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DEVELOPMENTAL CHANGES IN TASTE RESPONSE CHARACTERISTICS OF RAT SINGLE CHORDA TYMPANI FIBERS[1]

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Abstract

Integrated records show that multifiber responses from the chorda tympani nerve to NaCl and LiCl are of very small magnitude in young postnatal rats. When compared with NH_4Cl, KCl, and citric acid, the integrated responses to NaCl and LiCl gradually increase in magnitude as a function of development. To clarify the basis of changes in multifiber responses, we recorded from single fibers in the chorda tympani nerve in postnatal rats aged: 14 to 20 days, 24 to 35 days, and adult. Taste stimuli applied to the anterior tongue were: 0.1 and 0.5 M NaCl, LiCl, NH_4Cl, and KCl and 0.1 M citric acid.

Even in the youngest rats, at least 89% of the single fibers responded to all salts. Therefore, the small magnitude of the multifiber responses to NaCl and LiCl in young animals cannot be explained by an inability of young receptors to respond to these salts. However, single fiber response frequencies during lingual stimulation with NaCl and LiCl were low in young rats, and during development, the frequencies increased to approximately twice the youngest values. Concomitantly, the response frequencies for NH_4Cl did not change and those for KCl remained the same or progressively increased, depending on the concentration. The responses to citric acid were different from those to any of the salts because response frequencies decreased during development. We also found that more fibers responded maximally to NaCl and LiCl as a function of age and fewer fibers were maximally responsive to NH_4Cl. For all salts and citric acid, a developmental decrease in response latencies was observed.

Single fiber data have revealed that the previously observed changes in multifiber salt responses are attributable to differential changes in response frequencies and in the ranking of salts that are maximally effective. Developmental changes in taste response frequencies and latencies must relate to one or more processes at various levels of the peripheral gustatory system, including the taste pore, the receptor membrane, the synapses between receptor cells and chorda tympani afferents, and the chorda tympani fibers themselves.

Neurophysiological changes associated with development have been documented extensively in the visual and auditory systems (e.g., Movshon and Van Sluyters, 1981; Saunders and Brock, 1978). Very recently, it has become apparent that developmental changes also occur in the gustatory sense. Studies in sheep have demonstrated that neurophysiological taste responses in the peripheral and central nervous systems alter substantially during fetal development and after birth (Bradley and Mistretta, 1980; Mistretta and Bradley, 1978, 1980). Changes in peripheral taste responses now have been observed in the rat also (Ferrell et al., 1981; Hill and Almli, 1980; Yamada, 1980), a mammal with a much shorter developmental time course than sheep. The sheep has a lengthy gestation (~150 days) and structural development of the peripheral taste system is primarily a prenatal event (Bradley and Mistretta, 1973). In rats, taste buds appear toward the end of the short gestation (21 days) and major morphological changes occur postnatally (Farbman, 1965; Mistretta, 1972).

Developmental studies of the rat taste system have included multifiber recordings of neural responses to

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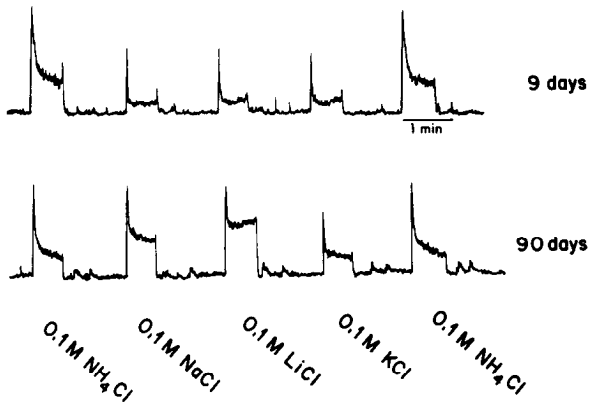

Figure 1. Integrated records of multifiber taste responses from the chorda tympani nerve in rats aged 9 and 90 days, respectively. Relative to NH_4Cl, stimulation of the tongue with NaCl and LiCl elicits small magnitude responses early in development. In adults, however, NaCl and LiCl elicit greater responses than NH_4Cl.

salts, acids, and sucrose. Among the specific changes that occur are those during stimulation of the tongue with four monochloride salts: NaCl, LiCl, NH_4Cl, and KCl. Relative to the responses to NH_4Cl and KCl, the responses to NaCl and LiCl are of very small magnitude early in development and gradually increase with age. As illustrated in Figure 1, these changes are so significant that the order of effective stimuli actually alters from NH_4Cl > LiCl > NaCl > KCl in young postnatal animals to LiCl > NaCl > NH_4Cl > KCl in adults (Ferrell et al., 1981).

One limitation of multifiber data is that the responses must be expressed relative to each other and thus, absolute response changes cannot be determined. Therefore, we have recorded from single fibers in the chorda tympani nerve in rats in three age groups that have different taste responses as demonstrated by multifiber recordings. Our objectives were: (*1*) to learn whether a large proportion of single fibers fail to respond to stimulation of the tongue with NaCl and LiCl in young rats; (*2*) to determine if response frequencies to NaCl, LiCl, NH_4Cl, and KCl increase with age, decrease, or remain the same; and (*3*) to study the response latency characteristics of single fibers as a function of age. Since NH_4Cl and KCl have a sour as well as salty taste component (McBurney and Schick, 1971), we also used citric acid as a stimulus for comparison with the various salts.

Materials and Methods

Surgery. Single fiber neurophysiological taste responses were recorded from the chorda tympani nerve in rats aged 14 to 20, 24 to 35, and 80 to 110 days old (adults). Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 to 60 mg/kg of body weight) and the trachea was cannulated. The head was secured in a nontraumatic holder and a water-circulating heating pad was wrapped around the torso to maintain body temperature between 36 and 38°C. The left chorda tympani nerve was exposed from the bifurcation with the lingual nerve to its entrance into the tympanic bulla. The nerve was cut at the bulla and the exposed length was freed of surrounding connective tissue. Small bundles of fibers were dissected from the nerve trunk for recording single fiber activity.

Neurophysiology. Each nerve bundle was placed on a 30 gauge platinum recording electrode and a platinum reference electrode was positioned in nearby tissues. Warm mineral oil was flowed into the cavity around the chorda tympani to prevent dehydration. The amplified neural activity was displayed on an oscilloscope, monitored on an audio amplifier, and stored on magnetic tape along with voice cues to note experimental procedures. Neural data were subsequently replayed and photographed to produce a permanent record for reviewing impulse amplitude and waveform. Impulses were characterized as originating from single fibers on the basis of uniform spike height and waveform. Using these criteria, data from 19 fibers in 10 rats aged 14 to 20 days, 16 fibers in 7 rats aged 24 to 35 days, and 20 fibers in 8 adult rats were analyzed.

Stimuli. Chemical stimuli were flowed over the tongue in this order: 0.1 M NH_4Cl, 0.1 M NaCl, 0.1 M LiCl, 0.1 M KCl, 0.1 M NH_4Cl, 0.5 M NH_4Cl, 0.5 M NaCl, 0.5 M KCl, 0.1 M NH_4Cl, 0.1 M citric acid, and 0.1 M NH_4Cl. The total sequence was applied at least once for each fiber, and the sequence was repeated in 35% of the fibers. Two concentrations of each monochloride salt were used so that developmental comparisons could be made of single fiber responses to concentrations that elicit approximately one-third to half-maximal (0.1 M) and maximal (0.5 M) responses from the whole nerve. The responses to a complete concentration series of NH_4Cl (0.01 to 1.00 M) also were recorded from 6 fibers in rats aged 14 to 20 days and from 6 fibers in adults.

The stability of the neural preparation was monitored by analyzing the responses to the four 0.1 M NH_4Cl applications. The average response frequencies to this standard stimulus varied somewhat for fibers within each of the three age groups (mean frequencies and standard deviations: 6.2 ± 1.9, 4.5 ± 1.6, and 5.4 ± 1.8, youngest to adult groups, respectively). For our developmental comparisons, it is important to note that the variability in response frequencies did not differ among age groups ($F(6, 156) = 1.45; p > 0.10$). Furthermore, the variability that we found *within* the adult age group is similar to that reported by Ogawa et al. (1973) for the average response frequencies of adult chorda tympani fibers during repeated lingual stimulation with 0.1 M NaCl.

All chemicals were reagent grade, dissolved in distilled water, and kept at room temperature. Two milliliters of each stimulus were applied to the anterior third of the tongue from a syringe and remained there for approximately 20 sec. The tongue then was rinsed for at least 1 min; the next stimulus was presented 15 to 20 sec after the final rinse (at least 1.5 min after the previous stimulus). Syringes were used to apply stimuli instead of a flow chamber in order to have an electrical artifact that occurred when the stimulus contacted the tongue (Fig. 2). This artifact was used to measure the response latencies (Beidler, 1953; Pfaffmann, 1941).

Data analysis. Single fiber data were analyzed by converting the action potentials to standard pulses using a window discriminator. Only impulses from single fibers

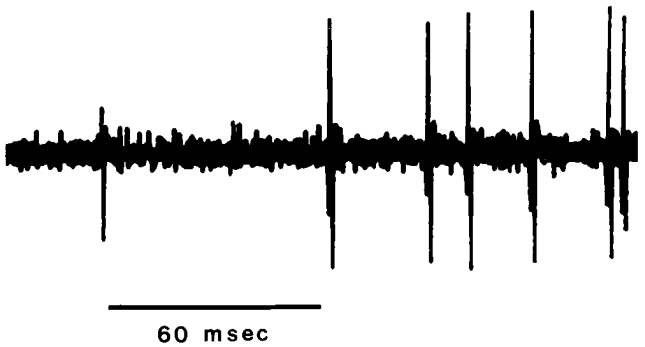

Figure 2. Stimulus artifact and neural impulses from a single chorda tympani fiber to illustrate the measurement of response latency. Latency was measured from the occurrence of the electrical artifact, produced when the stimulus contacted the tongue, to the occurrence of the first neural impulse (64 msec).

(based on amplitude and waveform), or from 2 fibers that could be clearly discriminated, were studied. A digital microcomputer (resolution = 1 msec) was used to measure the interpulse intervals and to store the intervals on magnetic disks (Bradley, 1982). These data were used to generate histograms of impulse frequency before, during, and after chemical stimulation of the tongue. The mean spontaneous activity for each fiber was calculated by averaging impulse frequencies during each 5-sec period preceding individual stimulations. The mean frequencies and standard deviations were 0.4 ± 0.3, 0.7 ± 0.5, and 0.4 ± 0.3 impulses/sec for fibers in rats aged 14 to 20, 24 to 35, and 80 to 110 days, respectively; these mean spontaneous rates were not significantly different ($F(2, 52) = 2.13$; $p > 0.10$). To yield response measures, the mean spontaneous frequency for each fiber was subtracted from the impulse frequency during chemical stimulation of the tongue. When stimuli were presented more than once, the responses were averaged over the multiple applications.

We have expressed the responses to chemical stimuli as average frequencies (impulses per sec) during the first 0.5 to 5.5 sec of impulse discharge. Exclusion of the first 500 msec of the response avoids including the electrical artifact and the transient high frequency portion that other investigators have described as "noisy" (Doetsch and Erickson, 1970). To be certain that the results were not biased by selecting one fixed portion of the taste response for data analysis, we also analyzed the response frequencies from the first 1 sec (0.5 to 1.5 sec) and second 5 sec (5.5 to 10.5 sec) of the response. Use of any one of the three response portions yielded the same major conclusions. Therefore, we will report data based on the first 0.5 to 5.5 sec only, since this response portion is used frequently in other published studies.

To ensure that the single fibers sampled in each age group were representative of the population of chorda tympani fibers, we compared the single fiber responses to 0.1 M salts with the multifiber data for these same chemicals published by Ferrell et al. (1981). A response ratio, defined as the single fiber response frequency for the salt divided by the response frequency for NaCl, was calculated for each salt relative to NaCl. Mean ratios then were calculated for all salts in each age group. Similar response ratios were obtained for both single and multifiber data (Hill et al., 1980).

Response and latency definitions. To learn whether the response frequencies to chemical stimulation of the tongue change as a function of development, we did not impose an arbitrary criterion in determining whether a response occurred. Rather, we simply analyzed frequencies (minus spontaneous activity) when a chemical was on the tongue. Therefore, the results were somewhat biased to observe responses to all chemicals at all ages.

However, to characterize fibers as responding maximally to either NH_4Cl or NaCl and LiCl, we did impose a criterion. Fibers categorized as responding maximally to NH_4Cl had response frequencies at least twice as great as those to NaCl or LiCl. Similarly, a maximal NaCl or LiCl response exceeded the NH_4Cl response frequency by at least 2 times. Therefore, we were conservative in labeling a fiber as maximally responsive to any chemical.

Response latency was defined as the period that elapsed from the time of stimulus contact with the tongue to the first neural impulse. This period was measured from the displays of neural activity on a storage oscilloscope screen (Fig. 2).

For statistical analysis of the data, we set a rejection level of $p < 0.10$; however, the actual p values are reported for the reader's information.

Results

Number of fibers responding to stimuli. To determine whether a large proportion of single fibers in young rats did not respond to NaCl and LiCl, histograms were drawn from the responses from all fibers (Fig. 3). As illustrated in Figure 3, only a few fibers in each age group failed to respond to one or more of the 0.1 M monochloride salts, and the frequency of these instances was not significantly different throughout development ($\chi^2 = 4.76$; $df = 6$; $p = 0.61$). Since 89% of all fibers in rats aged 14 to 20 days responded to 0.1 M NH_4Cl, NaCl, LiCl, and KCl, the small multifiber responses to NaCl and LiCl in rats as young as 14 days cannot be explained by an absence of fibers responding to these stimuli. NH_4Cl, NaCl, LiCl, and KCl at 0.5 M concentrations elicited responses from all fibers in each age group.

Mean response frequencies. To determine whether the response frequencies to NaCl, LiCl, NH_4Cl, and KCl increase, decrease, or remain the same as a function of age, we calculated the mean frequencies for the responses from fibers within each group (Fig. 4) and compared the means by analysis of variance. For both 0.1 and 0.5 M NaCl and LiCl, the response frequencies approximately doubled from 14 to 20 days of age to adulthood. The frequency increases were statistically significant for both 0.1 M (NaCl: $F(2, 52) = 2.73$; $p = 0.08$; LiCl: $F(2, 52) = 2.95$; $p = 0.06$) and 0.5 M concentrations (NaCl: $F(2, 52) = 3.26$; $p = 0.05$; LiCl: $F(2, 52) = 3.94$; $p = 0.03$).

The developmental trends in the response frequencies for the bitter-sour salts, NH_4Cl and KCl, and for citric acid were very different from those for NaCl and LiCl. For example, there was no change in the mean response frequency to 0.1 or 0.5 M NH_4Cl as age increased (0.1 M NH_4Cl: $F(2, 52) = 1.15$; $p = 0.32$; 0.5 M NH_4Cl: $F(2, 52) = 0.66$; $p = 0.52$). Even when the responses to an entire

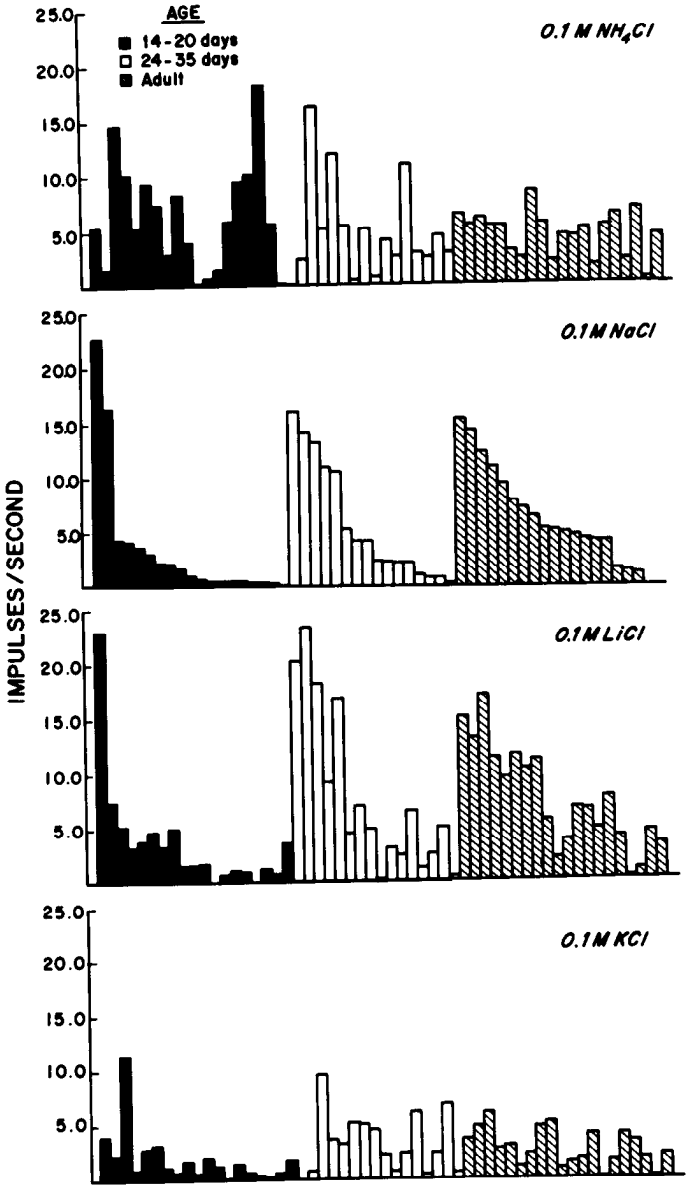

Figure 3. Response frequencies (impulses per sec) of individual chorda tympani fibers to 0.1 M salts in rats aged 14 to 20 days (*solid bars*), 24 to 35 days (*open bars*), and adults (*hatched bars*). The spontaneous activity rate has been subtracted from all responses. Individual fibers are arranged within each age group according to the order of NaCl response frequency. Therefore, the responses of any individual fiber to each of the salts can be read in the vertical columns of the histograms. Only five fibers did not respond to all four salts.

concentration series of NH_4Cl were compared between 14- to 20-day rats and rats older than 80 days, no differences were found in the mean frequencies (Fig. 5; repeated measures analysis of variance, main effect: $F(1, 10) = 0.009$; $p = 0.96$; age × concentration interaction: $F(6, 60) = 0.69$; $p = 0.53$).

KCl was similar to NH_4Cl in that there were no developmental changes in the response frequencies to the 0.1 M salt ($F(2, 52) = 0.95$; $p = 0.39$; Fig. 4). However, the responses to 0.5 M KCl clearly increased in frequency as age increased ($F(2, 52) = 5.49$; $p = 0.007$). Therefore, this salt is distinguished by the fact that different developmental trends are obtained at different concentrations.

Since NH_4Cl and KCl have a sour taste component, it could be informative to compare the responses to these salts with those to an acid. Interestingly, the responses to citric acid are different from those to any of the four salts, because the mean response frequencies *decreased* during development ($F(2, 52) = 6.19$; $p = 0.004$).

Thus, for NaCl and LiCl, the response frequencies increased during development; for NH_4Cl, they remained the same; for KCl, either no change or an increase was observed, depending on the concentration; and for citric acid, frequencies decreased.

Numbers of fibers responding maximally to monochloride salts. Thus far, we have described the mean response data. However, an examination of individual

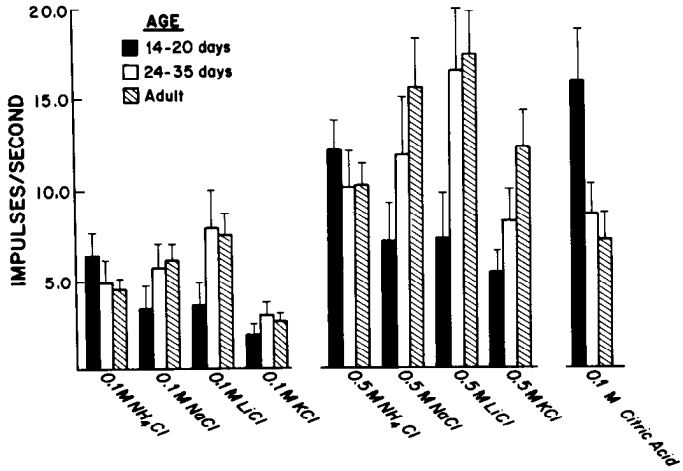

Figure 4. Mean response frequencies of chorda tympani fibers in the three age groups to 0.1 and 0.5 M salts and 0.1 M citric acid. Standard errors are noted above each *bar*. The frequencies in response to lingual stimulation with NH_4Cl remained constant during development, whereas the response frequencies to NaCl and LiCl increased and those to citric acid decreased. Although the response frequencies during 0.1 M KCl stimulation did not alter, the response frequencies to 0.5 M KCl increased as a function of age.

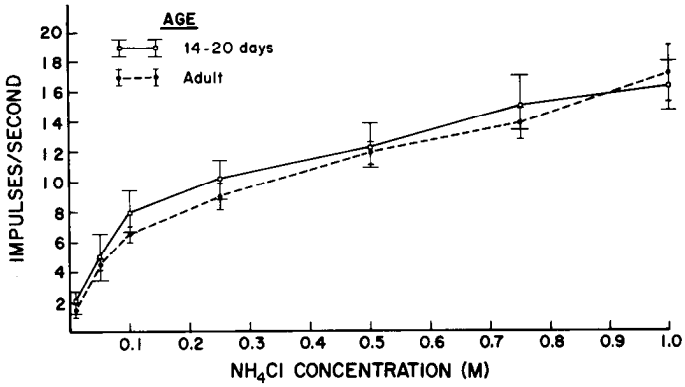

Figure 5. Response-concentration functions for NH_4Cl from chorda tympani fibers in rats aged 14 to 20 days and adults. The mean response frequencies and standard deviations are presented for the 6 fibers in each age group. The response frequencies of fibers in the two age groups are similar at each concentration.

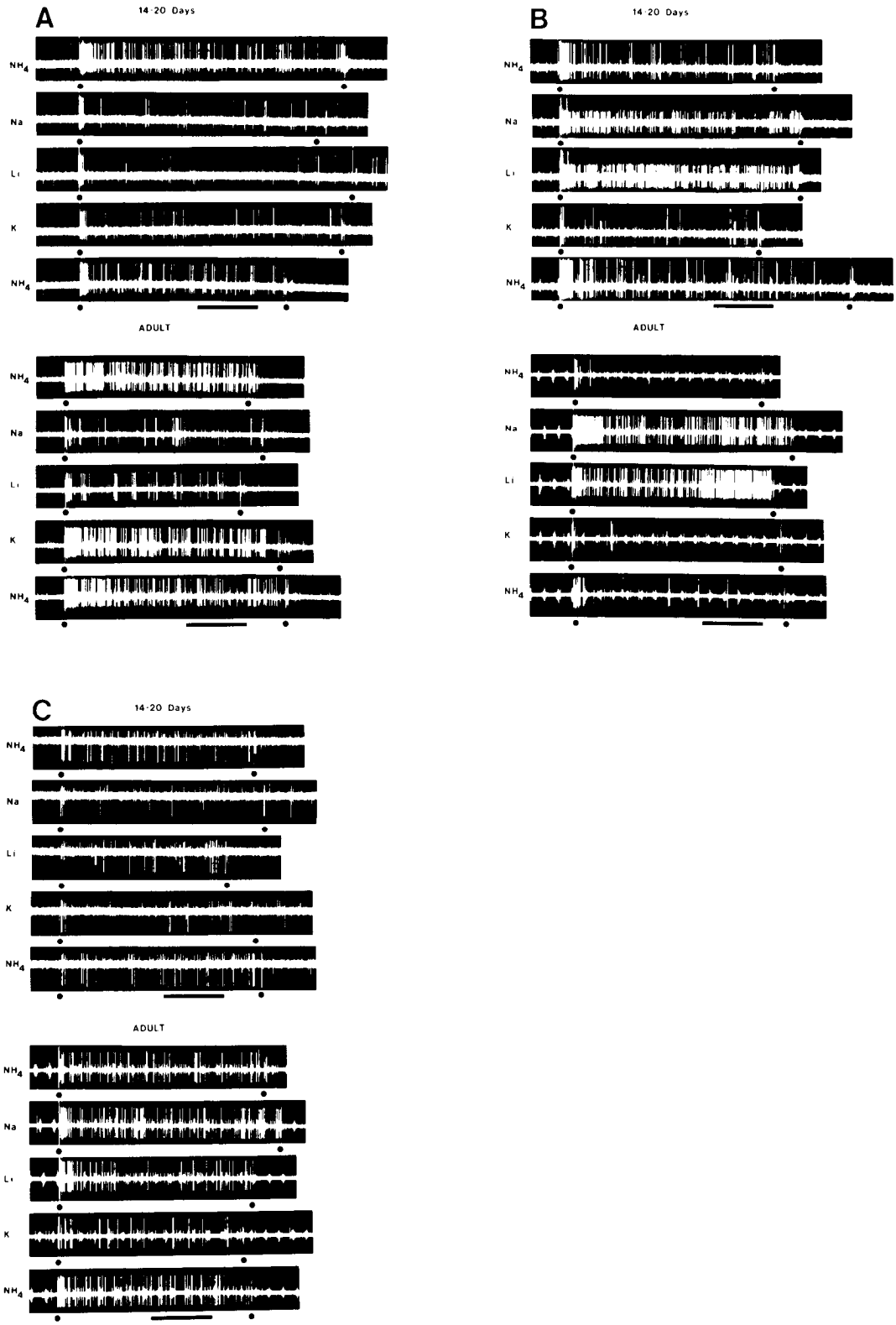

Figure 6. Neurophysiological records from single chorda tympani fibers in young and adult rats representing three response categories: (*A*) fibers that respond maximally to 0.1 M NH_4Cl, (*B*) fibers responding maximally to 0.1 M NaCl or LiCl, and (*C*) those that respond equally well to 0.1 M NH_4Cl, NaCl, and LiCl. For each set of responses, the *dots* denote application of the stimulus and distilled water rinses. The *solid bars* represent a 5-sec period. In *category B* example, 2 fibers were recorded simultaneously from a young rat; the fiber with a smaller impulse amplitude responded maximally to NaCl and LiCl.

fiber responses as illustrated in Figure 3 suggests an alternative approach to data analysis. Often a single fiber responded with a very high frequency to one particular salt; that is, the response frequency for this salt exceeded that for other salts by at least 2 times. A particular stimulus, therefore, was most effective for the fiber. Using this basis, we categorized fibers in three groups: (*1*) those in which NH_4Cl was the most effective stimulus and NaCl and LiCl were less effective (Fig. 6*A*), (*2*) those in which either NaCl or LiCl was most effective and NH_4Cl was less effective (Fig. 6*B*), and (*3*) those that responded equally well to NH_4Cl, NaCl, and LiCl (Fig. 6*C*). (No fiber responded maximally to KCl and the response frequencies to NaCl and LiCl were always similar for each fiber.) This scheme was applied using 0.1 and 0.5 M concentrations to categorize fibers within each of the three age groups.

When the responses to 0.1 M solutions were used to categorize individual fibers, the majority of fibers in rats aged 14 to 20 days responded maximally to 0.1 M NH_4Cl; most fibers in rats aged 24 to 35 days responded equally well to 0.1 M NH_4Cl, NaCl, and LiCl; and fibers in adult rats either responded maximally to 0.1 M NaCl and LiCl or equally to all salts (Table I). These developmental differences are statistically significant ($\chi^2 = 14.3$; $df = 4$; $p = 0.006$).

When the response frequencies to 0.5 M solutions were used to categorize individual fibers, the majority of fibers in rats aged 14 to 20 days again responded maximally to 0.5 M NH_4Cl; nearly an equal number of fibers in rats aged 24 to 35 days were placed in the three categories; and fibers in adult rats again responded maximally to NaCl and LiCl or equally well to 0.5 M NH_4Cl, NaCl, and LiCl (Table I). Although the developmental differences in categories for 0.5 M salts did not reach statistical significance ($\chi^2 = 6.4$; $df = 4$; $p = 0.17$), the changes are essentially similar to those obtained with 0.1 M salts. It can be concluded that the numbers of fibers responding maximally to NH_4Cl, NaCl, and LiCl alter during development. Further, fibers in the 24- to 35-day age group seem transitional since none of the salts is clearly maximally effective at this time.

Response latencies. To learn if taste response latencies change as a function of development, we measured the latencies for all four salts and citric acid. Variability among the latencies within any age group was high; therefore, median rather than mean values are used for the presentation of data (Table II). Also, since the variances of response latencies to the nine stimuli were not homogeneous across the three age groups (Box's test; $p < 0.05$), nonparametric statistics were used to compare latencies as a function of development. First, the Kruskal-Wallis analysis of variance was used to determine if an overall significant difference ($p < 0.10$) existed among the three age groups for response latencies to each chemical. The Mann-Whitney U test then was used to make pairwise comparisons on data in which a significant Kruskal-Wallis value occurred.

TABLE I

Number of fibers in three age groups categorized on the basis of a stimulus eliciting a maximal response

Age	Most Effective Stimulus					
	0.1 M			0.5 M		
	NH_4Cl	NaCl or LiCl	None	NH_4Cl	NaCl or LiCl	None
14-20 days	12	4	3	10	4	5
24-35 days	3	4	9	5	4	7
Adult	3	9	8	3	7	10

TABLE II

Median response latencies for fibers in three age groups

Stimulus	Latency		
	14-20 Days	24-35 Days	Adult
		msec	
0.1 M NH_4Cl	95[a]	76	57
0.1 M NaCl	89[a]	98[a]	59
0.1 M LiCl	105[a]	92[a]	62
0.1 M KCl	85[a]	108[a]	62
0.5 M NH_4Cl	76[a]	68[a]	48
0.5 M NaCl	71[a]	87[a]	54
0.5 M LiCl	90[a]	100[a]	51
0.5 M KCl	72[a]	62	52
0.1 M citric acid	188	163	115

[a] Significantly different from adult latency (Mann-Whitney U test; $p < 0.05$).

Using these statistical procedures, the latencies of salt responses for fibers in rats aged 14 to 20 and 24 to 35 days were longer than those in adult rats ($p < 0.05$) (Table II). The response latencies for fibers in the two younger age groups were not different from each other ($p > 0.10$). Although there were no statistical differences among groups for the latencies to 0.1 M citric acid ($p > 0.10$), a general pattern of decreasing latency across age was observed.

Age-related differences in latency were studied further by measuring the response latency as a function of NH_4Cl concentration for 6 fibers in rats aged 14 to 20 days and 6 fibers in adult rats (Fig. 7). In both age groups, the latencies decreased as the concentration increased up to about 0.1 M NH_4Cl, and then the function reached a plateau. Such a descending function also has been observed during stimulation of the adult rat tongue with 5 to 200 mM NaCl (Marowitz and Halpern, 1977) and has been interpreted as providing evidence that the response latency relates to the diffusion time required for the stimulus to reach threshold levels at the receptor site (DeSimone and Heck, 1980). Greater differences in the response latencies for the two age groups presented in Figure 7 are observed for concentrations from 0.01 through 0.25 M than at higher concentrations. This may indicate that developmental factors play a more significant role in influencing response latency at low salt concentrations.

Aside from comparisons among age groups, response latencies for 0.1 M citric acid were longer than those for the monochloride salts at any age (Table II). In comparison with published data, the median response latency of fibers in adult rats to 0.1 M NaCl (59 msec, Table II) is

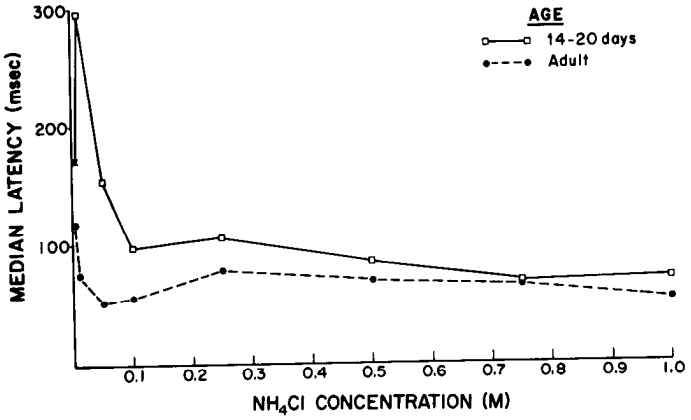

Figure 7. Median response latencies of 6 chorda tympani fibers in young rats and 6 fibers in adults as a function of NH_4Cl concentration (0.001 to 1.0 M). The median latency of fibers in rats aged 14 to 20 days to 0.001 M NH_4Cl (×) does not include the latencies from 2 fibers which did not respond to 0.001 M NH_4Cl. For both age groups, the response latencies decrease as the concentration increases to about 0.1 M.

similar to that reported by Beidler (1953) for responses to 0.2 M NaCl (45 to 55 msec).

It can be concluded from these results that salt response latencies in rats aged 14 to 20 days are similar to those in rats at 24 to 35 days but that shorter latencies occur in adults.

Discussion

Changes in single fiber response frequencies. By studying the responses from single chorda tympani fibers, we now know that the relatively small multifiber responses obtained in young rats during stimulation of the tongue with NaCl and LiCl *cannot* be explained by a general inability of single fibers to respond to NaCl and LiCl. Eighty-nine percent of all fibers in rats aged 14 to 20 days responded with an increased and sustained neural discharge that exceeded spontaneous frequency when NaCl and LiCl were used as stimuli. Even if arbitrary criteria are used to define a response, the majority of fibers in rats aged 14 to 20 days are still observed to respond to NaCl and LiCl. For example, 74% of the fibers in rats aged 14 to 20 days responded to NaCl and LiCl with a discharge frequency that exceeded the mean spontaneous frequency by 2 SD. Eighty-one and 85% of the fibers in rats aged 24 to 35 days and in adults, respectively, responded to NaCl and LiCl based on this criterion.

Although the taste system in young rats has the ability to respond to NaCl and LiCl, the response frequencies to these salts are generally low; they progressively increase during development to about twice the immature values. In contrast, the response frequencies to NH_4Cl, over a concentration range of 2 log units, do not alter as a function of age. If the response frequencies to NaCl and LiCl increase substantially during development while those to NH_4Cl remain rather constant, then the order of effective salt stimuli should reverse from NH_4Cl > NaCl and LiCl in young rats to NaCl and LiCl > NH_4Cl in adults. This reversal is, of course, just what is observed with whole nerve recordings (Ferrell et al., 1981).

The single fiber data also have revealed another component underlying the observed changes in multifiber salt responses. NH_4Cl is the most effective stimulus in the majority of fibers in young rats, whereas more fibers in adult rats respond maximally to NaCl or LiCl. Therefore, not only are the overall response frequencies to NaCl and LiCl increasing during development but also more fibers become maximally responsive to these salts.

The responses to KCl are quite different from those to the other salts since a concentration factor apparently determines whether the frequencies remain the same or increase developmentally. The frequencies in response to 0.1 M KCl do not change as a function of age. Since 0.1 M NaCl frequencies increase while KCl frequencies remain the same, the decreasing responses from the whole nerve for KCl relative to NaCl are to be expected (Ferrell et al., 1981). Citric acid is different in still another manner because the response frequencies decrease as age increases. Hill and Almli (1980) reported that the magnitude of the whole nerve response to 0.1 M citric acid decreased relative to 0.5 M NH_4Cl from 20 days postnatally to adulthood. This is partially explained by the single fiber data: citric acid response frequencies decrease, while those to NH_4Cl remain constant. Also, although Ferrell et al. (1981) used a lower concentration of citric acid in their experiments, the observation that whole nerve responses for citric acid relative to NaCl decrease developmentally is predicted by our single fiber data.

Relation of developmental changes to the maturation of the peripheral taste system. It is apparent that taste responses change during development in the rat. The changes probably relate to the maturation of various levels of the peripheral gustatory system, including the taste pore region, the receptor membranes, the synapses between receptor cells and chorda tympani fibers, and the chorda tympani fibers themselves. Even though there is little information on the development of any of these components, consideration of the kinds of response changes that do occur suggests that some levels are more important than others.

For example, unless the biochemical composition of extracellular substances in the pore acts as a selective filter for certain ions, it is not apparent how differential developmental responses to monovalent salts could derive from an altering taste pore substance. However, the development of substances in the taste pore could alter general access of stimuli to receptor microvilli and possibly relate to changing response latencies.

It is more likely that changing developmental taste responses relate to the maturation of taste receptor membranes. Investigators have proposed several mechanisms for taste membrane stimulation by salts (Beidler, 1967; Beidler and Gross, 1971; DeSimone and Price, 1976; Kamo et al., 1974; Mooser, 1980). Whatever the correct mechanism may be, our data suggest that key receptor components (for example, negatively charged portions of phospholipids or proteins) may alter in kind and/or in proportion to each other during development and that these alterations independently affect membrane sensitivities to NH_4Cl, to NaCl and LiCl, and to KCl.

The possibility of changing receptor membrane composition may be linked to the dynamic process of cell

turnover occurring in the taste bud. Taste cells are renewed constantly in the adult rat (Beidler and Smallman, 1965). Assuming that a population of immature taste bud cells is first acquired in early development and that the process of turnover is subsequently initiated, some predictions can be made. Before the turnover process begins, there may be a preponderance of receptor membranes with a distinctive biochemical composition that respond maximally to certain chemical stimuli (e.g., NH_4Cl). Then, as taste cells are renewed, relatively more cells with a different membrane composition that respond maximally to other chemical stimuli (e.g., NaCl) also may be present. To account for the observed, long term developmental changes in chorda tympani responses, different rates of taste cell maturity throughout development would be necessary; that is, more of the initial type of cell should be present in taste buds of young rats than in older rats.

The next obvious stage to consider in the taste system is that of the synapse between gustatory cells and the first order chorda tympani fibers. There is no study on the development of synapses in the taste bud, but there is evidence that the number of synapses at the rat neuromuscular junction decreases substantially from birth to 20 days postnatally (Bennett and Pettigrew, 1974; Brown et al., 1976; Redfern, 1970). Also, changes in the number and characteristics of synapses at outer hair cells in the rat cochlea occur postnatally through at least 16 days (Lenoir et al., 1980). If functionally distinct types of synapses exist in the taste system and if these change in number, it is possible that taste response characteristics could alter accordingly. Also, changes in synapse efficiency may relate to observed decreases in response latency during development.

Finally, changes in the physical properties of the chorda tympani fibers per se may relate to the changing taste responses. Alterations in fiber characteristics, however, cannot be logically used to explain the differential changes in the response frequencies but must be considered as a possible factor in introducing bias in the fibers sampled at different ages. For example, it may be suggested that specific fibers exist that are especially responsive to NaCl and LiCl, and other fibers may be highly responsive to NH_4Cl. If fibers maximally responsive to NaCl or LiCl are small in diameter and relatively unmyelinated in young rats, whereas fibers maximally responsive to NH_4Cl are larger, then more "NH_4" fibers would be selected during dissection and recording procedures in immature rats. If the *converse* were true in adult rats (large "Na" or "Li" fibers, small "NH_4" fibers), then more fibers maximally responsive to NaCl or LiCl would be selected in older animals. This argument is cumbersome at best. It is known, however, that the amount of myelination and the overall proportion of large diameter fibers increase through about 70 days in another nerve, the rat sciatic nerve (Friede and Samorajski, 1968). If similar changes occur in the chorda tympani, they probably relate to the decreasing response latencies that we observed during development.

Other factors related to the development of salt taste responses. The preceding discussion focuses on potential changes within the gustatory system itself that may relate to developing taste responses. Other possible influences on the taste receptors may derive from internal physiological changes. For example, altering electrolyte levels in saliva and blood may influence or modulate taste cell sensitivities throughout the rat's development. Sodium levels increase developmentally in rat saliva (Schneyer and Hall, 1968) and blood plasma (Jelinek, 1961); therefore, it may be expected that taste cells of adult rats are constantly bathed in higher concentrations of sodium than are those of young rats. Since Bradley (1973) has shown that the responses of the chorda tympani can be recorded after intravascular stimulation with NaCl, the increasing tonic levels of plasma sodium as well as salivary sodium provide an altered background level of sodium to which receptors are exposed. Alterations in the rat's feeding experience also may have an influence on taste cell responses. Since the taste system does not respond in a mature manner from the moment function begins, the gustatory sense may be susceptible to modifying influences from ingested stimuli (Mistretta, 1981).

In summary, changing single fiber responses may relate to an altering ability of stimuli to reach receptors, to changes in the composition of taste cell membranes, to synaptic development, and/or to changes in fiber size and degree of myelination. Of these factors, developmental changes in the composition of taste membranes probably relate most directly to differential salt responses. Possible changes in stimulus access, synaptic efficiency, and afferent fiber properties probably relate more directly to decreasing latencies. Information on the development of taste bud morphology and biochemistry will be essential for understanding the mechanisms through which neurophysiological taste responses mature.

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