Innervation of Single Fungiform Taste Buds During Development in Rat

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ABSTRACT

To determine whether the innervation of taste buds changes during postnatal development, the number of geniculate ganglion cells that innervated single fungiform taste buds were quantified in the tip- and midregions of the tongue of adult and developing rats. There was substantial variation in both the size of individual taste buds and number of geniculate ganglion cells that innervated them. Importantly, taste bud morphology and innervation were highly related. Namely, the number of labeled geniculate ganglion cells that innervated a taste bud was highly correlated with the size of the taste bud (r = 0.91, $P < .0003$): The larger the taste bud, the more geniculate ganglion cells that innervated it.

The relationship between ganglion cell number and taste bud volume emerged during the first 40 days postnatal. Whereas there was no difference in the average number of ganglion cells that innervated individual taste buds in rats aged 10 days postnatal through adulthood, taste bud volumes increased progressively between 10 and 40 days postnatal, at which age taste bud volumes were similar to adults. The maturation of taste bud size was accompanied by the emergence of the relationship between taste bud volume and number of innervating neurons. Specifically, there was no correlation between taste bud size and number of innervating geniculate ganglion cells in 10-, 20-, or 30-day-old rats, whereas taste bud size and the number of innervating ganglion cells in 40-day-old rats were positively correlated (r = .80, $P < .002$). Therefore, the relationship between taste bud size and number of innervating ganglion cells develops over a prolonged postnatal period and is established when taste buds grow to their adult size. J. Comp. Neurol. 398:13–24, 1998. © 1998 Wiley-Liss, Inc.

Indexing terms: taste bud; geniculate ganglion; sensory afferents; fluorescent tracers; tongue

Animals undergo substantial changes in size, form, and function as they mature. Accordingly, adjustments must occur in the connectivity of the peripheral nervous system that result in coordination between the developing peripheral target and the neurons that innervate it (Purves, 1988). Simply stated, neural innervation often adjusts to and becomes coordinated with its changing target during development.

The taste system is an ideal system in which to examine issues related to nerve/target interactions during development, because substantial age-related changes occur in the morphology and function of taste buds. During development of the gustatory system, there is a prolonged period of continued addition of receptor cells and organs (Bradley et al., 1980; Mistretta, 1991). Even in the adult, the receptor organ is not static because receptor cells continuously turn over (Beidler and Smallman, 1965). Therefore, innervation of these structures must constantly be remodeled. Functionally, taste sensitivity to some stimuli develops over a prolonged developmental period. The most impres-

sive functional increase in sensitivity during development occurs to sodium salts (Ferrell et al., 1981; Hill and Almli, 1980; Hill and Bour, 1985; Hill et al., 1982; Yamada, 1980). It is possible that these functional and morphological changes either result in, or are caused by, developmental adjustments in the innervation patterns of taste buds (Mistretta et al., 1988). Therefore, it is necessary to examine the manner in which single taste buds are innervated throughout development to determine relationships about structure and function.

One way to determine such relationships is to study the development of receptive fields of single peripheral taste

neurons. However, the only information concerning developmental adjustments in taste bud innervation patterns derives from studies of the receptive fields of single taste neurons in sheep (Mistretta et al., 1988; Mistretta, 1991; Nagai et al., 1988). The number of taste buds in fungiform papillae innervated by an individual chorda tympani neuron increases in perinatal sheep as compared to fetal sheep and then decreases through postnatal development. This increase and subsequent decrease in receptive field size is accompanied by concomitant developmental changes in taste bud numbers. In addition, the developmental refinement in the receptive fields of chorda tympani neurons occurs in parallel with developmental increases in sodium taste sensitivity and is limited to neurons that become highly responsive to sodium. Thus, in the sheep taste system, both development of sodium sensitivity and changes in the number of taste buds per papilla are developmentally coincident with an apparent modification of branching characteristics of peripheral chorda tympani nerve fibers.

Examination of the way that single taste buds are innervated in the rat offers several advantages over sheep. First, taste buds are initially formed the day before birth in the rat (Farbman, 1965; Mistretta, 1972). Therefore, their development is largely a postnatal event which allows greater experimental access to the developing animal. Second, although taste buds continue to increase in size throughout postnatal development (Farbman, 1965), it is unlikely, based upon indices of mature taste buds, that significant numbers of additional fungiform taste buds are added after 12 days postnatal (Mistretta, 1972). Thus, unlike taste buds in fungiform papillae of sheep, which increase in size and in number during prolonged periods of development, rat taste buds only increase in size.

Although it is clear that substantial changes are made in the morphology and function of taste buds during rat postnatal development, it is not clear to what extent adjustments are made in the way that taste buds are innervated during this period. For example, it is possible that the numbers of neurons that innervate taste buds are established early in development and that taste bud size increases with age to match the number of innervating neurons. Conversely, the mature numbers of innervating neurons may match the mature taste bud size. Finally, some combination of these developmental sequences may occur; both the number of innervating neurons and taste bud size increase concomitantly during development. To test these hypotheses, the number of geniculate ganglion cells that innervated individual taste buds was determined throughout postnatal development and compared with taste bud size.

MATERIALS AND METHODS

The number of geniculate ganglion cells that innervate single taste buds was assessed by labeling individual fungiform papillae with fluorescent neuronal tracers that were then transported from the corresponding taste bud to the cell bodies of the chorda tympani nerve in the geniculate ganglion. The number of fluorescently labeled geniculate ganglion cells were subsequently counted. Because each fungiform papilla usually contains only one taste bud in the rat, the total number of labeled geniculate ganglion cells can be assumed to be the number of neurons that innervate a single taste bud.

Individual fungiform papillae located in the mid-region of the tongue were fluorescently labeled in adult rats (>60 days; n = 13 papillae in 13 rats) and in developing rats aged 10 days (n = 9 papillae in eight rats), 20 days (n = 11 papillae in 11 rats), 30 days (n = 13 papillae in six rats), or 40 days (n = 12 papillae in six rats). These ages were chosen because taste bud structure and function change rapidly between 10 and 20 days postnatal (Hill, 1987), and postnatal days 30 and 40 correspond to the age range when mature functional taste responses emerge in the rat (e.g., Hill et al., 1982). To extend the findings from the tongue mid-region to other tongue regions, papillae at the tip of the tongue were labeled in adults (n = 10 papillae in nine rats) and rats aged 10 (n = 8 papillae in six rats) and 20 (n = 9 papillae in eight rats) days.

Labeling single fungiform taste buds

Adult rats were anesthetized with sodium pentobarbital (50 mg/kg; i.p.) or sodium Brevital (60 mg/kg, i.p.) and 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% aqueous solution of methylene blue (Fisher Scientific, Pittsburgh, PA) painted on the tongue surface. By using a micromanipulator and surgical microscope, a glass pipette (150 µm diameter) was placed over a single papilla without penetrating the epithelium, yet firmly 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, Quincy, MA; 0.5–1.0 µA positive current, 4 seconds on/4 seconds off for 5–10 minutes), one of two fluorescent dyes was iontophoresed into papillae: True Blue chloride (Molecular Probes, Eugene, OR; 2% in distilled water) or Fluoro-Gold (Fluorochrome, Inc., Englewood, NJ; 2% in distilled water). Animal care, anesthesia, surgery, and euthanasia were conducted in compliance with guidelines set forth by the Animal Research Committee at the University of Virginia.

The distances between labeled taste buds and the intermolar eminence and between labeled taste buds and the midline were measured to record the location of taste buds. Thus, a map was made of the locations of labeled taste buds on the tongue surface.

During preliminary observations, a total of 26 taste buds on the tongue mid-region and 19 taste buds on the tongue tip were labeled in adult, 10-, 20-, and 30-day-old rats. No labeled ganglion cells were observed in the geniculate ganglion on the contralateral side of the labels. Therefore, taste buds on both sides of the tongue were examined independently.

Histological procedures

Rats were killed with a lethal dose of sodium pentobarbital 4 days after application of the label, perfused intracardially with physiological saline, followed with 4% paraformaldehyde (pH 6.5 followed by pH 9.5). The tongue and geniculate ganglia were removed and placed in 30% sucrose overnight. Each block of tissue was positioned in a plastic embedding mold, covered with Histotech, and frozen at −70°C until sectioned. Serial 10-µm sections of the geniculate ganglion and 20-µm tongue sections were
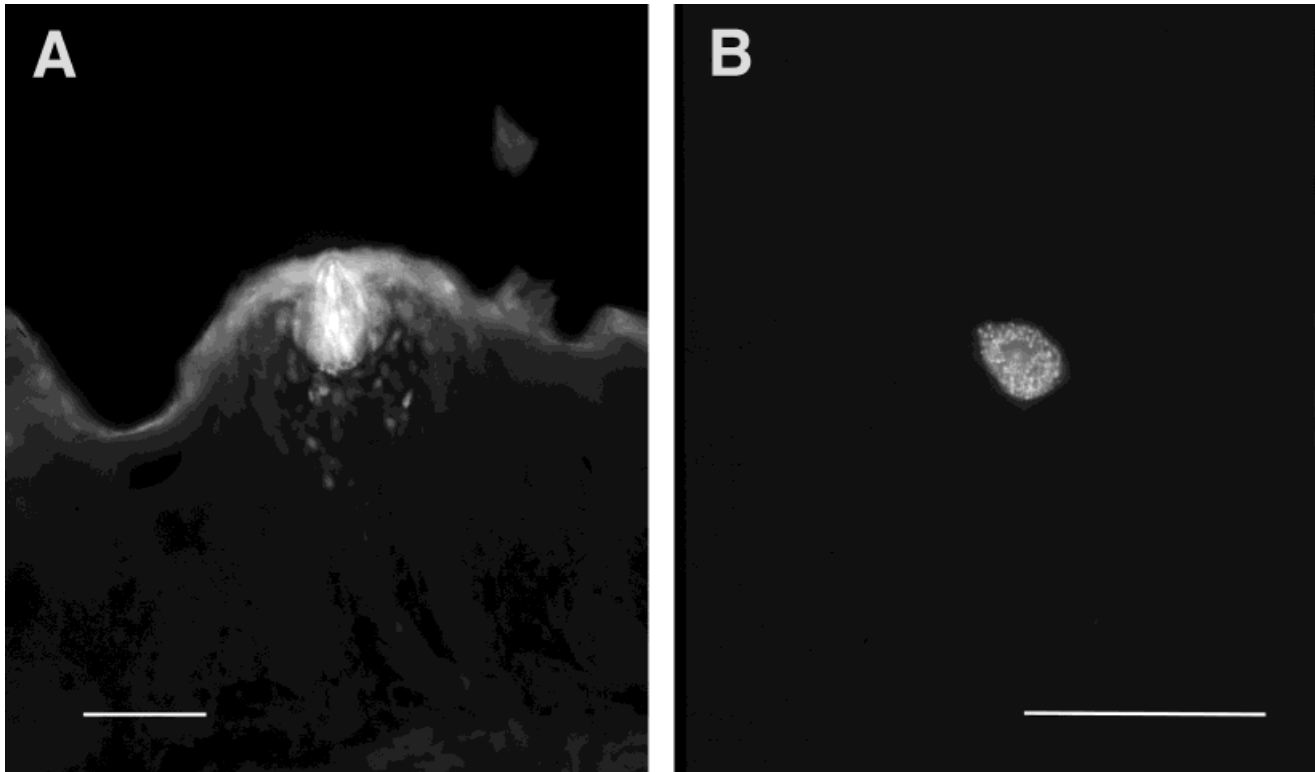
Fig. 1. **A:** Photomicrographs of a coronal section through a fungiform papilla in which Fluoro-Gold was iontophoretically applied. **B:** Photomicrograph of a Fluoro-Gold labeled geniculate ganglion cell that innervated the fungiform papilla shown in A. Prints were made from photomicrographs and then scanned with Adobe Photoshop. The images were only sharpened with Photoshop. Scale bars = 100 µm.

obtained with a cryostat. Sections were thaw-mounted on gelatin-coated glass slides, cleared with xylenes, and cover-slipped with DPX. After determining the extent of fluorescent label within the taste bud, coverslips were removed, and slides containing tongue sections were stained with hematoxylin and eosin.

Data analysis

All tissue was examined under a microscope with an epifluorescent illuminator. True Blue and Fluoro-Gold labels were visualized by using wide-band ultraviolet (UV) excitation. The resultant labels from both tracers in the tongue and geniculate ganglion were robust and were easily distinguished from each other and from background fluorescence (Fig. 1). Therefore, an "all-or-none" criteria was used to determine if a cell was fluorescently labeled with True Blue or with Fluoro-Gold. Tongues were inspected initially to determine that the label filled the entire taste bud but was contained only within the dorsal half of the papilla (Fig. 1A) to prevent labeling chorda tympani fibers that course ventral to fungiform papillae. Only ganglia from labels that met these criteria were sectioned and analyzed.

Analyses of ganglia were accomplished by serial reconstruction of each ganglion with a computer microscope system (Neurolucida, Microbrightfield, Inc., Colchester, VT). Digital information of X, Y, and Z coordinates was fed on-line to a computer as tracings were made of the borders of the ganglion and of labeled cells. Fluorescently labeled ganglion cells were drawn and counted without experimenter knowledge of taste bud size.

Although there was not a way to determine the quantitative limits of the labeling procedure directly, it was apparent that invasion of the neural plexus ventral to fungiform papillae by the fluorescent dye resulted in many more labeled ganglion cells than if the dye was confined to the dorsal half of papillae. Thus, there is indirect evidence that excessively large dye injections, which were not included in the data analysis, labeled large numbers of geniculate ganglion cells. In contrast, there were no apparent differences in number of ganglion cells labeled due to variability in amount of dye injections in the area ventral to the taste bud but dorsal to the base of the fungiform papilla (see above for criteria used to include labels).

In order to determine whether there was a topographical representation of the tongue surface in the geniculate ganglion, the locations of labeled ganglion cells from computerized three-dimensional (3-D) reconstructions were replotted onto a template, consisting of a 2-D drawing of the geniculate ganglion (Coreldraw 3.0). A dorsal view and a lateral view of the geniculate ganglion were constructed for 10-day-old and adult rats. Two of the ganglia could not be reconstructed with the computer microscope; therefore, the composite drawing in adult rats contained locations of labeled ganglion cells from 11 mid-region taste bud labels and ten tip-region taste bud labels. Composites of 10-day-old rats contained the locations of labeled ganglion cells from a total of 16 taste bud labels.
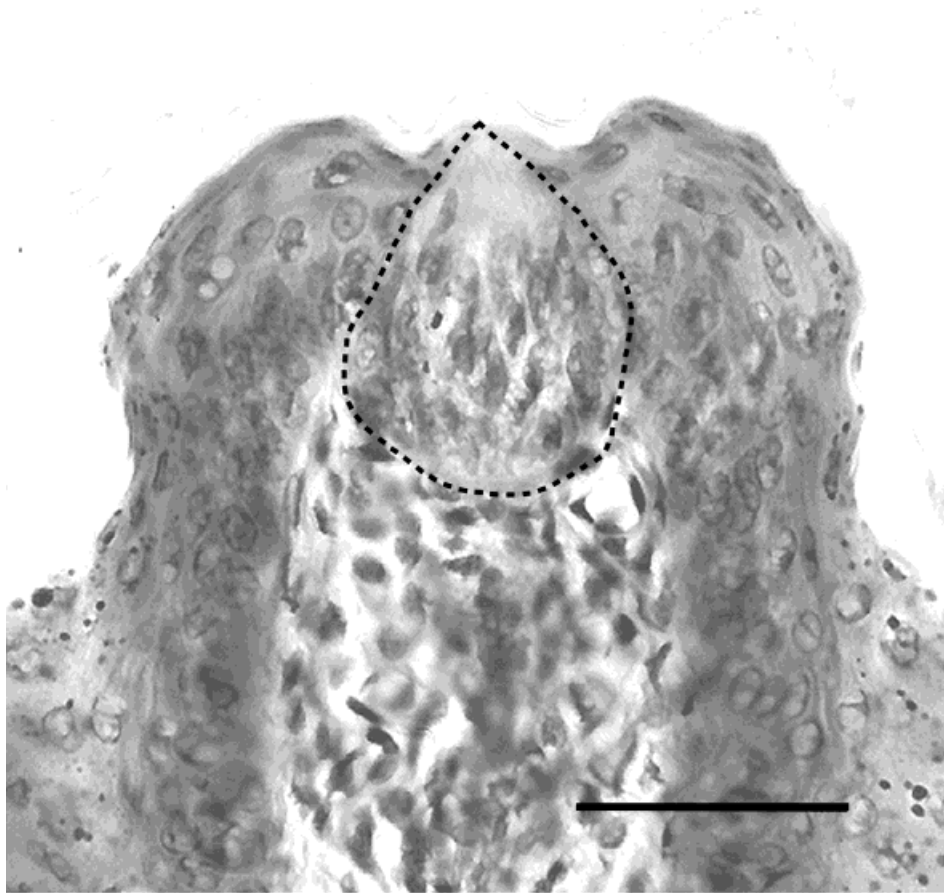
Fig. 2.   Photomicrograph of a coronal section through fungiform papilla in a 40-day-old rat stained with hemotoxylin and eosin. The taste bud is outlined with dashes to illustrate how the borders were defined for the area measurements. The image was captured digitally through the microscope and then sharpened with Adobe Photoshop. Scale bar = 50 µm.

To measure taste bud areas, the perimeter of the taste bud was outlined in each section that contained a portion of the taste bud (Fig. 2), and the corresponding area was computed by an Olympus Cue-2 image analysis system. To achieve consistency in measurement with other reports, the borders were drawn such that peripheral cells of the taste bud were included in the measurement (Whitehead et al., 1985). 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. In addition to the labeled taste buds, a sample of 50 unlabeled taste buds for each group was stained with hematoxylin and eosin and measured to determine if labeled taste buds were representative of the total population of taste buds (Fig. 2). In addition, analysis of a larger number of taste buds allowed a more reliable examination of changing taste bud size during development.

Statistics

Volumes of labeled taste buds were compared to the volumes of an additional 50 taste buds measured from the same region by using t-tests. Analysis of variance (ANOVA) was used to compare mean taste bud size and mean number of ganglion cells across groups. Following a significant ANOVA, multiple comparisons were made by using the Bonferroni t-test. 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.

RESULTS
Labeled taste buds are representative
of the population

Mean taste bud volume of the 13 fluorescently labeled mid-region taste buds in adult rats were not significantly different from the mean volume of the 50 additional taste

buds measured from this same tongue region ($P = .5$). Likewise, there were no significant differences in taste bud volume between the labeled taste buds and the 50 unlabeled taste buds measured from the mid-region of the tongue for any of the four developmental groups (10 days old, $P = .8$; 20 days old, $P = .4$; 30 days old, $P = .5$; 40 days old, $P = .06$). Thus, the taste bud volumes for the labeled mid-region taste buds were representative of mid-region taste bud volumes. There also was no difference in taste bud volumes between the fluorescently labeled tip-region taste buds and the additional 50 taste bud volumes measured from the same region in either adult ($P = .08$) or 10-day-old rats ($P = .06$). However, in 20-day-old rats, the labeled taste buds were significantly smaller than the additional 50 taste buds measured from the same region ($P = .03$). Therefore, labeled taste buds on the tongue tip of 20-day-old rats may not accurately represent the taste bud size of the population.

Numbers of innervating neurons in adults

To determine if the number of ganglion cells innervating single taste buds varied with papilla location, labeled fungiform taste buds were analyzed by region (tip- and mid-region; Fig. 3). Mid-region taste buds in adults were defined as those located between 2.5 and 7.5 mm rostral to the intermolar eminence. Labeled taste buds on the tongue tip were defined as those located between 9.5 and 13 mm rostral to the intermolar eminence. The rostralmost 2 mm of tongue tip was not studied because of difficulties in labeling taste buds due to the curvature of the tongue. Because fungiform papillae density is greater in the tip region compared to the mid region of the tongue (Miller, 1976), labeling both of these regions provided an opportunity to examine the relationship between numbers of innervating ganglion cells and taste bud density. To examine regional differences in innervation further, taste buds located laterally more than 1.2 mm from the midline were defined as lateral taste buds and those located within 1.2 mm of the midline were defined as medial taste buds.

There was substantial variation in the number of innervating geniculate ganglion cells that innervated single taste buds in both the mid-region and tip-region of the tongue (Fig. 3). Individual fungiform taste buds in the mid-region of an adult rat tongue were innervated by geniculate ganglion cells ranging in number from 3 to 14. Taste buds on the tongue tip were innervated by three to 11 ganglion cells. Although it appears that slightly fewer ganglion cells innervated fungiform taste buds of the tongue tip (7.1 ± 0.9) compared to taste buds of the tongue midregion (9.0 ± 1.1), this difference was not statistically reliable ($P = .09$). Therefore, the difference in taste bud density between the tip- and mid-regions of the tongue probably is not related to the number of innervating neurons. However, it appears that the number of ganglion cells innervating fungiform taste buds may depend on the taste bud's medial–lateral location on the tongue surface. Lateral taste buds were innervated by significantly fewer mean ganglion cells (5.4 ± 0.6) than medial taste buds (10.2 ± 0.8; t = 4.67, d.f. = 21, $P = .0001$) when examined across both tip and midregion. Therefore, although there is no difference of innervation along the rostral to caudal extent of the tongue surface, the amount of innervation per taste bud decreases along the medial to lateral extent of the tongue surface.
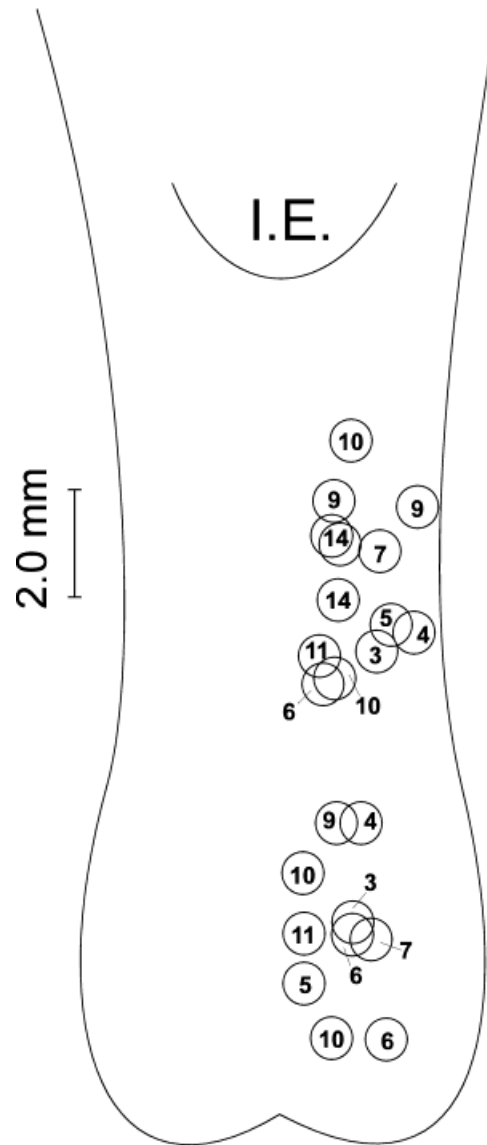

Fig. 3. Locations of the fungiform papillae (circles) that were labeled in adult rats. The location of each papilla was determined by measuring its distance from the intermolar eminence (I.E.) and from the midline. The numbers inside the circles represent the number of labeled ganglion cells that innervated the taste bud in the respective papilla location.

Adult taste bud volumes

Taste bud volumes on the tip-region of the tongue in adult rats (4.74×10^4 μm^3 $\pm 0.46 \times 10^4$) were significantly smaller than taste bud volumes on the tongue mid-region (7.35×10^4 μm^3 $\pm 0.75 \times 10^4$; t = 2.75, d.f. = 21, $P = .006$). In addition, lateral taste buds (4.62×10^4 μm^3 $\pm 0.31 \times 10^4$) were significantly smaller than medial taste buds when examined across both tongue tip and mid-region (7.42×10^4 μm^3 $\pm .77 \times 10^4$; t = 2.97, d.f. = 21, $P = .004$). Much more striking than these regional differences in average taste bud size is the variation in taste bud size within a tongue region. For example, the 50 taste buds measured on the mid-region of the tongue ranged in volume from 2.89×10^4 μm^3 to 13.45×10^4 μm^3.
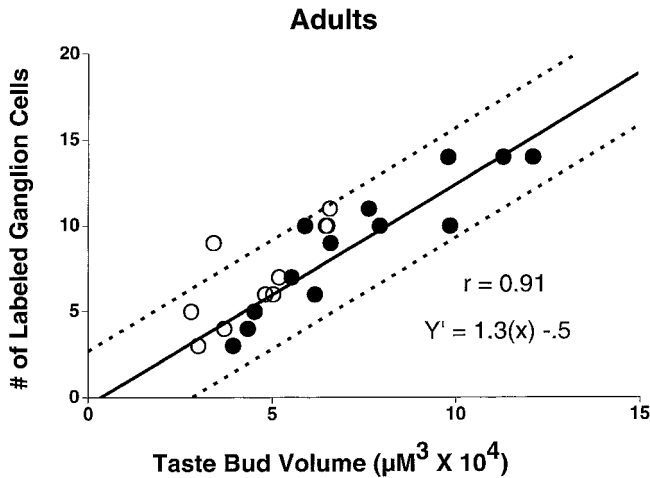
Fig. 4. The number of labeled geniculate ganglion cells plotted against taste bud volume for taste buds sampled from the mid-region (solid dots) and the tip (open dots) of an adult tongue. The regression line for the mid-region data is shown as a solid line, and the correlation coefficient and regression equation are shown in the lower right portion of the figure. The 95% confidence intervals for the mid-region are shown as dotted lines on either side of the regression line.
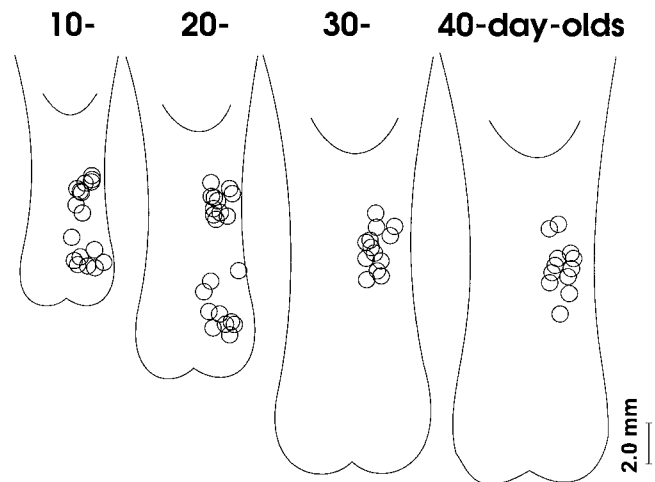

Fig. 5. Locations of the fungiform papillae (circles) that were labeled in developing rats. The lengths of the tongues are representative of measured tongue lengths of rats at the indicated ages. Widths of the tongues were not measured, but were assumed to be proportional to the changes in tongue length.

Interestingly, the largest taste bud was 4.65 times larger than the smallest, and the most innervated taste bud was innervated by 4.66 times as many ganglion cells as the least innervated taste bud. Thus, it appears that there is as much variation among individual taste buds in size as there is in number of innervating ganglion cells.

Relationship between taste bud size and amount of innervation in adults

As the range of taste bud volumes appears to be proportional to the range in the number of ganglion cells that innervate single taste buds, it is possible that these two variables may be more precisely related. Indeed, in normal adult rats, the number of geniculate ganglion neurons that innervated fungiform taste buds in the tongue midregion was positively correlated with the corresponding taste bud size (r = 0.91, P = .0003; Fig. 4). That is, taste bud size explained 84% of the observed variation in ganglion cell innervation of the tongue mid-region. Simply stated, the larger the taste bud, the more geniculate ganglion cells that innervate it. Similarly, the number of ganglion cells that innervated taste buds on the tongue tip was also predicted by its volume (r = 0.78, P = .007).

Numbers of innervating neurons in developing rats

The labeled taste buds of the tongue mid-, tip-, medial, and lateral regions of the tongue in all developmental groups were located in the same relative regions as in adult rats (Fig. 5). To determine if the amount of innervation for single taste buds change during development, the average number of ganglion cells innervating individual taste buds was compared among groups. The mean number of ganglion cells innervating fungiform taste buds in the mid-region of the rat tongue from postnatal day 10 to postnatal day 40 did not differ (F[4,53] = 0.32). Thus, there was no change in the average number of ganglion cells that innervated individual fungiform taste buds in

the tongue midregion after postnatal day 10. However, there was a significant difference in the number of ganglion cells that innervated taste buds of the tongue tip during development (F[2,24] = 3.46; P = 0.04). Significantly, fewer mean ganglion cells innervated fungiform taste buds on the tongue-tip of 10-day-old rats (5.1 ± 0.8) compared with those on the tongue tip of 20-day-old rats (9.0 ± 1.2; t = 2.94, d.f. = 15, P = .005). Thus, it appears that taste buds on the tongue tip are not fully innervated by postnatal day 10.

As in adult rats, there was substantial variation among individual taste buds in the number of innervating ganglion cells for all postnatal ages examined. For example, taste buds in 10-day-old rats were innervated by three to 14 ganglion cells. To determine if the medial to lateral difference in taste bud innervation noted in adults was present in young rats, medial and lateral taste buds were compared in 10-day-old rats. As in adults, taste buds near the midline were innervated with significantly more mean ganglion cells (8.1 ± 1.4) in 10-day-old rats than those located more laterally (5.1 ± 0.61; t = 1.9, d.f. = 15, P = .04).

Taste bud volume during development

Although taste buds in 10-day-old and adult rats are innervated by similar mean numbers of ganglion cells, the taste buds of 10-day-old rats are smaller than those of adults (Fig. 6). In fact, there is a progressive increase in taste bud size throughout postnatal development (F[4,256] = 60.3, P = .0001), with adult sizes seen between 30 and 40 days. Taste buds in 30-day-old rats had significantly smaller volumes than adult taste buds (t = 2.78, d.f. = 98, P = .003), whereas taste bud volumes in 40-day-old rats did not differ from those in adults (P = .16; Fig. 6).

In contrast to adults, the volumes of taste buds near the midline in 10- and 20-day-old rats (mean ± SEM; 2.19 × 10^4 μm³ ± .31 × 10^4 μm³; 6.13 × 10^4 μm³ ± 1.21 × 10^4 μm³, respectively) were not significantly different from those more laterally located (2.13 × 10^4 μm³ ± .36 × 10^4 μm³; P = .89; 4.52 × 10^4 μm³ ± .55 × 10^4 μm³, respectively; P = .18). How-
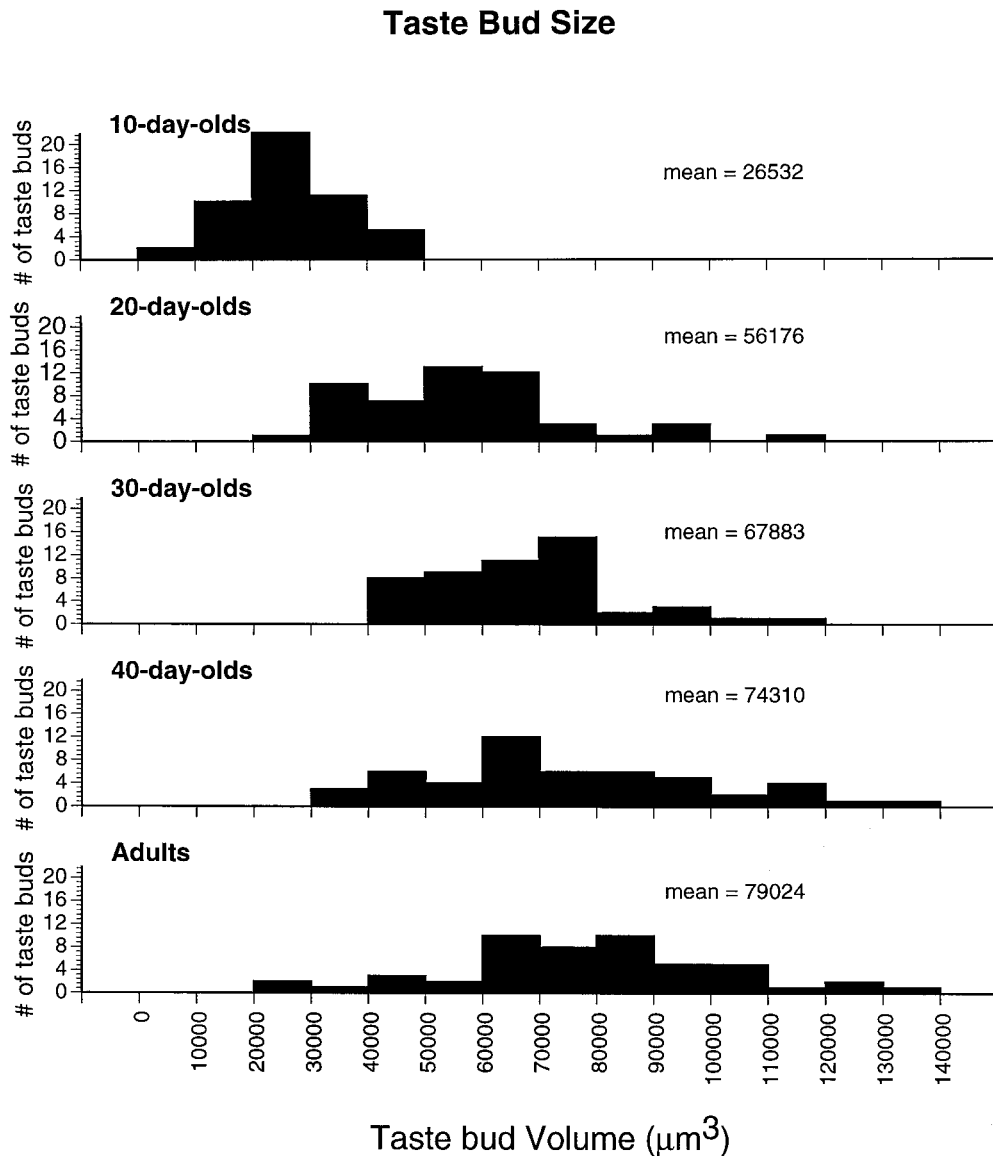
Taste Bud Size

Fig. 6. Histograms showing the number of taste buds of different volumes as a function of age. Taste buds increased in both mean volume and the range of observed volumes as age increased until postnatal day 40.

ever, by postnatal day 30, taste buds near the midline (7.38×10^4 µm³ ± $.52 \times 10^4$ µm³) were significantly larger than those more laterally located (4.56×10^4 µm³ ± $.55 \times 10^4$ µm³; $P = .0076$). Thus, the medial to lateral difference found in the number of innervating ganglion cells, as noted in the previous section, precedes the medial to lateral difference in taste bud volume observed during development.

Relationship between taste bud size and innervation during development

In adult rats, the number of innervating ganglion cells was predicted by the volume of the taste bud. However, there was no correlation between taste bud volume and number of innervating ganglion cells in 10-, 20-, or 30-day-old rats ($P > .05$; Fig. 7). In fact, when data from 10-, 20-, and 30-day-old rats were replotted on the adult function, it

was evident that taste bud sizes were smaller than predicted by the number of ganglion cells that innervated them. It appeared that taste buds in young rats were smaller and more uniform in size than those in adults, although the number of innervating ganglion cells in young rats was similar to those in adults. By comparison, 40-day-old rats had a significant correlation between taste bud volume and the number of innervating ganglion cells ($r = 0.80$, $P = .002$) with a function similar to that in adults (Fig. 4). Thus, the relationship between taste bud size and number of innervating ganglion cells developed between postnatal days 30 and 40.

Ganglion topography

Geniculate ganglion reconstructions in adult rats indicated that the ganglion is conical in shape and extends at
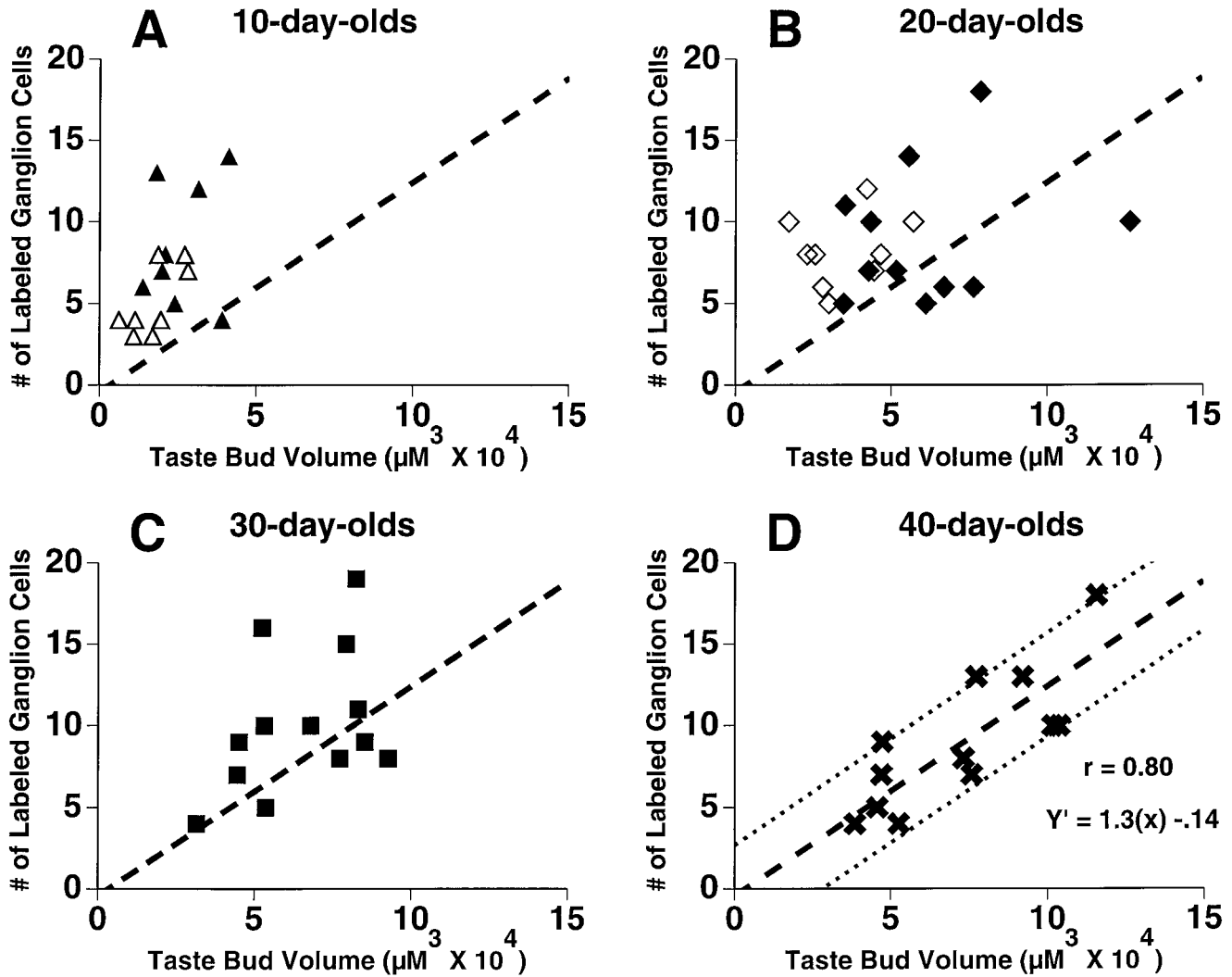
Fig. 7. The number of labeled geniculate ganglion cells plotted against taste bud volumes of taste buds sampled from the tongue mid-region (solid symbols) and tip (open symbols) in 10-day-old (**A**), 20-day-old (**B**), and in 30-day-old rats (**C**). The regression line is replotted from Figure 4 and is shown as a dashed line. There was no relationship between taste bud volume and number of labeled ganglion cells at any of these postnatal ages. In **D**, the number of labeled ganglion cells are plotted against taste bud volumes of taste buds sampled from the tongue mid-region in 40-day-old rats. The regression line is replotted from Figure 4 and is shown as a dashed line. The 95% confidence intervals for the adult mid-region data are shown as dotted lines on either side of the regression line. There was a significant positive correlation between taste bud volume and number of labeled ganglion cells for 40-day-old rats. The data for this age group fall within the 95% confidence intervals of adult taste buds in the tongue mid-region. The correlation coefficient and equation for the 40-day-old regression line are shown in the lower right-hand corner.

its longest axis, which is approximately 1,200 µm along the greater superficial petrosal nerve (GSP). The geniculate ganglion is approximately 500 µm in diameter at its widest point, which is located at its junction with the VIIth nerve.

When individual taste buds were labeled with a fluorescent tracer, the locations and distribution of labeled cells showed as much variation as the number of cells labeled. For example, a taste bud labeled in the adult tongue mid-region with Fluoro-Gold resulted in 14 labeled geniculate ganglion cells, all of which were located near the VII nerve and medial to the nerve core (fibers from the GSP and CT located in the center of the ganglion; Fig. 8A). However, another adult taste bud labeled with Fluoro-Gold and also located in the tongue mid-region had seven labeled corresponding ganglion cells that were distributed

throughout the ganglion (Fig. 8B). Within this ganglion, ten labeled ganglion cells were observed resulting from a True Blue–labeled taste bud located at the tongue tip and were also seen distributed throughout the ganglion. In general, labeled cells that resulted from the injection of a single taste bud were observed to be scattered throughout the geniculate ganglion rather than clumped together in close proximity.

All of the labeled ganglion cells in adult rats were pooled into a 2-D template for the purpose of comparing the locations of the labeled ganglion cells among animals (Fig. 9A,B). Labeled ganglion cells were found in all parts of the geniculate ganglion. From the composite figure, it can be seen that most labeled ganglion cells were near the junction of the VIIth nerve and medial to the nerve fiber
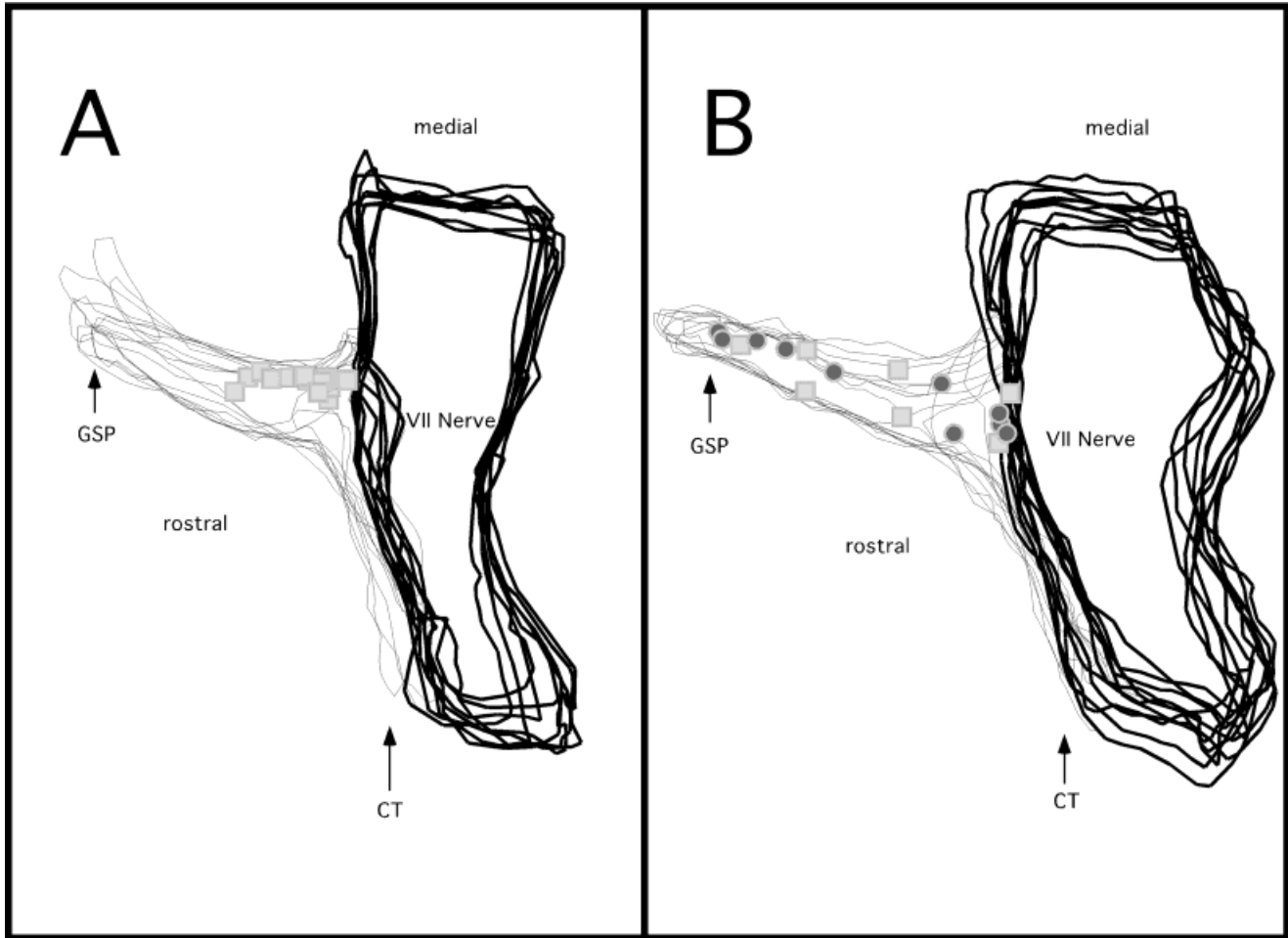
Fig. 8. Computerized 3-D reconstructions of two adult geniculate ganglia. The locations of geniculate ganglion cells that innervated a single fungiform taste bud located in the tongue mid-region (squares) are shown for two different rats (**A,B**). In A, the 14 ganglion cells that innervated a single taste bud were relatively close together, whereas in B the seven ganglion cells that innervated a single taste bud were distributed throughout the ganglion. In B, the ten ganglion cells that innervated a single taste bud near the tongue tip (circles) in the same animal were also distributed throughout the ganglion. The facial nerve (VIIth nerve) and the locations where the greater superficial petrosal (GSP) and chorda tympani (CT) nerves enter the ganglion are also indicated.

core of the ganglion (Fig. 9A). Moreover, there was no observable topography based on the location of the labeled taste buds. Ganglion cells that innervated either the tip-region or the mid-region of the tongue were distributed equally throughout the ganglion (Fig. 9A,B). Similarly, ganglion cells that innervated taste buds near the midline had approximately the same distribution as those that innervated taste buds more laterally. In addition, the distributions of ganglion cells innervating the mid- and tip-regions and medial and lateral regions in 10-day-old rats were similar to those in adults. That is, there was no apparent topography based on the location of the labeled taste buds in 10-day-old rats.

DISCUSSION

There is substantial variation in both the size of individual taste buds and number of geniculate ganglion cells that innervate them. However, these variations can be accounted for by relating taste bud size to geniculate ganglion cell number. In adult rats, the number of gan- glion cells that innervate a fungiform taste bud is directly related to the size of the taste bud. The relationship between taste bud size and number of innervating ganglion cells does not emerge until taste buds reach their adult size, which is between postnatal days 30 and 40. Therefore, this relationship appears to require a relatively long postnatal developmental period.

Development of taste buds

Although information exists on early postnatal development of fungiform taste buds (Farbman, 1965; Mbiene and Farbman, 1993; Mistretta, 1972), little is known about the morphological maturation of taste buds after postnatal day 10. The current results show that taste buds gradually increase in size after postnatal day 10, and that rat fungiform taste buds reach adult volumes between postnatal days 30 and 40. However, it is not known whether such an increase in taste bud volumes results from increased numbers and/or size of taste bud cells. Future experiments involving analyses of morphological and cell cycle charac-
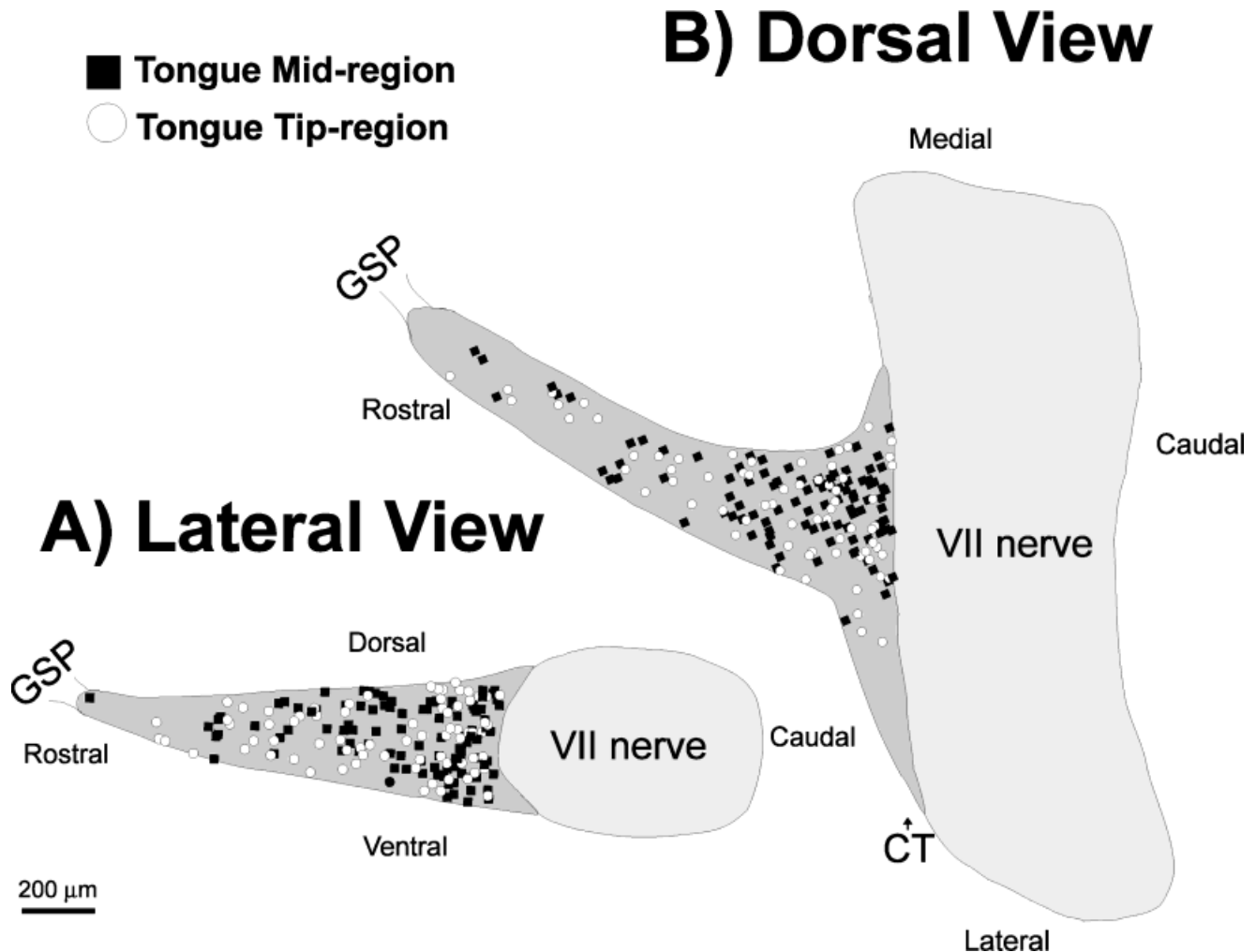
B) Dorsal View

■ Tongue Mid-region
○ Tongue Tip-region

Medial

GSP

Rostral

Caudal

A) Lateral View

VII nerve

Dorsal

GSP

Rostral

VII nerve

Caudal

Ventral

CT

200 μm

Lateral

Fig. 9. Composite illustrations from 18 different computer reconstructions of adult geniculate ganglia viewed from the lateral side (**A**) or the dorsal side (**B**). Both the geniculate ganglion cells that innervated the taste buds at the tongue tip (open circles) and those that innervated taste buds of the tongue mid-region (solid squares) were dispersed throughout the ganglion. The locations of the greater superficial petrosal (GSP), the facial nerve (VIIth nerve), and the chorda tympani (CT) nerves are indicated.

teristics of taste bud cells will allow a determination of what underlies the changes in volume with age.

Development of innervation patterns

Although the relationship between taste bud size and number of innervating ganglion cells did not mature until postnatal day 40, there was no change in the mean number of ganglion cells labeled with age from 10 days postnatal through adulthood. This suggests that the development of the relationship between taste bud size and number of innervating neurons must not be due to a substantial increase or elimination of chorda tympani neuron branches. Such a conclusion assumes that no changes occur in the total number of geniculate ganglion cells after postnatal day 10. This assumption is reasonable, because geniculate ganglion cell proliferation ends around E15 (birth = E21; Altman and Bayer, 1982), and developmental cell death in the geniculate ganglion could be expected to peak when the tongue epithelium is first innervated (Ganchrow and

Ganchrow, 1989; Vogel and Davies, 1991), between E15 and E17 in the rat (Farbman and Mbiene, 1991).

The development of rat taste bud innervation appears to differ considerably from that observed in sheep, the only species in which receptive fields of fungiform taste buds have been examined. During sheep development, there is an overall increase followed by a decrease in the number of papillae innervated by individual chorda tympani neurons (Nagai et al., 1988). If the average number of taste buds innervated by each chorda tympani neuron increases, then the number of geniculate ganglion cells innervating single taste buds should increase also. However, in the rat, there is no increase or decrease in the average number of geniculate ganglion cells innervating individual papillae throughout development. Therefore, either the number of innervating ganglion cells do not change during development, or adjustments in the innervation of rat fungiform taste buds must consist of equal numbers of additions and retractions of chorda tympani fibers at each age.

Developmental mechanisms: Potential role of neurotrophic factors

From examination of the timing that occurs in the maturation of taste bud size and numbers of innervating neurons, it appears that taste bud size adjusts to the numbers of neurons that innervate them early postnatally. For example, differences in taste bud innervation between medial and lateral regions of the tongue develop before postnatal day 10, yet taste bud size differences do not occur until later. Thus, regional differences in ganglion cell number precede regional differences in taste bud size. An explanation for such an outcome is that the "program" of taste bud size is established by the early numbers of ganglion cells that innervate them. This would imply, therefore, that there is coordination of taste bud size with numbers of innervating neurons by way of signaling molecules from innervating neurons to the taste bud. The more innervating neurons, the more of the factor(s) that regulate taste bud size. Although the specific factors have not been identified, it is clear that signals originating in taste neurons maintain the integrity of taste buds. This is most clearly demonstrated by the loss or significant morphological change of taste buds following sectioning of the taste nerve that innervates them (Cheal and Oakley, 1977; Farbman, 1980; Guth, 1957; Oakley et al., 1993; Whitehead et al., 1987). Moreover, blocking axoplasmic transport in a taste nerve with colchicine results in a loss of normal-appearing taste buds (Sloan et al., 1983). Thus, factors released from taste nerves similar to (or the same as) those that maintain taste bud morphology may also regulate the size of the taste bud during development.

An alternative to the hypothesis that the number of innervating neurons with their associated trophic factors regulate taste bud size (i.e., the number of fibers determines size) is that early-developing taste buds may produce differential amounts of neurotrophins that recruit a corresponding number of geniculate ganglion cells. This neurotrophic-dependent process would be followed by the growth of taste buds to their predetermined size. Neurotrophins produced by target cells, such as developing taste cells, serve to promote survival of innervating sensory neurons by way of competition (Davies, 1994; Klein, 1994). Neurons that are exposed to an appropriate amount of neurotrophic factors survive, whereas neurons that are not die. In the taste system, there is emerging evidence that brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) are neurotrophins involved in survival of geniculate ganglion cells (Jones et al., 1994; Liu et al., 1995). In addition, BDNF is expressed at the apex of developing fungiform papillae (Nosrat and Olson, 1995) and is important for normal taste bud and papillae development (Nosrat et al., 1997; Zhang et al., 1997). Therefore, it is possible that abundant amounts of BDNF and or NT-4 produced by developing taste buds would ultimately be innervated by more ganglion cells than taste buds that produce relatively small amounts of the neurotrophins.

Finally, some combination of these processes could occur and account for the present findings. That is, differential amounts of neurotrophins in early developing taste buds may recruit proportional numbers of neurons, even though the taste buds have not achieved their mature size. The differential numbers of neurons innervating individual taste buds may, in turn, determine the final size of the taste buds. In short, the types of developmental relationships shown here between taste bud size and number of innervating neurons make the gustatory system an ideal system to explore potential mechanisms related to neurotrophins, trophic influences from the nerve onto target cells, and the coordination of nerve/target morphologies.

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