first mimetic response (mouth movements) following stimulus onset. After the first lateral tongue movements, mouth movements and tongue protrusions reoccurred in no apparent order between subsequent lateral movements. Both the number of lateral tongue movements and total response duration (first mimetic response to the onset of a 4 sec period without activity) increased with increasing sucrose con-

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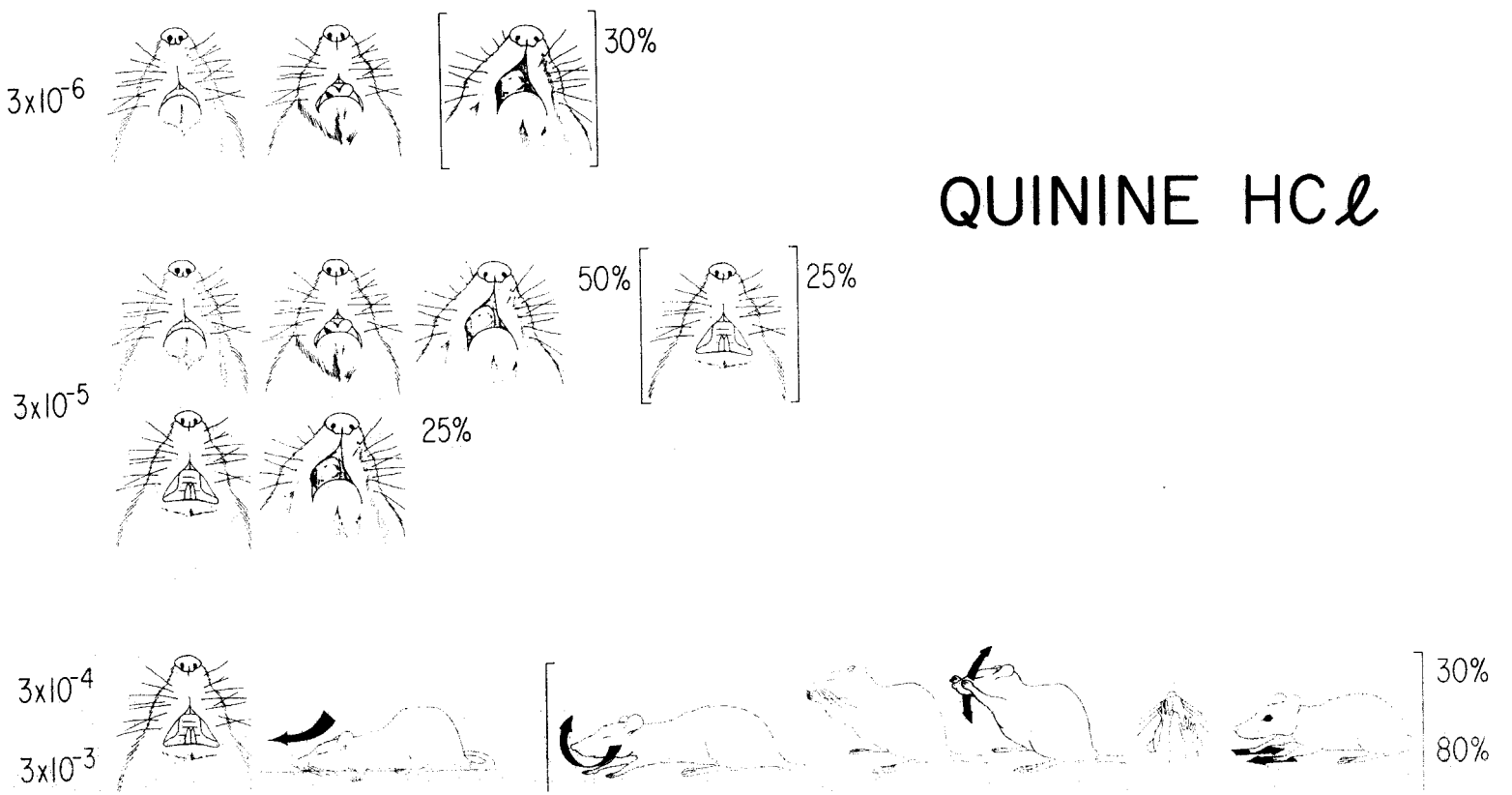

centration (Fig. 4). The initial sequence of mouth movements and tongue protrusions however, had a relatively constant duration across concentrations (Fig. 4).

The weakest sucrose (0.03 *M*) elicited the complete stereotyped response sequence in only 50% of the trials. In the remaining instances only the initial burst of mouth movements and tongue protrusions occurred. At all concentrations, the mimetic response rarely led into further activity. In the vast majority of trials the rat abruptly returned to quiescence without even shifting position.

Strong quinine (3×10^{-4} and 3×10^{-3} *M*) produced a pattern strikingly different from the sucrose response. A series of gapes initiated the response. A stereotyped sequence of chin rubbing, head shaking, face washing, forelimb flailing and paw pushing followed in 80% of the trials at 3×10^{-3} *M* and in 30% of the trials at 3×10^{-4} *M* QHCl (Fig. 5). Rhythmic bursts of gaping frequently reoccurred between execution of the successive behaviors of this sequence. In the remaining instances just chin rubbing followed the initial gaping burst. The mouth movements–tongue protrusions sequence was not seen in response to strong quinine, but lateral tongue movements were periodically observed. After the initial sequence (Fig. 5), gape bursts, individual body responses and lateral tongue movements reoccurred, without pause, until all responding ceased abruptly. Gape sequences were observed during body movements, but lateral tongue movements and body movements were mutally exclusive. Both the number of gapes per response and the total response duration increased markedly with increasing concentration (Fig. 4).

The threshold for gaping was 3×10^{-5} *M* QHCl. In 25% of the trials the initial gaping burst was followed by lateral tongue movements (latency 7.2 sec) while body movement and the initial mouth movements–tongue protrusions pattern was never seen (Fig. 5). In an additional 25% of the instances, the response began with mouth movements, tongue protrusions, and lateral movements, but ended with gaping. With this exception, gaping never occurred late in the response if it did not initiate responding. In the remaining trials, only the stereotyped sequence of mouth movement, tongue protrusions and lateral tongue movements was elicited. The characteristics of this response did not differ from the sucrose response.

The weakest QHCl (3×10^{-6} *M*) elicited the complete stereotyped pattern of mouth movements, protrusions and lateral movements of the tongue in 30% of the trials. In the remaining observations, only the initial sequence of mouth movements and tongue protrusions occurred (Fig. 5). In contrast to the active body responses

Fig. 5. Quinine responses are concentration dependent, a completely different response sequence is elicited by concentrations above 3×10^{-5} *M* QHCl. At 3×10^{-4} *M* the sequence in brackets (head shaking, face washing, forelimb flailing and paw pushing) follows gaping and chin rubbing in 30% of the trials whereas the more concentrated 3×10^{-3} *M* elicits the bracketed responses in 80% of the trials. In the remaining trials at these concentrations, just gaping and chin rubbing are elicited. The threshold for gaping is 3×10^{-5} *M* QHCl. In 25% of the trials, gaping (bracketed) follows mouth movements, tongue protrusions and lateral tongue movements and in another 25% gaping initiates the response and is followed by lateral tongue movements. Mouth movements and tongue protrusions are elicited in 70% of the trials at 3×10^{-6} *M* QHCl; lateral tongue movements (bracketed) follow these components in 30% of the cases.

suprathreshold to gaping (3×10^{-3} *M*), threshold (3×10^{-5} *M*) and subthreshold (3×10^{-6} *M*) quinine concentrations, like sucrose, did not provoke a change in the rat's position.

The stereotyped sequence of mouth movements, tongue protrusions and lateral tongue movements characterized the response to all concentrations of NaCl and most concentrations of HCl (0.03, 0.1, 0.3 *M*). The sequence of the response to salt and acid did not differ from the sucrose response. The weakest acid (0.003 *M*) elicited the complete stereotyped response in 66% of the trials and only the initial sequence of mouth movements and tongue protrusions in the remaining trials. As in the sucrose condition, rats did not alter their position in response to NaCl and HCl concentrations. The number of lateral tongue movements and the total response duration increased with increasing NaCl and HCl concentration (Fig. 4).

Responses to taste stimuli were remarkably uniform in the rats tested. Conversely, the response to water rinses was variable between and with rats. A large part of this variability was explained by the percentage of the observations (35%) which elicited no response at all. Of all the remaining instances 45% consisted of just mouth movements and 55% consisted of mouth movements followed by tongue protrusions. The duration of both forms of the water response was 2–3 sec, whereas response durations of the lowest concentration of any of the taste stimuli tested were 4–6 sec.

While the sequence of response components was similar for sucrose, NaCl and HCl, the duration and form of lateral tongue movements differed among these stimuli. Differences in the duration of lateral tongue movements could be a function of stimulus concentration within a stimulus category or stimulus category itself. To examine whether differences were related to concentration, lateral tongue movement duration for most and least concentrated stimuli were compared (*t*-test) and no significant differences were found within sucrose, NaCl and HCl categories. To examine whether lateral tongue movement duration was a function of stimulus category, the mean lateral movement duration for all observations at each concentration were averaged by stimulus

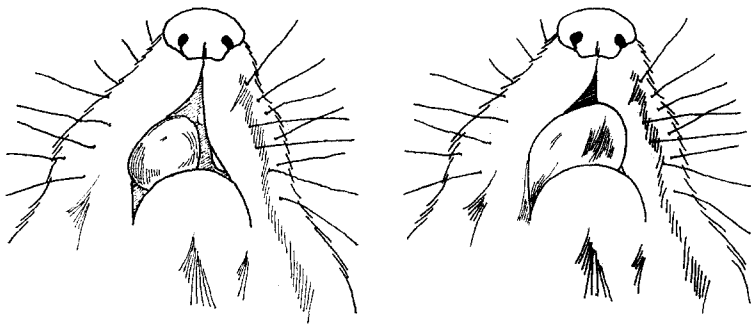

Fig. 6. The maximal protrusion of the tongue is greater in response to 0.03 *M* HCl or 1.0 *M* NaCl (b) than to 1.0 *M* sucrose (A). In response to the NaCl or HCl stimulus the tongue arcs medially over the midline cleft to cover a portion of the contralateral lip; the tongue remains ipsilateral in response to sucrose.

category and compared (t-test). The mean duration of lateral tongue movements for sucrose (127.8 msec) was significantly different ($P < 0.001$) from the mean durations of NaCl (151.89 msec) and HCl (168.9 msec). The mean duration of NaCl and HCl differed significantly ($P < 0.05$). The form of the lateral tongue movement was correlated with its duration. That is, increases in duration were generally visualized as increases in the magnitude of the tongue protrusion. Fig. 6 shows that the extent of the maximal protrusion of the tongue is greater in response to 1.0 *M* NaCl and 0.03 *M* HCl than to 1.0 *M* sucrose. In response to sucrose the tongue remains ipsilateral but after NaCl or HCl it arcs medially over the cleft in the upper lip.

DISCUSSION

The taste reactivity test analyzes discriminative responses to small intraoral injections of taste stimuli using frame by frame videotape analysis. The pattern of mimetic and body responses, or taste reactivity elicited by the four tastes examined took two distinct forms in all rats tested. Responses elicited by the 4 taste categories examined were composed of specific subunits. The arrangement of these components was initially fixed (initial sequence); the same components then reappeared with no apparent order. For example, the response to sucrose, NaCl and HCl began with mouth movements which were followed systematically by tongue protrusions. Appearance of the first lateral tongue movement following tongue protrusions signalled an apparently random reoccurrence of mouth movements, tongue protrusions and lateral tongue movements. Responses proceeded without pause and terminated abruptly.

Taste reactivity to sapid solutions of quinine differed strikingly from the response to sucrose, NaCl and HCl. Gaping initiated quinine responses while a pattern of mouth movements and tongue protrusions initiated responses to the other tastes. Body movement dramatically increased in response to quinine while rats did not alter their position following intraoral injections of sucrose, NaCl and HCl. The mouth movement–tongue protrusion pattern characterized the response to the other tastes but was only observed in response to the lowest concentrations of quinine. Lateral tongue movements were not systematically observed in response to strong quinine while they were consistently observed to follow tongue protrusions in response to sucrose, NaCl and HCl. The stereotyped sequence of taste reactivity, mouth movements, tongue protrusions and lateral tongue movements, was the same for sucrose, NaCl and HCl, however, the duration and form of the lateral tongue movements were different.

Taste reactivity to quinine was concentration dependent; responses took one form at low, and another at high concentrations. No such concentration gradient was apparent in response to sucrose, NaCl or HCl. Responses proceeded in the same manner (mouth movements to tongue protrusions) regardless of concentration. Quinine responses differed qualitatively; the two forms of the response consisted of different component parts, arranged in concentration dependent ways. Sucrose and HCl responses differed quantitatively; at the lowest concentration responding terminated with tongue protrusions but at higher concentrations it continued into lateral tongue movements and a replay of the initial response sequence. NaCl reponses took one form regardless of

concentration. To test the hypothesis that a wider range of concentrations would reveal a different form of taste reactivity, the concentration of NaCl was increased in one molar steps to 4.0 *M* and the volume of salt and acid stimuli was doubled (100 μl). To examine whether a different sodium salt might elicit a quinine-like response, sodium acetate was used. In concentrations through 1.3 *M* this sodium salt approximated NaCl reactivity. Changes in stimulus strength, size and anion did not alter the form of taste reactivity from that obtained under normal testing conditions. The different taste reactivity elicited by QHCl on the one hand, and by NaCl and HCl on the other must be extended to other bitter, salt and acid stimuli.

Taste reactivity was compared to results from other behavioral tests of taste acceptance and rejection. These other behavioral tests are not homogeneous and are grouped on the basis of dependent variable: amount ingested (two bottle, long term preference test[16]; single stimulus, brief exposure acceptance test[19]; single stimulus, esophageal and intragastric fistulae acceptance test[12]), or single and multiple lick (two bottle, single lick preference test[20]; single stimulus, 10 sec lick analysis test[17]). The brief duration (single lick through 10 sec) and non-ingestive nature of single and multiple lick tests best approximate the parameters of the taste reactivity test. These comparisons are therefore most heuristically interesting. Unfortunatley, however, single and multiple lick tests have only been applied to sugars and NaCl. Comparisons of taste reactivity to QHCl and HCl must be drawn from behavioral tests using ingestion as a dependent variable.

Results for sucrose are similar for taste reactivity and non-ingestive behavior tests. There is a monotonically increasing relationship between preference[20] or acceptance[3] and sucrose concentration. Davis[3] suggests that increasing sucrose concentration stimulates the activity of the tongue in the absence of negative feedback from the consequences of ingestion. Similarly, the total duration of the sucrose response and the number of lateral tongue movements within it, monotonically increased as a concentration gradient. In behavioral tests that use ingestion as a dependent variable, quinine consumption falls off rapidly as concentration exceeds 10^{-5} *M*, the absolute behavioral threshold for quinine[10]. The threshold of gaping is only 1/2 log step above this, reflecting the sensitivity of the taste reactivity test.

The major difference between taste reactivity and ingestion tests are the NaCl and HCl results. Taste reactivity to sapid solutions of NaCl (1.0, 1.3 *M*) and HCl (0.1, 0.3 *M*) gives no immediate impression of aversion, but instead looks similar to the sucrose response. Nevertheless, in fluid consumption tests sucrose is avidly consumed, but little if any concentrated acid or salt is ingested. In other words, salt and acid approximate quinine in tests of fluid consumption, but look like sucrose in the taste reactivity test.

To understand how salt and acid can share characteristics of two behaviorally opposite tastes, it may be necessary to differentiate between aversion and avoidance. Aversive stimuli are innately noxious. A single presentation of an aversive stimulus immediately elicits behavior(s) designed to reduce stimulus intensity[1]. Stimuli are avoided on the other hand, only after they have been associated with an aversive consequence. As a result of association, an avoided stimulus may elicit behaviors characteristically evoked by aversive stimuli.

Quinine seems to fit the criteria for an aversive stimulus. The sequence of motions comprising the quinine response appears to facilitate removal of fluid from the oral cavity. Gaping collects fluid anteriorly in the oral cavity. Chin rubbing passively retracts the lower lip allowing fluid to flow out of the oral cavity. The rapid lateral head shake scatters oral fluid within the test chamber. Face washing with its accompanying forelimb flailing rids the perioral region of accumulated fluid. Paw pushing terminates the sequence by rubbing the wet forepaws on the substrate. Although the expulsion of fluid from the oral cavity was witnessed repeatedly during the quinine response, its functional significance requires more direct quantification of the amount of fluid ingested and rejected during repeated exposure. Teitelbaum and Epstein[18] describe what they call a highly aversive response pattern, consisting of chin rubbing, forelimb flailing and paw pushing, elicited by 2.5×10^{-2} *M* (1 %) quinine placed in the mouth of intact rats with an eyedropper.

Neither strong NaCl nor HCl elicits immediate rejection. Nevertheless, it is possible that both are inherently noxious. No a priori reason demands that the quinine response be the sole concommitant of aversion. If there are several varieties of aversion, however, an organism's rejection of a sapid stimulus after association with illness should not necessarily be accompanied by a quinine-like mimetic response. Nevertheless, when either sucrose or NaCl stimuli are paired with LiCl injection (3.0 mEq/kg, i.p.), subsequent presentations induce a response which is indistinguishable from the quinine response (Grill[4] and unpublished observations). If strong salt and HCl are not inherently aversive but their ingestive consequences are, then the avoidance of these stimuli during fluid consumption could result from an associate process. Assuming the post-ingestional consequences of NaCl or HCl are similar in some respects to the effects of LiCl poisoning, then a quinine-like mimetic response might develop with repeated sampling of either of these sapid stimuli. This notion could be examined using lick pattern analysis[3,6,17]. The pattern and duration of licking in response to these stimuli may reveal the development of post-ingestive effects.

In contrasting the NaCl results obtained using ingestive and non-ingestive dependent variables it is clear that what is meant by acceptability is dependent upon how it is measured. For example, salt ingestion is a monotonically decreasing function of concentration[12], while the more concentrated of pairs of salt stimuli is consistently selected in single contact tests[21]. Obviously, the consequences of ingestion change what is meant by acceptability. The taste reactivity test is basically free of post-ingestive consequences and therefore reflects the organism's immediate impression of the stimulus. While taste reactivity to NaCl and HCl are strikingly different from quinine, responses to these stimuli are not identical to sucrose either. The form and duration of lateral tongue movements to NaCl and HCl are observably different than sucrose.

Analysis of the mimetic response has not provided a quantification of swallowing, but expulsion or ingestion of orally injected fluids was readily observable. Expulsion of sapid quinine was repeatedly observed. Fluid dribbled from the mouth, soaked the fur of the mandible and dripped to the cage floor. Conversely, no such expulsion was seen in response to the other taste stimuli tested; both mandible and substrate remaining dry. The most concentrated sucrose, NaCl and HCl stimuli were ingested. Swallowing has

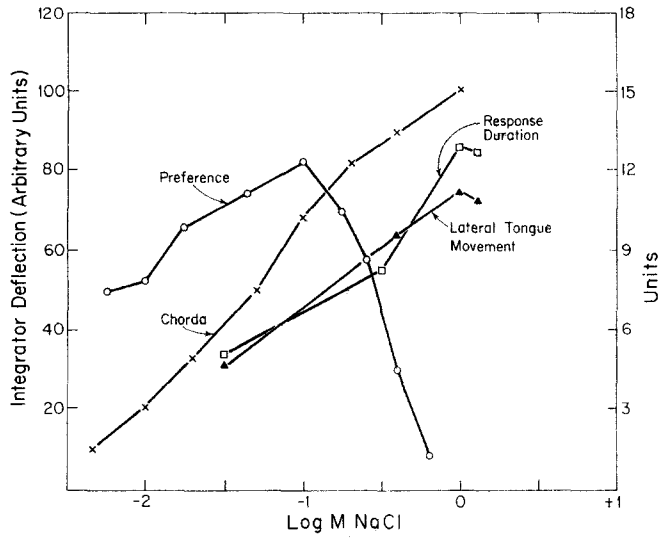

Fig. 7. The electrophysiological response of the chorda tympani increases monotonically as a function of concentration. Unlike fluid consumption which begins declining at approximately 0.1 *M* (−1 log M), response duration and number of lateral tongue movements increase monotonically through 1.0 *M* (0 log M), like the chorda tympani response. The taste reactivity test which minimizes post-ingestive influences, seems to reflect the range of afferent gustatory events more closely than does fluid consumption.

been quantified using the EMG record of either mylohyoideus[8] or genioglossus[11]. An EMG analysis synchronized with the videorecord of chronic ingestive behavior, such as the technique of Zweers[22], would enable a quantification of swallowing for the taste reactivity test.

Pfaffmann et al.[14] have compared NaCl preference behavior (2 bottle, 48 h ingestion test) with the afferent discharge of primary and secondary gustatory neurons. They were unable to find any change in sensory afferent events, beyond magnitude, that might correspond to the point of behavioral aversion (ingestion to avoidance) to NaCl. Fig. 7 compares the electrophysiological response of the chorda tympani nerve, behavioral preference (ingestion), and two taste reactivity dependent variables (total response duration and mean lateral tongue movements per response) as a function of NaCl concentration. Unlike preference data, taste reactivity variables and afferent gustatory events increase monotonically as a function of NaCl concentration through 1.0 *M*. The taste reactivity test seems to reflect the range of afferent gustatory events and may be a more sensitive index of the organism's interpretation of the stimulus than fluid consumption tests. The lack of electrophysiological and preference data beyond 1.0 *M* NaCl does not allow for comparisons with taste reactivity variables which appear to level off between 1.0 and 1.3 *M* NaCl.

It is not known whether the mimetic aspects of taste reactivity are communicative in rats as they have been assumed to be in man[2,13]. Our working hypothesis for the present is that these responses reflect the economy of the individual. It is important in this regard to examine the neurological basis of taste reactivity. Are the discriminative

responses to taste, comprising the final act of feeding behavior, a function of the integrated action of all levels of the CNS or are these fundamental response sequences subserved by more caudal neural levels?

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