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Developmental Decrease in Size of Peripheral Receptive Fields of Single Chorda Tympani Nerve Fibers and Relation to Increasing NaCl Taste Sensitivity

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During development in rats, sheep, and humans, the taste system acquires increasing responsiveness to NaCl, compared with a variety of other salts and chemicals. To better understand the neural basis of changes in salt taste responses, we studied receptive field size and response properties of single chorda tympani nerve fibers in fetal, perinatal, and postnatal sheep. Individual fungiform papillae were stimulated electrically with 5 μA anodal current to determine the location and number of papillae in receptive fields. Response characteristics of NH_4Cl, NaCl, and KCl were determined for the entire field. Receptive fields were dissected for later histological reconstruction and taste bud identification.

Median receptive field size decreased during development. Field sizes in lambs were smaller than those in younger animals. This decrease was accompanied by an increase in the $NaCl/NH_4Cl$ response ratio of single fibers and an increase in the proportion of fibers and associated fields that responded with higher frequency to NaCl, compared with NH_4Cl. In addition, for fibers across all age groups, receptive field size correlated negatively with the $NaCl/NH_4Cl$ response ratio; that is, fields most responsive to NaCl had fewer papillae than those most responsive to NH_4Cl. For all fibers, receptive field size correlated with response frequencies to NH_4Cl and KCl but not NaCl. For NaCl-best fibers, receptive field size correlated with the response frequencies to all 3 salts. There was no relation between number of taste buds in a single fungiform papilla and the response frequency elicited during electrical stimulation of the papilla.

We conclude that chorda tympani fibers not only have characteristic salt responses but are also distinguished by receptive field size. Salt response properties and field size are interrelated and acquired gradually during development. Specifically, there is a developmental acquisition of small, highly NaCl-responsive receptive fields in the peripheral taste system.

During development, nerve fibers must make precise qualitative and quantitative connections with sensory receptor organs. Single afferents must find the "correct" sensory cells and appropriate number of these cells, and establish synapses. For example, in the adult auditory system an inner hair cell is innervated by about 10 afferent fibers, and each fiber innervates only 1 inner hair cell; an outer hair cell is innervated by several afferent fibers, and each fiber innervates many outer hair cells. These patterns of divergence and convergence are established progressively during development and in the process of forming specific receptive fields, neural rearrangements take place (Pujol et al., 1978).

In the gustatory system, single fibers of the chorda tympani nerve innervate taste buds that are located in fungiform papillae distributed over the anterior two-thirds of the tongue. It is known that a single afferent fiber innervates from one to several gustatory papillae on the tongue of the adult rat (Miller, 1971), cat (Boudreau et al., 1970; Oakley, 1975), and goat (Boudreau et al., 1982). However, most investigators have not reported measures of receptive field size in studies of single fiber taste responses, and there has been no developmental study to determine how these taste receptive fields are established.

We hypothesized that taste response characteristics relate to receptive field properties and that the development of specific salt taste response characteristics relates to formation of receptive fields. Early in development in the sheep and rat, response magnitudes to NaCl are small compared to those for NH_4Cl and KCl (Ferrell et al., 1981; Hill et al., 1982; Mistretta and Bradley, 1983). Subsequently, response frequencies of single fibers to NaCl increase and the number of fibers most responsive to NaCl increases. For responses to NH_4Cl and KCl, these parameters either remain the same or decrease during development.

The differences in salt taste responses during development may reflect changes in the receptive field properties of single taste fibers. Therefore, in the present study, receptive fields were mapped in sheep fetuses, perinatal animals, and lambs at ages that had been studied previously in our laboratory. The present investigation was to determine for 3 age groups (1) the location and number of fungiform papillae innervated by single chorda tympani nerve fibers and (2) the responses to salt stimuli from mapped receptive fields. Individual fungiform papillae were stimulated electrically while recording from single afferent fibers to determine location and number of papillae in receptive fields. Response characteristics to salt stimuli were determined for the entire field. Receptive fields were dissected for later histological reconstruction and taste bud identification.

Several null hypotheses were studied. (1) There is no change in receptive field size during development; that is, the number of fungiform papillae innervated by a single chorda tympani

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nerve fiber remains constant. (2) There is no change in salt response characteristics of receptive fields during development. (3) There is no relation between receptive field size and salt response characteristics. (4) There is no relation between number of taste buds in a papilla and the electrical response frequency generated by that papilla.

Materials and Methods

Animals and surgical preparation. Recordings were made from Suffolk sheep in 3 age groups: *fetal* or *130 d of gestation,* which included 12 fetuses aged 129–134 d of gestation (term, 147 d); *perinatal,* which included 16 animals aged 145 d of gestation to 9 d postnatal; *lamb,* which included 16 lambs aged 35–83 postnatal d. Pregnant ewes and lambs were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg for ewes; 20 mg/kg for lambs), tracheotomized, and given supplemental O_2 (1.5–2.5 liters/min). An indwelling catheter was placed in the jugular vein for subsequent anesthetic administration. Fetuses (anesthetized via the pregnant ewe) were delivered onto a table at the ewe's side and wrapped with a heating pad and cotton blankets (Bradley and Mistretta, 1973). The umbilical and placental circulation remained intact. Rectal temperature was monitored continuously.

Receptive field electrophysiology. The fetal or lamb head was secured in a metal, atraumatic holder, and the mouth was dissected so that all fungiform papillae on the ipsilateral tongue could be stimulated easily with chemicals or an electrical probe. The chorda tympani nerve was dissected by a lateral approach through the cheek and ramus of the mandible and was cut near the point at which it enters the tympanic bulla. Surrounding connective tissues were dissected from the nerve, and small bundles of fibers were separated from each other by cutting. Then, very fine bundles of fibers were dissected with needles or forceps and placed on a platinum wire electrode for single-fiber recording. An indifferent electrode was positioned in nearby tissues. In perinatal sheep and lambs, a mixture of Vaseline and mineral oil was applied in the tissue cavity holding the dissected fiber so as to maximize stability in neural recordings (Kitada et al., 1984). Neural activity was recorded with a preamplifier, oscilloscope, and audio monitor and was stored on magnetic tape for later analysis.

Once a single fiber was isolated, the general receptive field was located by stimulating small areas of the tongue with a coarse electrical probe. Then, 3–5 ml of 0.5 M NH_4Cl, NaCl, and KCl were flowed over the field in sequence, remaining on the tongue for 10–15 sec each. These chemicals were dissolved in distilled water and used at room temperature. Between application of each salt stimulus, distilled water was used to rinse the tongue for 20–30 sec. The salt stimuli were chosen because we had data on developmental changes in neural responses to these stimuli from sheep at various ages. The concentration was chosen to elicit reproducible, near-maximum neural responses. In the fetal age group, it was difficult to hold isolated single-fiber preparations for long periods, and so we limited salt stimuli to NH_4Cl and NaCl only.

After chemical stimulation, the number of papillae in the receptive field was determined. Using a dissecting microscope, single papillae were stimulated individually for 1–2 sec with 5 μA anodal current delivered from a monopolar platinum electrode connected to a Grass stimulator. An indifferent electrode was placed under the tongue. Onset and offset of electrical stimulation were recorded. Current intensity was monitored continuously with an ammeter.

Once the field was mapped, salt stimuli were again applied to the tongue. Then, the field was mapped a second time at 5 μA to establish reproducibility. Some fields were mapped 3–4 times. After a map was established, a drawing was made of fungiform papillae in the field and location of the field was noted on a diagram of the sheep tongue. Finally, select fungiform papillae were tatooed with a tungsten microelectrode that had been dipped in India ink to mark papillae for later field reconstruction from histological sections.

Although electrical stimulation of single papillae has been used by other investigators to determine receptive field size of taste neurons (Boudreau et al., 1970, 1982, 1985), the method has not been applied in a developmental study, and some notes about validity and reliability are therefore included.

First, the electrical probe was small enough (250 μm) to stimulate papillae individually. Fungiform papillae in 130 d fetuses (youngest age group) are about 250–350 μm in diameter and are separated from each other on the tongue by about 400–1000 μm. Papillae in older animals are larger and further apart on the tongue. Figure 1 illustrates fungiform papillae on the fetal tongue and electrical probe size.

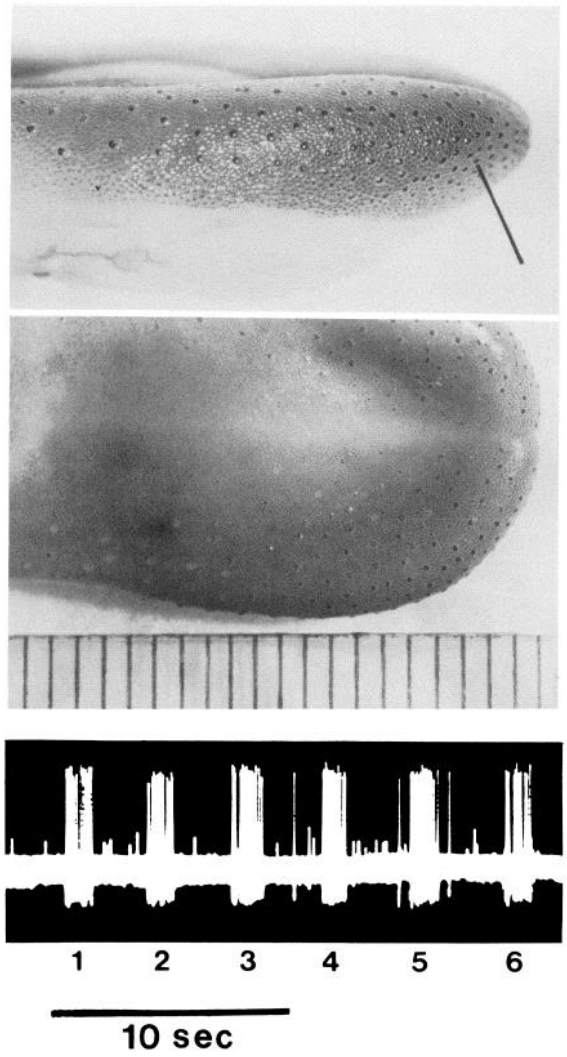

Figure 1. Photographs of anterior tongue edge and dorsum from a sheep fetus 130 d of gestation and examples of recordings during electrical stimulation of 6 single papillae. The line drawn in the top photograph represents the actual size of the electrode used for electrical stimulation of single papillae. Scale under photograph of the dorsal tongue is in millimeters. Many papillae in this sheep breed are pigmented and therefore easily visible.

The majority of fungiform papillae on the tongues of most of our Suffolk sheep were pigmented. Based on contrast provided by the pigmentation, the large size of the papillae and tongue, and topographical landmarks on the tongue, it was a reasonable task to stimulate up to about 30 individual papillae and then re-map the receptive field by stimulating the same papillae in the same sequence. All papillae in the general field area were stimulated systematically with the electrical probe in an effort to locate all possible responsive papillae; thus, many unresponsive as well as responsive papillae were stimulated. We mapped 78% of the receptive fields at least twice, and the average reproducibility of receptive field size for the first 2 maps was 87%. Examples of maps and reproducibility for small and large receptive fields are illustrated in Figure 2.

To map fields, we chose a stimulating intensity of 5 μA based on published literature indicating that this intensity is not sufficient to directly stimulate chorda tympani nerve terminals (see discussion and reviews by Bujas, 1971; Herness, 1985) and on preliminary experiments in our laboratory to establish a near-maximum electrical stimulus intensity for fetuses and lambs. In addition, response/intensity functions for 1–10 μA anodal current were studied for all papillae in the receptive

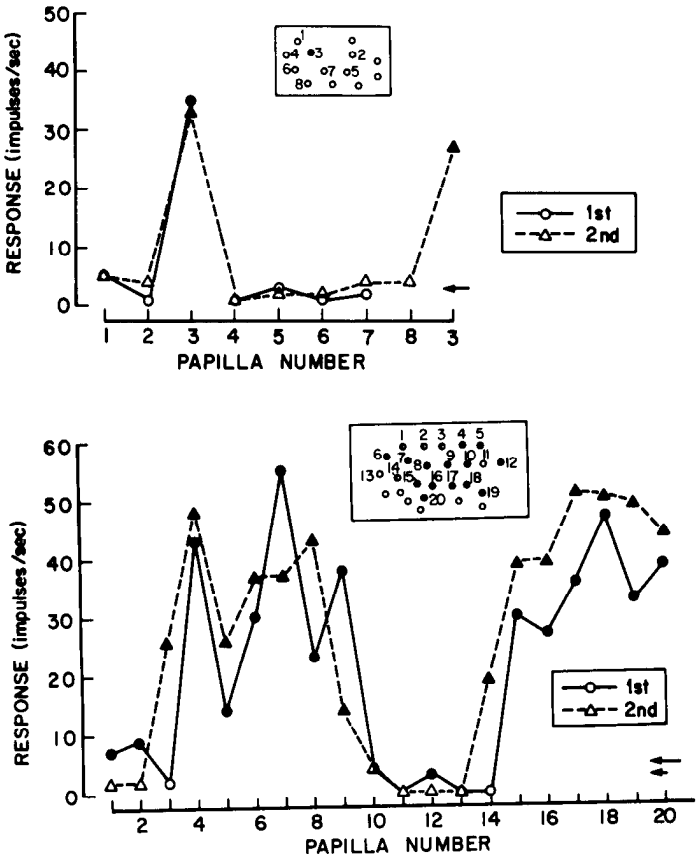

Figure 2. Response frequency elicited during electrical stimulation of individual fungiform papillae in receptive fields of 2 single fibers (*top* and *bottom*). For each fiber receptive fields were mapped twice (*1st, 2nd*). *Arrows* at the bottom of each graph indicate spontaneous frequency level. Papillae were counted as part of the receptive field when the electrical response frequency generated from the papilla exceeded the mean plus 2 SD of spontaneous frequency. These responsive papillae are noted with *filled symbols* in the graphs. For the *top graph,* one papilla was counted in the receptive field; for the *bottom,* 16 papillae were counted (average of 16 for the 1st map and 15 for the 2nd). *Insets* illustrate topographical arrangement of papillae in receptive field areas. *Filled circles* represent responsive papillae and *open circles,* nonresponsive; *half-filled circles* represent papillae that responded during one mapping sequence, but not the other.

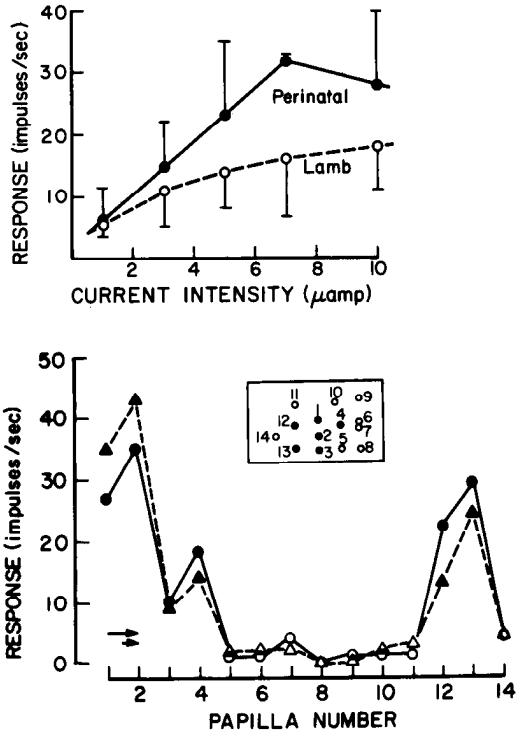

Figure 3. Top, Response frequency as a function of current intensity used to stimulate papillae in receptive fields of perinatal and lamb fibers. Data points are means and SDs for 6 perinatal fibers (innervating 76 papillae) and 7 lamb fibers (innervating 102 papillae). *Bottom,* Response frequency elicited during electrical stimulation of single fungiform papillae in the receptive field of one fiber. One map was made using 5 μA stimulating intensity (*circles*) and one map made using 10 μA (*triangles*). *Arrows* indicate spontaneous frequency for the 2 map sequences. *Filled symbols* denote responsive papillae. Six papillae were counted in each map, and the topographical arrangement of these and other, nonresponsive papillae is illustrated in the *inset.* Note that an increase in current from 5 to 10 μA did not recruit papillae numbered 5–11 into the receptive field, even though they were located near other highly responsive papillae (e.g., numbers 1, 2, 4, and 12).

fields of 13 fibers (6 perinatal fibers innervating 76 papillae; 7 lamb fibers innervating 102 papillae) to ensure adequacy of 5 μA for stimulus intensity. Curves of average response frequencies for all responding papillae in the receptive fields of these fibers are illustrated in Figure 3 (top) and indicate that 5 μA elicited response frequencies more than ⅔ maximum. Furthermore, when the sizes of the receptive fields obtained with 5 and 10 μA stimulation were compared, there was no difference (mean and SD for 5 μA = 13.5 and 7.1; 10 μA = 15.4 and 7.3; $t = 0.68$, $df = 24$, $p = 0.50$). Therefore, stimulating with 10 μA did not result in larger receptive fields than 5 μA. This is illustrated in Figure 3 (bottom), in which 10 μA current did not recruit papillae numbered 5–11 into the receptive field even though they were located near other highly responsive papillae (i.e., numbers 1, 2, 4, 12).

It is unlikely that 5 μA was too intense a stimulus, resulting in apparent stimulation of noninnervated papillae through electrotonic spread of current to nearby, innervated papillae. Nonresponsive papillae were found regularly in close proximity to highly responsive ones, as illustrated in the geographical arrangements of papillae in receptive fields on Figures 2 and 3. At all ages, very small fields were encountered as well as large ones. Of the 74 receptive fields that we mapped, 19 contained 5 or fewer papillae per field; if current spread were occurring regularly, such small fields would not be found.

Data analysis and definition of terms. Single-fiber data (based on amplitude and waveform) were analyzed by converting action potentials to standard pulses using a window discriminator. A digital microcomputer was used to measure interpulse intervals, store the intervals on magnetic disks, and generate response frequency data (Bradley, 1982).

Spontaneous activity was measured during the 5 sec period preceding stimulation with each of the 3 salts and is expressed as an average of these 3 measures. Average spontaneous activity (impulses/5 sec) and SD for fetal, perinatal, and lamb groups were 9.8 (6.1), 4.8 (5.0), and 4.4 (6.0). These were significantly different [$F(2,73) = 4.81$, $p = 0.01$], and Scheffé posttests indicated that spontaneous activity in fetuses was higher than in perinatal or lamb groups ($p < 0.01$).

Salt taste response frequencies were measured during the first 5 sec of chemical stimulation and are expressed as impulses/5 sec. When stimuli were presented more than once, responses were averaged over the multiple applications. In addition to NaCl, NH_4Cl, and KCl response frequencies, we calculated the NaCl/NH_4Cl response ratio, defined as the response frequency to NaCl divided by the response frequency to NH_4Cl. If that ratio exceeded 1.00, we categorized the single fiber as "Na-best"; if the ratio was less than 1.00, the fiber was "NH_4-best."

To measure receptive field size, the number of papillae innervated by a single fiber was determined with electrical stimulation of single papillae. First, spontaneous activity was measured for the 5 sec period preceding electrical stimulation of papillae in a field. Then the electrical response frequency generated during the initial 1 sec of stimulation of each papilla was measured. A fungiform papilla was counted as part of the receptive field for a given fiber when the electrical response frequency generated from the papilla exceeded the mean plus 2 SD of spontaneous frequency. In addition, we took the highest frequency generated during

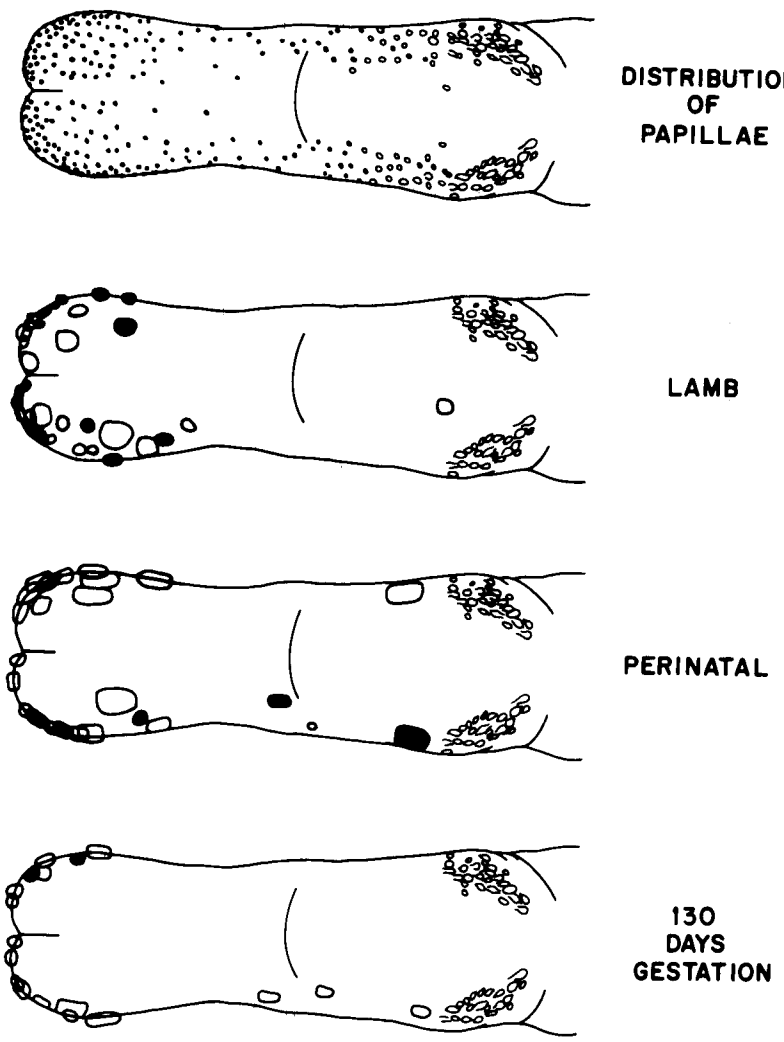

Figure 4. Distribution of fungiform papillae on the sheep tongue (*top*) and locations of receptive fields in each age group. Oval-shaped papillae drawn on each side of the posterior tongue represent circumvallate papillae. Receptive field locations are drawn on each side of the tongue for ease in illustration, but all fields were innervated ipsilaterally. *Filled* receptive fields are those innervated by fibers that responded to NaCl with a higher response frequency than to NH_4Cl.

stimulation of any one papilla in the receptive field as a measure of maximum electrical response frequency.

Thus, measures were made of chemical response frequencies for the entire receptive field to NaCl, NH_4Cl, and KCl; $NaCl/NH_4Cl$ response ratios; numbers of Na-best or NH_4-best fibers; receptive field size; electrical response frequency for each papilla in the field; maximum electrical response frequency; and spontaneous activity.

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

Receptive field reconstruction. At the end of each experiment the pregnant ewe or lamb received an overdose of sodium pentobarbital, and receptive fields were dissected from the fetal, perinatal, or lamb tongue and placed in 10% neutral buffered formalin. Tissues containing receptive fields were processed for light microscopy, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin.

Using a light microscope and drawing tube, serial coronal sections of each receptive field were traced to locate and quantify fungiform papillae and taste buds within papillae. Locations of tatoo marks also were noted. After all sections were traced, a map of the receptive field was constructed. By comparing this map with drawings made during the experiment and by matching the locations of marked papillae, specific papillae in each field could be reidentified. This made it possible to analyze data for a possible relation between number of taste buds in a papilla and the response frequency generated by electrical stimulation of that papilla. In addition, the histology yielded morphological data on taste buds and papillae that could be used in determining possible neural rearrangements during receptive field development (see following paper, Mistretta et al., 1988).

Results

Location and size of receptive fields

Diagrams of locations of all 74 receptive fields are presented in Figure 4 and indicate reasonable consistency in sampling across age groups. Although there is an insufficient number of receptive fields on more posterior tongue areas to allow analysis of detailed locations, receptive fields located on the tongue tip (defined as the region anterior to the widest portion of the tongue on diagrams) were larger (mean = 13, SD = 8, $n = 51$) than fields located behind the tip (mean = 5, SD = 5, $n = 23$) ($t = 4.08$, $df = 72$, $p = 0.0001$). It is apparent from Table 1 that receptive fields on the tip tended to be larger in each age group individually, also. Thus, in more posterior areas of the tongue with a lower density of papillae, receptive fields contained fewer papillae. The percentages of receptive fields in more posterior tongue areas were 29% in fetuses, 36% in perinatal animals, and 28% in lambs. Therefore, no location bias was introduced through sampling a disproportionate number of posterior fields at any age.

Distributions of receptive field sizes for each age group are presented in Figure 5. At all ages a wide range of sizes was observed, from 2–40 papillae per field in fetuses, 1–33 in peri-

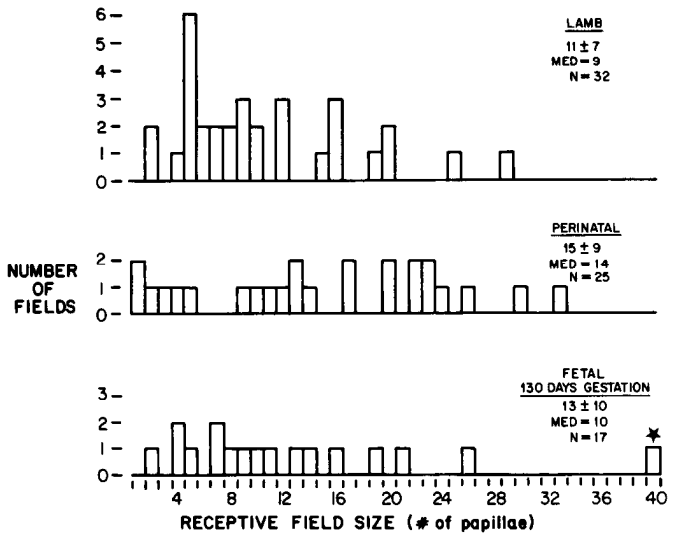

Figure 5. Distributions of number of receptive fields of a given size in each age group. For each group, data are included on mean and SD of field size, median field size (*MED*), and number (*N*) of receptive fields mapped. Median field size differed significantly as a function of age; lamb receptive fields were smaller than perinatal fields.

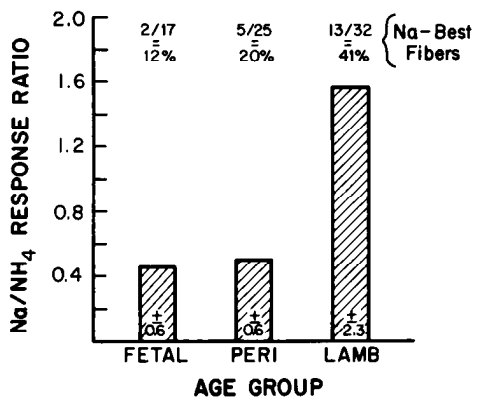

Figure 6. The mean NaCl/NH_4Cl response ratio for each age group. The SDs are noted within the histogram bars. At the *top* of the graph are noted numbers and percentages of fibers within each group that responded with a higher frequency to NaCl than to NH_4Cl (Na-best). With age, both the response ratio and the proportion of "Na-best" fibers increased significantly. (Note: the lamb response ratio data presented in this figure were calculated excluding one extremely high ratio of 30; see Fig. 7.)

natal animals, and 2–29 in lambs. Mean receptive field sizes were 13 in fetuses (SD = 10, n = 17), 15 in perinatal sheep (SD = 9, n = 25), and 11 in lambs (SD = 7, n = 32); medians were 10, 14, and 9, respectively. Because field sizes in the 3 age groups were not normally distributed, data were analyzed with the median test, a nonparametric statistic that does not require the assumption of normality. A significant difference was observed across groups (median test = 5.23, df = 2, p = 0.07).

In addition, one receptive field size in the fetal age group was identified as a potential outlier (field size = 40 papillae, asterisk in Fig. 5). Analysis of variance was used to reanalyze data without the outlier, and a significant difference was found [$F(2,72)$ = 2.56, p = 0.08]. Assumptions about equality of variance across groups were not violated. Means for the 3 age groups were 11, 15, and 11 with the outlier removed.

Posttests (2 sample comparison median tests or Scheffé allowances for the ANOVA) indicated that the receptive field size in lambs was smaller than that in perinatal animals (p = 0.03. There were no differences between other age groups. As noted above, the observation of smaller receptive fields in lambs is not likely to relate to any possible bias in receptive field locations. Therefore, we conclude that there is an age-related difference in receptive field size and that fields in lambs are smaller than those in perinatal sheep.

Table 1. Receptive field size as a function of tongue location in 3 age groups

Age group		Location: Tip	Location: Posterior	t	df	p
All	$\bar{x}$	13	5	4.08	72	0.0001
	SD	8	5			
	n	51	23			
Fetal	$\bar{x}$	14	8	1.28	15	0.22
	SD	9	8			
	n	12	5			
Perinatal	$\bar{x}$	18	5	4.59	23	0.0001
	SD	8	4			
	n	16	9			
Lamb	$\bar{x}$	9	4	2.13	30	0.04
	SD	7	3			
	n	23	9			

Salt taste responses as a function of age

During development in sheep there is an increase in response magnitude from the whole chorda tympani nerve to NaCl, compared with NH_4Cl (Mistretta and Bradley, 1983). Therefore, we analyzed data to discover whether there is a developmental difference in the NaCl/NH_4Cl response ratio for single fibers and respective receptive fields. Figure 6 illustrates an increase in average ratios across age groups; this increase is significant [$F(2,72)$ = 2.58, p = 0.02]. Scheffé posttests indicate that the means for both fetal and perinatal groups are smaller than the mean for lambs (p < 0.05).

To understand the basis of the increasing NaCl/NH_4Cl response ratio, we examined 2 other factors. First, single fibers were categorized as responding "best" to NaCl (NaCl/NH_4Cl response ratio > 1.00) or "best" to NH_4Cl (NaCl/NH_4Cl ratio < 1.00). As presented in Figure 6 (top), the proportion of Na-best fibers increased with age (χ^2 = 5.63, df = 2, p = 0.06).

Second, average response frequencies to NH_4Cl and NaCl were analyzed for fibers from the 3 age groups. Mean frequencies for NH_4Cl were 129 in fetuses (SD = 82), 136 in perinatal animals (SD = 67), and 106 in lambs (SD = 72). There was no developmental difference in these frequencies [$F(2,73)$ = 1.37, p = 0.26]. Average frequencies for NaCl were 50 (SD = 59), 45 (SD = 48), and 65 (SD = 56), respectively, and these were not significantly different [$F(2,73)$ = 1.08, p = 0.34].

Thus, the developmental increase in the NaCl/NH_4Cl response ratio is attributable to an increase in the proportion of fibers that respond with highest frequency to NaCl compared with NH_4Cl. Although there is a trend for NaCl average response frequencies to increase and a trend for NH_4Cl frequencies to decrease, the differences are not significant over these age groups.

Response frequencies for KCl were also compared for the 2 age groups (perinatal and lamb) in which this salt was used as an additional stimulus. The average frequency in lambs ($\bar{x}$ = 55, SD = 39) was significantly lower than that in perinatal animals ($\bar{x}$ = 153, SD = 73) (t = 5.34, df = 37, $p < 0.0001$).

Receptive field size and salt taste responses

Data from the present study indicate that there is a developmental decrease in receptive field size and an increase in the $NaCl/NH_4Cl$ response ratio for single fibers. We compared receptive field size and the $NaCl/NH_4Cl$ response ratio for all fibers to learn whether there is a relation between these variables. Field size correlated negatively with the $NaCl/NH_4Cl$ ratio; that is, receptive fields most responsive to NaCl had fewer papillae than fields most responsive to NH_4Cl (Fig. 7). The data can be fit by an exponential function (r = 0.32, df = 72, $p < 0.01$). The shape of the function in itself is not biologically important but derives from the response properties of fibers and fields at different ages (denoted by different symbols in Fig. 7). For example, only 2 fields have $NaCl/NH_4Cl$ ratios exceeding 1.0 in fetuses and only 5 in perinatal animals, whereas 13 ratios exceed 1.0 in lambs. This reflects the developmental increase in the proportion of fibers most responsive to NaCl.

We also tested for a possible relation between field size and the salt response category of fibers in all age groups. The average receptive field size for NaCl-best fibers was 8 (SD = 6, n = 20) and the average for NH_4Cl-best fibers was 14 (SD = 9, n = 54), a significant difference (t = 2.81, df = 72, p = 0.007). The relation between field sizes and chemical responses could be quite striking, as illustrated in Figure 8 by chemical and electrical neural responses from 2 lamb fibers, one which responded best to NH_4Cl and one to NaCl.

Receptive field size and response frequency

As well as comparing receptive field size with the $NaCl/NH_4Cl$ response ratio, we tested for relations with absolute response frequency measures. As presented in Table 2, for all fibers, field size correlated positively with NH_4Cl and KCl response frequencies for the entire field and with the maximum electrical response frequency ($p < 0.0001$). There was no correlation between size of field and response frequency to NaCl (p = 0.65). However, if NaCl-best fibers were analyzed separately, there was a positive correlation between NaCl response frequency and field size ($p < 0.02$); for these fibers, field size also correlated with maximum electrical and with NH_4Cl and KCl response frequencies.

Relation between number of taste buds per papilla and the electrical response frequency elicited from the papilla

Because we could reconstruct receptive fields and accurately identify specific papillae in the field, we were able to test for a relation between number of taste buds *in a particular papilla* and the electrical response frequency elicited during stimulation of that papilla. There was no relation between number of taste buds and response frequency (r = 0.03, df = 491, p = 0.50), nor was there any relation when taste buds in either NH_4-best or Na-best fields were analyzed separately ($p > 0.10$).

Discussion

From studies of peripheral receptive fields of chorda tympani nerve fibers in fetal, perinatal, and postnatal sheep, we can draw some major conclusions about receptive field development and

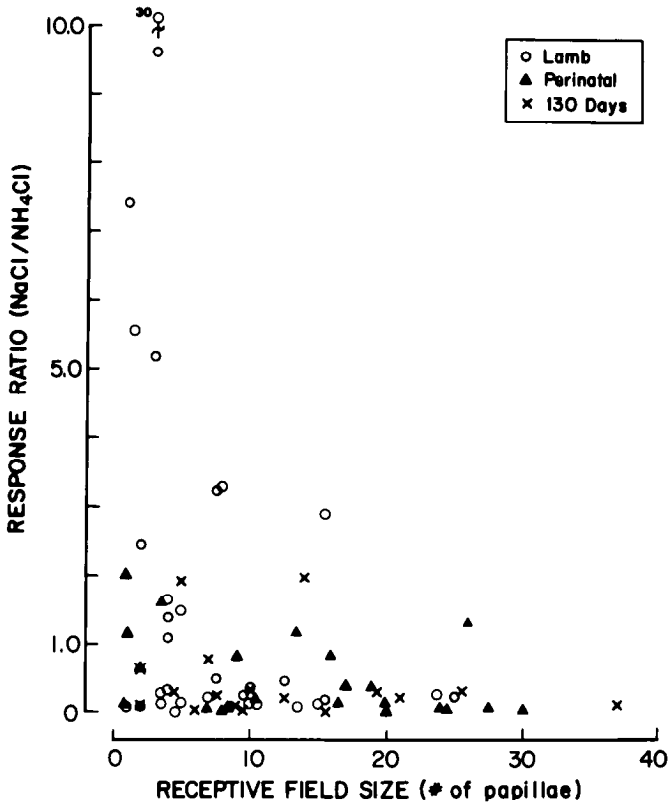

Figure 7. The $NaCl/NH_4Cl$ response ratio for each fiber as a function of receptive field size. Data for each age group are represented with different symbols. There is a significant, negative correlation between the response ratio and field size. That is, smaller fields are more responsive to NaCl than larger fields.

taste function. First, receptive fields decrease in size developmentally; that is, there are fewer fungiform papillae in lamb receptive fields than in fields of younger animals. Second, there is a concomitant increase in the $NaCl/NH_4Cl$ response ratio of single fibers. Third, the proportion of single fibers most responsive to NaCl, compared with NH_4Cl, increases. Fourth, there is a significant negative correlation between receptive field size and the $NaCl/NH_4Cl$ response ratio.

These results demonstrate that chorda tympani fibers not only have characteristic chemical response properties, but also related receptive field sizes. Both of these characteristics change during development and in relation to each other. Thus, there is a developmental acquisition of the small, highly NaCl-responsive, receptive fields in the peripheral taste system that complement existing, larger, highly NH_4Cl-responsive receptive fields. One implication of differential receptive field size is that in the small, NaCl-sensitive receptive fields relatively few papillae presumably exert a powerful effect on activity in the afferent innervating fiber, whereas in very large, NH_4Cl-sensitive fields, many papillae converge in activating the innervating afferent.

Taste responses to NaCl generally have been thought to involve different receptor systems at the membrane level than responses to KCl and NH_4Cl, based on response/concentration data for various salts (Beidler, 1961), developmental data on emerging neural responses to salts (Hill et al., 1982; Mistretta and Bradley, 1983), data on blocking effects of amiloride on salt taste responses (Schiffman et al., 1983; DeSimone et al., 1984; Brand et al., 1985; DeSimone and Ferrell, 1985; Hill and Bour,

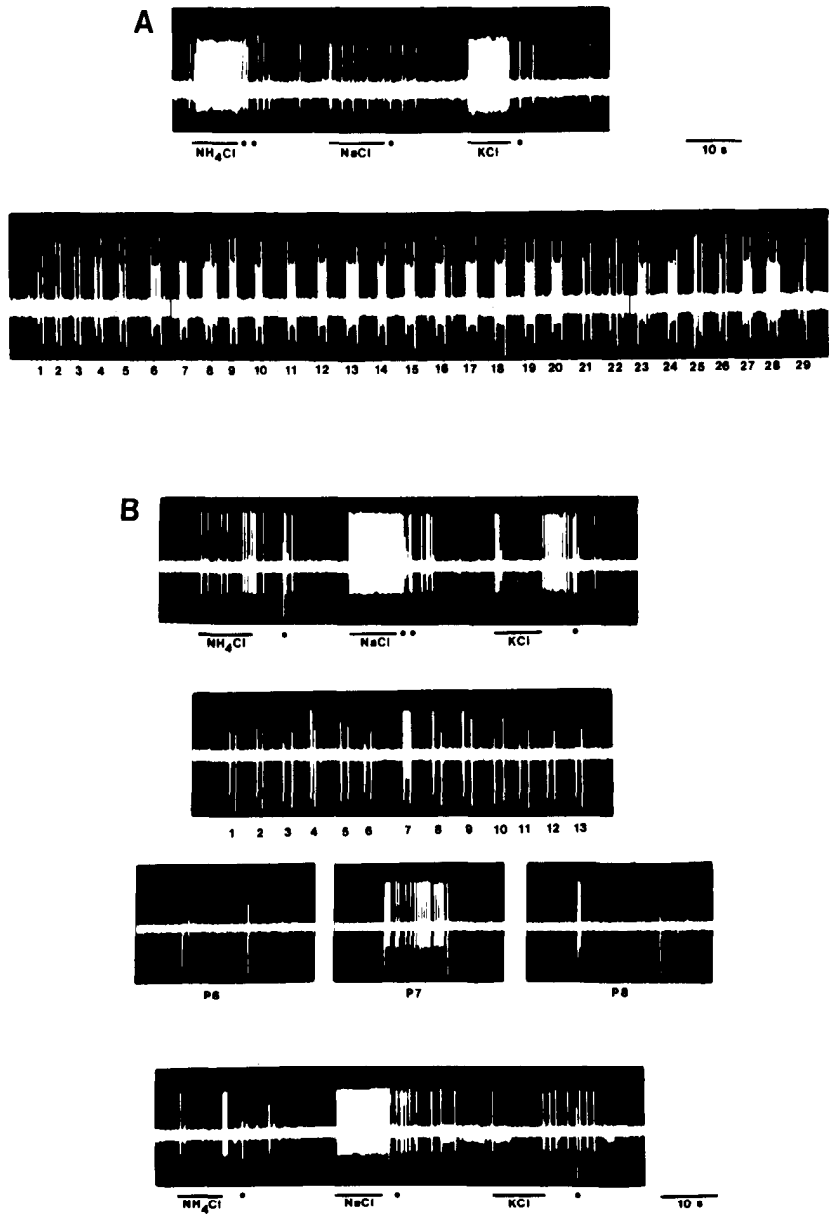

Figure 8. Neural responses from a fiber with a large receptive field (*top*) and a fiber with a small field (*bottom*). For each fiber, responses to 0.5 M NH_4Cl, NaCl, and KCl are presented first. Chemicals were applied to the tongue for the period indicated by a line under the data; dots denote water rinses. Responses to electrical stimulation of single papillae are presented next, with numbers under the data representing each papilla. Artifacts delimit onset and offset of stimulation for each papilla. In the *bottom* figure the oscilloscope sweep speed has been increased in the third row of data, to demonstrate the high-frequency neural response during stimulation of papilla 7, compared with papillae 6 and 8.

1985), and effects of early, dietary NaCl deprivation on neural responses to various salts (Hill et al., 1986). In addition, some investigators have proposed different afferent, taste fiber types based on average response frequencies of single neurons to NaCl, KCl, and NH_4Cl (Boudreau et al., 1985) and to NaCl and HCl (Frank et al., 1983). The present study augments this literature by providing evidence for different neuron types based not only on response frequency, but also on receptive field size. Neurons that are highly responsive to NaCl innervate fewer fungiform papillae than neurons that are highly responsive to NH_4Cl.

Table 2. Correlation coefficients for receptive field size and response frequencies of all fibers and Na-best fibers

	Correlation coefficient(r)	
	All fibers	Na-best fibers
Field size	1.00	1.00
NH_4 frequency	0.58[a]	0.60[b]
K frequency	0.60[a]	0.80[b]
Electrical frequency	0.56[a]	0.54[b]
Na frequency	0.02	0.53[b]

[a] $p < 0.0001$; [b] $p < 0.02$.

In a study of taste responses from goat geniculate ganglion cells, Boudreau et al. (1982) distinguished among 3 types of neurons: (1) most responsive to Na and Li salts; (2) most responsive to acidic salts, including NH_4Cl; (3) not consistently responsive to any class of stimuli. Average receptive field sizes for types 1, 2, and 3 were 5.0, 5.1, and 3.0 papillae, respectively. The investigators concluded that there was no apparent difference in field size between type 1 and type 2 cells. However, stimulus concentrations for salts were 50 mM compared with 0.5 M in our study. Therefore, geniculate ganglion cells might have been typed differently if maximal, rather than near-threshold, concentrations had been used. In addition, since the primary objective of the goat study was not to investigate receptive field size, the number of reported field sizes was rather small

(n = 11 for type 1 cells; 18 for type 2 cells).

Although comparison of the chemical aspects of the goat and sheep studies is obscured by use of very different stimulus concentrations, electrical aspects are in close agreement. Our reported range of field sizes in lambs is broader (1–29 papillae) than that in goats (1–19), but most of the goat receptive fields contained 4–8 papillae, which compares well with our data (Fig. 5). Also, in both goat and lamb, receptive fields in more posterior tongue locations contained fewer papillae than those located more anteriorly.

Other investigators have reported measures of receptive field sizes for chorda tympani neurons in other species; however, they usually have not been concerned specifically with determining field size, but with chemically stimulating individual papillae in a field to learn about single papilla responses. Miller (1971) first reported that one chorda tympani fiber can innervate several fungiform papillae and found a range of receptive field sizes of 1–12 papillae in rat. Using electrical stimulation (Boudreau et al., 1970) and chemical stimulation (Oakley, 1975) in independent studies, other investigators have reported an average of 3 papillae per receptive field in cat. Oakley (1975) reported that if 2 papillae in a receptive field are stimulated separately with several chemicals, the response profile of the innervating fiber across the various chemicals is similar during stimulation of each papilla. This supports the idea that fibers do not innervate papillae randomly. These orderly, chemically related innervation patterns must be established during development.

In the present study, receptive field size correlated with the NH_4Cl and KCl response frequencies from the entire field but not with NaCl response frequencies. On the other hand, if data from NaCl-best fibers are analyzed separately, receptive field size correlates with NH_4Cl, KCl, and NaCl responses. This is further evidence for fiber types, based on field size and salt responses.

Although evidence related to fiber specificity emerges from the present study of receptive fields, it must be remembered that such specificity ultimately derives from the taste buds within papillae in the receptive fields. In most mammals a single papilla contains multiple taste buds. Within a taste bud there are 40–60 cells, and individual taste cells have different, intracellular response profiles (Kimura and Beidler, 1961). Therefore, to understand the response characteristics of a particular papilla, intracellular recordings would be necessary, from several cells within a bud and from all buds within a papilla. This task is not feasible at present. Thus, chemical or electrical stimulation of a single papilla with multiple taste buds does not permit direct understanding of how individual receptor cells are responding.

In addition, one cannot assume that all taste buds within a papilla are innervated by one and the same afferent fiber or that all taste cells within a bud are innervated by the same fiber. It is known that one papilla can be innervated by more than one chorda tympani fiber (Miller, 1971), and we observed some overlapping receptive fields in this study. Therefore, taste buds within a papilla might be innervated by different afferents, or some buds might be innervated by more than one afferent. By reconstructing receptive fields, we determined that there is no relation between the electrical response frequency elicited by stimulating a single papilla and the number of taste buds within the papilla. This could indicate that one or a few taste buds provide the principal input to the afferent fiber and that other buds do not augment the response proportionately, or that not all taste buds within the papilla are innervated by the afferent. Either, or both, of these hypotheses are equally tenable. However, Miller (1971) has demonstrated that, in rat, where one papilla contains one taste bud only, the most sensitive papilla in a receptive field provides about 50% of the total input in generating chemical response frequencies. Thus, in rat, a single taste bud in a field has substantial input to the fiber.

Even though multiple taste buds within a papilla and possible overlapping afferent innervation impose limitations on any study of single papilla stimulation, the study of receptive fields provides important insight into peripheral innervation patterns and their relation to chemical responses. In addition, from the present study we now know that these patterns are acquired gradually during development and the acquisition involves an increase in proportion of small receptive fields. The following paper (Mistretta et al., 1988) discusses proposed neural rearrangements during receptive field development.

Total sequence of development of taste function in sheep

This study of receptive field development essentially spans the last fifth of gestation (term, about 150 d) and perinatal and early postnatal life. From previous studies we know that lingual taste buds begin to form in sheep at about 45–50 d of gestation and function from at least 84 d of gestation (Bradley and Mistretta, 1973, 1980). Limitations of PNS and CNS extracellular recording techniques constrain our ability to obtain responses from the taste system in early fetal life. In the present series of experiments we attempted to record from single chorda tympani fibers and map receptive fields in fetuses at 110 d of gestation, an age for which we have multifiber taste response data. It was not possible to maintain recordings for periods sufficient to ensure accurate receptive field size determinations. The chorda tympani nerve is very fragile at this age.

Therefore, although we have mapped fields over an age range during which we already knew that there are substantial changes in taste nerve function (Mistretta and Bradley, 1983), it is a rather narrow part of total taste development. Other differences in peripheral innervation of receptive fields might take place during early fetal life.

Development of taste buds in human fetuses (Bradley and Stern, 1967) has a similar time course to that in sheep. Therefore, the increase in NaCl responsiveness observed in the peripheral taste system in sheep could provide a biological explanation for the developmental emergence of behavioral taste responsiveness to NaCl in humans. Human infants less than 4 months of age ingest water and moderate concentrations of NaCl in equal amounts, whereas infants 4–24 months of age accept NaCl solutions in preference to water, and children aged 31–60 months reject NaCl relative to water (Beauchamp et al., 1986). Although experience presumably plays some role in these changing NaCl ingestation patterns, maturation of neurophysiological responses provides a basis for interactions between experience and the nervous system. It is interesting that in sheep there are substantial modifications in receptive fields and in neurophysiological salt responses that take place during the first few postnatal months. By extrapolation, this suggests that there could be biological changes in the human taste system for several months after birth.

Conclusions

We have measured receptive field sizes of afferent taste fibers during development in sheep and demonstrated that there is an

increase in proportion of small receptive fields in older animals. At the same time, there is an increase in the proportion of chorda tympani fibers that respond with highest frequency to NaCl, compared to NH_4Cl. Since there is a negative correlation between receptive field size and responsiveness to NaCl, we conclude that there is a developmental acquisition of small, highly NaCl-responsive receptive fields in the peripheral taste system. To understand the neural basis of this acquisition of small fields, other data are needed on morphology of the tongue, fungiform papillae, and chorda tympani nerve. These data and a discussion of models for neural rearrangements in the peripheral taste system during development are contained in the following paper (Mistretta et al., 1988).

The present paper clearly demonstrates that chorda tympani afferent fibers not only have characteristic salt responses, but are also distinguished by receptive field size. Furthermore, salt responses and receptive field size are related, and these interrelated characteristics are acquired gradually during development.

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