Neuron/Target Matching Between Chorda Tympani Neurons and Taste Buds During Postnatal Rat Development

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ABSTRACT: During postnatal development, a relationship is established between the size of individual taste buds and number of innervating neurons. To determine whether rearrangement of neurons that innervate taste buds establishes this relationship, we labeled single taste buds at postnatal day 10 (P10) and again at either P15, P20, or P40 with retrograde fluorescent neuronal tracers. The number of single- and double-labeled geniculate ganglion cells was counted, and the respective taste bud volumes were measured for the three groups of rats. The current study replicates findings from an earlier report demonstrating that the larger the taste bud, the more geniculate ganglion cells that innervate it. This relationship between taste bud size and number of innervating neurons is not apparent until P40, when taste bud size reaches maturity. These findings are extended here by demonstrating that the number of neurons that innervate taste buds at P10, when taste bud size is small and relatively homogeneous, predicts the size that the respective taste bud will become at maturity. Moreover, while there is some neural rearrangement of taste bud innervation from P10 to P40, rearrangement does not impact the relationship between taste bud size and innervating neurons. That is, the neurons that maintain contact with taste buds from P10 through P40 accurately predict the mature taste bud size. Therefore, the size of the mature taste bud is determined by P10 and relates to the number of sensory neurons that innervate it at that age and the number of neurons that maintain contact with it throughout the first 40 days of postnatal development. © 2000 John Wiley & Sons, Inc. J Neurobiol 43:98–106, 2000

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There is a highly ordered relationship between morphology and innervation of taste buds located in fungiform papillae on the anterior two thirds of the rat tongue. Specifically, the size of single taste buds in normal adult rats can be reliably predicted by the number of geniculate ganglion cells that innervate it. The larger the taste bud, the more innervating neurons (Krimm and Hill, 1998). This relationship, however, does not emerge until taste buds reach their adult size, between postnatal day 30 (PN30) and PN40. Therefore, there is a relatively prolonged postnatal developmental period where gustatory neurons match their targets.

A candidate mechanism for the matching of taste bud size with the number of innervating neurons relates to neural rearrangement. Stated simply, branches may be eliminated from some target taste bud cells following their initial innervation, whereas others are added to innervate new target cells (Pujol et al., 1978; Hume and Purves, 1989). Indeed, it is reasonable to expect that branches could be eliminated from some taste buds and reinnervate other taste buds.
buds because of the normal, continual turnover of taste receptor cells that occurs approximately every 9 days (Beidler and Smallman, 1965; Farbman, 1980; Delay et al., 1986). Thus, taste receptor cells continually undergo proliferation, differentiation, and death. Accordingly, synaptic contacts must be formed, broken, and reformed throughout life, and therefore, long-term synaptic stabilization in the peripheral taste system does not occur even in adulthood. Given the constant remodeling of synapses that must be present in the peripheral taste system, similar adjustments may also be present in the branching characteristics of individual sensory fibers, such that increased size of taste buds (via increased cellular volume and/or numbers) may support increased numbers of synapses, branches, and neurons. Conversely, greater numbers of neuronal branches may support more taste receptor cells. Since gustatory neurons have a trophic influence on taste bud morphology (e.g., Sloan et al., 1983), it is reasonable to expect that the more trophic support supplied by innervating neurons, the more sensory receptor cells in the target taste bud. For fungiform taste buds, the fibers that would presumably make these adjustments are chorda tympani nerve fibers, which have their cell somas in the geniculate ganglion.

The concept that neural rearrangement occurs during peripheral gustatory development is not a new one. During sheep development, there is an increase and subsequent decrease in the size of receptive fields from prenatal through postnatal ages (Mistretta et al., 1988; Nagai et al., 1988; Mistretta; 1991). That is, the number of taste buds innervated by single chorda tympani fibers change significantly in the developing sheep gustatory system. This suggests that neural rearrangement involves increases and then decreases in branching of single neurons to innervate multiple papillae. These changes in innervation are accompanied by similar changes in the number of taste buds per fungiform papilla. As receptive field size increases, so do the numbers of taste buds per fungiform papilla; as receptive field size decreases, so do the number of taste buds. Thus, there must be some developmental rearrangement of peripheral taste organs and associated innervation. Most importantly, however, sodium taste sensitivity changes occur concomitantly with decreases in receptive fields of single fibers (Nagai et al., 1988). Specifically, there is an increasing proportion of small receptive fields that correspond to high sodium salt responses during development (Nagai et al., 1988). By contrast, relatively large receptive fields that are highly responsive to ammonium chloride are retained with age (Nagai et al., 1988). It is not clear if changes in taste bud morphology and/or function result from, or cause changes in, peripheral innervation.

While neural rearrangement may seem like the most plausible mechanism used in matching chorda tympani fibers with taste buds, there are other equally plausible mechanisms. In fact, at the other extreme, there may be no neural rearrangement if proliferation rates are low and/or if taste bud receptor cells do not turnover during early development. That is, there may be no need for neural plasticity until their targets turnover (i.e., when taste buds reach mature volumes). Although there are no data about turnover rates during development, there is a reported lack of proliferating cells in fungiform papillae in late-gestational rats (Farbman and Mbiene, 1991).

To test the hypothesis that neural rearrangement is responsible for establishing the relationship between taste bud size and number of innervating ganglion cells, we used a double-fluorescent labeling method to determine the degree to which neurons that innervate taste buds at PN10 are constant through PN40. Therefore, unlike developmental studies of receptive field organization in which neural rearrangement is defined in terms of how many papillae are innervated by a single neuron (e.g., Nagai et al., 1988), we examined neural rearrangement in terms of how many neurons innervate a single taste bud. Techniques used here also allowed us to determine if the mature taste bud size could be predicted by the number of neurons that innervate it at PN10.

**METHODS**

Initially, single fungiform taste buds located in the midregion of the tongue in P10 rats were injected with a blue fluorescent tracer (P0 = day of birth). Upon completion of the label, a map of the dorsal tongue was made using measurements and lingual landmarks so the papillae could be reliably identified later to make a second injection of the same taste bud (see next). On either P15, P20, or P40, rats were anesthetized, and the previously labeled papillae were identified and labeled again with a red fluorescent tracer. These ages were chosen because taste bud structure and function changes rapidly between P10 and P20 in the rat, because taste bud structure in P10 and P20 rats are similar to fetal (110 days gestation) and perinatal sheep, respectively (Hill, 1987), and because three of these ages (P10, P20, and P40) have been used in our initial work on the development of taste bud innervation (Krimm and Hill, 1998). P40 was chosen because the relationship between taste bud size and number of innervating ganglion cells occurs by that age (Krimm and Hill, 1998). The number of geniculate ganglion cells labeled with only the first fluorescent tracer, only the second fluorescent tracer, and double-
labeled with both tracers was counted for each labeled taste bud.

**Labeling Single Fungiform Taste Buds**

Rats P10, P15, or P20 were anesthetized with sodium Brevital (60 mg/kg, i.p.). Rats 40 days of age were anesthetized with sodium pentobarbital (50 mg/kg; i.p.) to maintain proper levels of anesthesia throughout the taste bud injection procedure. All rats were placed on a water-circulating heating pad to maintain body temperature at 36°C. The dorsal, anterior half of the tongue was exposed from the mouth by gently pulling on the ventral tongue. The tongue was stabilized by pressing the ventral surface to a glass slide covered with putty. Fungiform papillae were visualized with the aid of a 0.5% solution of methylene blue (Fischer Scientific) painted on the tongue surface. Using a micromanipulator and surgical microscope, a glass pipette (150 μm diameter) was placed over a single papilla without penetrating the epithelia, yet firm enough to create an electrical seal. A small wire (0.3 mm diameter), inserted into the ventral tongue, served as the reference electrode. By applying a square, anodal pulse (Grass Electronics; 0.5–4.0 μA positive current, 4 s on/4 s off for 5–8 min). True-Blue chloride (Molecular Probes; 2% in distilled water) was iontophoresed into the papillae of P10 rats, and a 3-kD tetramethylrhodamine dextran (Molecular Probes; 10% in 0.01 M phosphate buffer, pH 7.0) was iontophoresed into the papillae of P15, P20, or P40 rats (Krimm and Hill, 1998). True-Blue was chosen as the first label because of our previous success with the label in reliably producing the relationship between taste bud size and numbers of innervating geniculate ganglion cells in mature rats (Krimm and Hill, 1998) and because of its success in long-term labeling studies (Garrett et al., 1991; Houle, 1991).

Tetramethylrhodamine dextran was chosen as the second label because it also reliably produced the relationship between taste bud size and number of innervating geniculate ganglion cells in pilot experiments (also see Results) and because the emission wavelength (580 nm) is sufficiently different from that produced by True-Blue (404 nm). Therefore, the excitation wavelengths are sufficiently distinct so that single- and double-labeled ganglion cells can be visualized unambiguously.

Although it is possible that there are some long-term effects of applying True-Blue to taste buds, it has been used in a variety of long-term studies with no reported effects on injection site or decrease in the number of labeled neurons (Garrett et al., 1991; Houle, 1991). Therefore, it is unlikely that there is substantial effect of True-Blue on the taste bud or changes in innervation over time. However, True-Blue is typically pressure injected or placed on the cut end of a nerve; thus, it is possible that iontophoretic application of the tracer has some effect on taste buds or innervation. If iontophoretic application of the tracer results in long-term effects on the growth of taste buds or innervation, the correlation between taste bud size and number of labeled ganglion cells might be disrupted. We found no evidence for this in pilot work. Furthermore, we will show that the number of innervating ganglion cells at both 10 and 40 days was highly predictive of taste bud size (see Results), arguing against a substantial long-term effect of iontophoresis.

**Histological Procedures**

Rats were killed with a lethal dose of sodium pentobarbital 4 days after application of the second label and perfused intracardially with physiological saline, followed with 4% paraformaldehyde (pH 6.5 followed by pH 9.5). Tongues and geniculate ganglia were removed and placed in 30% sucrose overnight. The distances between the labeled papillae and the intermolar eminence and between papillae and the midline were measured to determine the location of labeled papillae on the tongue surface and subsequently were used to locate labeled papillae once the tissue was processed (see next). Each block of tissue was positioned in a plastic embedding mold, covered with Histotech, and frozen at −70°C until sectioning. Serial 10-μm sections of the geniculate ganglion and 20-μm tongue sections were obtained with a cryostat, thaw-mounted on gelatin-coated glass slides, cleared with xylenes, and coverslipped with DPX. After examination of the amount of fluorescent label within a papilla, coverslips were removed, and slides containing tongue sections were stained with hematoxylin and eosin so that taste bud volumes could be measured.

**Data Analysis**

All tissue were examined under a microscope with an epi-fluorescent illuminator. True-Blue was examined using wide band UV excitation (330–380 nm), and tetramethylrhodamine dextran was examined under green light excitation (465–550 nm) (see Fig. 1). In addition, a supplementary barrier filter (D460/50; Chroma Technology Corp., Brattleboro, VT) that blocked all wavelengths lower than 455 nm was used along with UV excitation to verify double-labeled cells. The difference in emission wavelengths in combination with the barrier filter allowed an unambiguous determination of blue-only, red-only, and double-labeled geniculate ganglion cells. Tongues were examined first to determine that the label was successful. Successful labels were ones in which the second label filled the entire taste bud but was contained within the papilla (Fig. 1(B) and in which only the dorsal half of the papilla core contained some of the first label (Fig. 1(A)). Labels (blue and red) that extended out of the base of the papillae were not used because of the likelihood that chorda tympani neurons passing near, but not into the papillae, were also labeled (also see Krimm and Hill, 1998). Only ganglia from successful labels were sectioned and analyzed (Fig. 1(C,D)).

Analyses of ganglia were accomplished by serially re-constructing each ganglion with a computer microscope system (Neurolucida; Microbrightfield, Inc., Colchester, VT). Digital information of X, Y, and Z coordinates were fed on line to a computer as tracings were made of the borders of the ganglia and of labeled cells. Ganglion cells were
drawn and counted without experimenter knowledge of taste bud size. The appearance of geniculate ganglion cells that only contained the blue label (i.e., the label at P10) was interpreted as the corresponding taste bud losing this innervation between the first and second injection. Conversely, the appearance of ganglion cells that only contained the red label (i.e., the label at P15, P20, or P40) was interpreted as the corresponding taste bud gaining this innervation between the first and second injection. Finally, a double-labeled ganglion cell was interpreted as the taste bud being innervated by the neuron at both ages.

To measure taste bud areas, the perimeter of the taste bud was outlined in each section that contained a portion of the taste bud, and the corresponding area was computed by an Olympus Cue-2 image analysis system. To achieve consistency in measurement between taste buds, the borders were drawn such that the peripheral cells of the taste bud were included in the measurement (Whitehead et al., 1985; Krimm and Hill, 1998). Three taste bud area measurements were computed from each section, and the average value was used to estimate taste bud volume. The area values were multiplied by section thickness and summed across all sections containing a taste bud to derive an estimate of the total taste bud volume. Taste buds were measured without experimenter knowledge of ganglion cell number.

STATISTICS

Proportions of papillae innervated by ganglion cells containing only the first label, only the second label and containing both labels were compared using the Chi-square distribution. Numbers of single- and double-labeled ganglion cells were compared among groups using analysis of variance. Pearson product-moment correlations were used to analyze the relationship between taste bud size and number of innervating ganglion cells. The alpha level was set at $p < .05$; however, the actual $p$ values are reported for the reader’s information.

RESULTS

Successful Papillae Labels

More than 30 days after papillae were originally injected, True-Blue (i.e., the label used at P10) was present in the dermal core of most injected papillae and was limited to the papilla’s dorsal half (Fig. 1(A)). However, it was not observed in the taste buds of double-labeled papillae in any of the three groups. This is consistent with our earlier work in which we failed to observe significant taste bud labeling after approximately 4 days postinjection (Krimm and Hill, 1998). We believe that this is due, in part, to loss of taste bud cells through turnover. In unsuccessful labels at P10, blue was not observed in either the ganglion or the dermal core of fungiform papillae examined. Since, these data could not be analyzed further, only successful labels are reported. Successful tetramethylrhodamine dextran labels (i.e., the red label used at P15, P20, or P40) were visible in the entire taste bud for all three groups. Unsuccessful red labels were where either no red was seen in fungiform papillae and corresponding ganglia or where red-labeled cells were seen below the base of fungiform papillae. For three of the papillae in the group labeled on P10 and then on P40, the first and second labels

Figure 1 Photomicrographs of a fungiform papilla visualized with wideband UV excitation (A) or green light excitation (B) in which True-Blue was iontophoretically applied on P10 and tetramethylrhodamine dextran was iontophoretically applied on P40. The blue label can be observed within the papilla core. The red label fills the taste bud and can be seen in fibers and cells in the papilla core. Although the epithelial surface appears red in the photomicrograph due to methylene blue staining, it is easily distinguished from the tetramethylrhodamine dextran label. Arrows point to the taste bud. Photomicrographs of a geniculate ganglion cell, under UV excitation (C) and under green light excitation (D). This ganglion cell is double-labeled. A supplementary barrier filter (D460/50; Chroma Technology Corp.), which was not used for photography, blocked all wavelengths $<455$ nm during data analysis. Scale bars, 100 $\mu$m.
were not located within the same papillae; data from these labeled papillae were not used in the double-label portion of the analysis but were used for single-label analyses.

**Innervation on P10 Predicts Taste Bud Size on P40**

There is no correlation between number of geniculate ganglion cells labeled at P10 and taste bud volume at P15 (\( r = .46; p = .23 \)) or at P20 (\( r = .40; p = .10 \)). In contrast, the number of ganglion cells labeled at P10 is significantly correlated with the size of the taste bud at P40 (Fig. 2; \( r = .75, p = .0002 \)). Thus, the number of ganglion cells that innervated taste buds on P10 did not successfully predict the size of taste buds until P40.

**Relationship between Taste Bud Size and Number of Innervating Ganglion Cells on P15, P20, and P40**

By 40 days of age, the number of innervating ganglion cells is related to the volume of the taste bud (Krimm and Hill, 1998). Similarly in the current study, we found that the number of ganglion cells labeled and measured at P40 was correlated with the size of the injected taste bud (Fig. 3; \( r = .77, p = .0001 \)). However, there was no correlation between taste bud volume and number of red ganglion cells in P15 (\( r = .46; p = .36 \)) or P20 (\( r = .40; p = .18 \)) rats. Therefore, consistent with Krimm and Hill (1998), the relationship between taste bud size and number of innervating ganglion cells develops after P20, but before P40.

**NUMBER OF DOUBLE- AND SINGLE-LABELED GANGLION CELLS**

In all three age groups, every taste bud was innervated by double-labeled neurons (Fig. 4). However, only four taste buds (14% of total labeled taste buds) were exclusively innervated by double-labeled neurons. Thus, some changes in ganglion cell innervation occurred in all three experimental groups.

In the group labeled at P10 and again at P15, 7 of the 9 taste buds were innervated by ganglion cells that only had the first label (i.e., blue-labeled; Fig. 4). Thus, a few neurons ceased to innervate most of the labeled taste buds between P10 and P15. Of the 9 labeled taste buds in this group, 4 were innervated by geniculate ganglion cells that contained only the second label (i.e., red-labeled; Fig. 4). Thus, although some taste buds lost innervation from some neurons, other taste buds received innervation from additional neurons between P10 and P15 (Fig. 4). In rats labeled at P10 and P20, 5 of the 6 taste buds were innervated by ganglion cells that only had the first label, whereas 4 of the 6 were innervated by ganglion cells with only the second label (Fig. 4). Finally, 10 of the 14 taste buds were innervated by ganglion cells with only the first label, and 9 were innervated by neurons with only the second label (Fig. 4).

In order to examine the degree of neural rearrange-
ment over time, we compared both the proportion of taste buds undergoing changes in innervation and the degree to which the change occurred through the different injection intervals. The proportions of taste buds innervated only by the first label compared to those innervated only by the second label were used to determine if the number of taste buds losing or gaining innervation was related to time between injections. The numbers of taste buds innervated by ganglion cells containing only the first label ($p = .84$) or only the second label ($p = .58$) did not differ between groups. Therefore, the same proportion of taste buds both gained and lost innervation between days 10 and 15 and between days 10 and 40. This indicates that most of the neural rearrangement occurred between P10 and P15.

The amount of change in innervation for taste buds that had neural rearrangement was also compared among groups. For taste buds that underwent change after the initial injection, the number of double-labeled ganglion cells decreased significantly from the group with the shorter interval between injections (P10–P15) through the group with the longer interval (P10–P40) $F(2,22) = 5.94; p = .009$. Thus, the overall change in innervation increased as the length of time between injections increased. In contrast, the number of single-labeled ganglion cells did not differ among the three groups ($p = .25$).

**Net Changes in Innervation**

Since individual taste buds gained innervation and lost innervation, the net gain or loss of innervation was determined for each taste bud in the group labeled at P10 and again at P40 (Fig. 5). Five taste buds had no net change in innervation, 5 taste buds showed decreases in innervation ranging from 1 to 3 geniculate ganglion cells, and 4 taste buds showed increases in innervation ranging from 1 to 6 geniculate ganglion cells. Thus, although a few taste buds showed substantial changes in innervation between postnatal days 10 and 40, 9 of the 14 labeled papillae showed a net change in innervation of one or fewer, suggesting that amount of reorganization during this period is not large.

**Double-Labeled Cells Predict Taste Bud Size**

To test if neural rearrangement had a significant impact on the ability to predict taste bud size from innervating neurons, the number of double-labeled neurons was analyzed alone to see how well it predicted taste bud size at P40. That is, are the neurons that maintain connections with a taste bud throughout development good predictors of mature taste bud size? As seen in Figure 6, the number of double-labeled neurons that innervate a taste bud do just as well in predicting taste bud size as do the total number of innervating neurons (all double- and single-labeled neurons) at P40 (i.e., cf. Fig. 6 with Figs. 2 and 3). Therefore, even though there is some neural rearrangement from P10 to P40, it is the neurons that continue to innervate taste buds through this period that accurately predict mature taste bud size.

**DISCUSSION**

The match between number of innervating geniculate ganglion cells and taste bud size occurs over the first 40 postnatal days (Krimm and Hill, 1998). Findings from the current study extend these findings by demonstrating that the number of neurons that innervate a taste bud at P10 predicts the size the taste bud becomes at P40. This indicates that taste bud size is...
predetermined on or before P10, when taste buds have a mature complement of innervating neurons (Krimm and Hill, 1998). Although there is some rearrangement of innervation from P10 through P40, it is not substantial enough to predict taste bud size. In fact, the neurons that innervate taste buds at P10 and are maintained through P40 (i.e., double-labeled neurons) are as good of predictors of taste bud size as are the neurons that innervate taste buds at P40, when taste buds are mature in size.

It is tempting to attribute a causal relationship between numbers of innervating neurons and taste bud size; however, such a mechanism cannot be determined from the current study because the data are correlational. Therefore, it is possible that mechanisms other than that determined directly by the number of innervating ganglion cells could result in the match between innervation and taste bud size. Factors influencing both neuronal number and taste bud size may be responsible for the development of this relationship. For example, the size of individual taste buds varies considerably, but systematically, across the tongue surface (e.g., Krimm and Hill, 1998). Brain-derived neurotrophic factor (BDNF), which is expressed at the apex of developing fungiform papillae (Nosrat and Olson, 1995; Nosrat et al., 1996) could be made in disparate amounts in different papillae and taste buds, depending on their size. Because this factor likely prevents developmental cell death of geniculate ganglion neurons (Liu et al., 1995; Vogel

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**Figure 5** The net change in innervation plotted for individual papillae in the group injected on P10 and injected on P40. Hatched bars denote a net decrease in number of innervating neurons, open bars denote a net increase in number of innervating neurons, and asterisks denote no change. Papillae are ordered by size of corresponding taste bud.

**Figure 6** The number of geniculate ganglion cells (●) double-labeled with True-Blue (first label) and tetramethylrhodamine dextran (second label) plotted against taste bud volume at P40. The regression line for these data is shown as a solid line, and the correlation coefficient and equation are shown in lower right portion of the figure.
and Davies, 1991; Nosrat et al., 1997; Mistretta et al., 1999; Ringstedt et al., 1999), it may determine the number of neurons that initially innervate a taste bud. The number of surviving neurons would be proportional to the amount of BDNF produced by the emerging taste bud. The amount of BDNF could also limit the size taste buds become during development by regulating taste bud cell cycle dynamics (e.g., taste bud cell birth and/or cell death). Therefore, some feature of the system could be responsible for both the number of ganglion cells innervating a particular taste bud and could provide a limitation on the final size taste buds become.

While noncausal relationships between innervation and taste bud size cannot be ruled out, it is possible that future studies may ultimately show that neural innervation directly influences taste bud growth and maintenance. That is, the number of ganglion cells that innervate a particular taste bud by P10 could direct taste bud growth. Indeed, taste buds depend on neural innervation in order to maintain their structure and integrity in adulthood (Whiteside, 1928; Guth, 1957; Fugimoto and Murray, 1970; Oakley et al., 1990; Seta et al., 1999) and for the completion of their normal development (Hosley et al., 1987; Oakley et al., 1998; Morris-Wiman et al., 1999). Although it appears that cellular proliferation rates in fungiform papillae are unchanged following loss of innervation (Oliver and Whitehead, 1992), it is apparent that either taste bud cells die and/or dedifferentiate after denervation. Therefore, gustatory neurons have a trophic influence on taste bud cells (Sloan et al., 1983). It is possible in the developing system that the number of innervating neurons determines the upper limit of the number of taste bud cells that can be supported by the amount of trophic material available. Once the limiting number of taste bud cells is reached, the taste bud may then begin increased rates of turnover.

In addition to the initial neurotrophic factor related events that determine the number of geniculate ganglion cells that survive, the amount of neurotrophic factor(s) produced by taste buds postnatally may be directly related to the size of the taste bud and/or number of taste bud cells and, therefore, have a maintenance influence on numbers of neurons that innervate the taste bud. That is, the differential amount of neurotrophic factors produced may support corresponding numbers of innervating ganglion cells, even at maturity. Although this implies that differential numbers of ganglion cells innervating a specific taste bud may be maintained, depending on the amount of neurotrophic factors produced by that taste bud, the total number of neurons available to innervate all taste buds probably do not change during the ages studied here (Ferrell et al., 1985).

Although neural rearrangement is not responsible for the development of the relationship between innervation and taste bud size, it appears that some neural rearrangement occurs during postnatal development. For many taste buds, innervation was both removed and added between postnatal days 10 and 15, when the taste system undergoes significant functional development. More specifically, response magnitudes of the chorda tympani nerve to sodium and lithium salts begin to increase during this period (Hill and Almli, 1980; Ferrell et al., 1981; Hill et al., 1982). Although the chorda tympani nerve of the newborn rat is relatively insensitive to sodium and lithium salts, these are the most effective stimuli for the adult chorda tympani nerve. Therefore, it could be that changes in peripheral function relates to this withdraw and addition of neurons. Indeed, changes in receptive fields of chorda tympani fibers in developing sheep are coincident with changes in response profiles (Mistretta et al., 1988; Nagai et al., 1988; Mistretta; 1991).

Findings from the present study further demonstrate that the developing gustatory system is an ideal system to examine neuron/target interactions, because of the apparent establishment of the mature complement of innervating neurons early in development and the prolonged postnatal development of the sensory organ. Therefore, even after the sensory organs are formed embryonically and early postnatally, there is an extensive interplay between neurons that innervate taste buds and the cellular dynamics that determine structure and function.

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