

Early Prenatal Critical Period for Chorda Tympani Nerve Terminal Field Development

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ABSTRACT

In order to determine whether the developing central gustatory system responds to dietary manipulation during restricted developmental periods, terminal fields of the chorda tympani nerve within the nucleus of the solitary tract were investigated via anterograde transport of horseradish peroxidase in control rats and in rats in which a low sodium diet was systematically fed during specific periods of development. Rats fed a low sodium diet (0.03% NaCl) from embryonic day 3 (E3) to day E12 and then fed a sodium replete diet to at least 60 days postnatal exhibited enlarged and irregularly shaped chorda tympani terminal fields. Specifically, the dorsal zone of the field was the smallest in controls, whereas it was the largest in restricted rats, occupying more territory within the nucleus. This alteration in the terminal field was apparent in all groups of rats fed the low-NaCl diet beginning at E3, and continuing beyond E12. In contrast, no effects of the dietary manipulation on the developing chorda tympani field was evident when it occurred from E3 to day E9, from E0 to day E9 or when it occurred at adulthood only. Therefore, only 9 days of maternal exposure to a sodium-restricted diet is required for a permanent expansion of the chorda tympani terminal field in the offspring. Moreover, a brief period from E9 to E12 must be included within the 9-day dietary restriction to yield the expanded field. Since this period is before taste receptors appear on the tongue, it is likely that nonactivity-dependent factors determine the formation of the chorda tympani terminal field during later development. *J. Comp. Neurol.* 378:254-264, 1997. © 1997 Wiley-Liss, Inc.

Indexing terms: taste; development; horseradish peroxidase; sensory afferents; nucleus of the solitary tract

For many sensory systems, it is apparent that normal functional and morphological sensory maturation depends upon proper stimulation during well-defined periods of development. During these developmental periods of susceptibility, the neural apparatus can be modified easily (Aslin, 1981; Mistretta and Bradley, 1978). An extensive literature exists which examines the consequences of sensory restriction during development and the reversal of such effects in the olfactory, auditory, visual, and somatosensory systems (e.g., Brunjes and Frazier, 1986; Deitch and Rubel, 1984; Hubel and Weisel, 1970; Renehan et al., 1989). These studies have been important not only in elucidating the capacity of the respective sensory system to respond to abnormal environmental conditions, but also to understand the processes necessary for normal development. That is, the goal of much of this work is to learn about normal development by perturbing the system.

In comparison to other sensory systems, little emphasis has been placed on clarifying the role of sensory experience in the developing gustatory system. One strategy to under-

stand such a role has been to alter dietary components during early periods of development. This experimental procedure has yielded many important insights about the effects of dietary manipulation, as well as providing clues about normal development. For example, restriction of maternal dietary sodium, beginning on or before embryonic (E) day 8 and continuing throughout development, results in dramatically reduced neurophysiological responses selectively to sodium salts in the chorda tympani nerve (Hill et al., 1986; Hill, 1987; Hill and Przekop, 1988; Ye et al., 1993b). Since sodium salts are the "best stimuli" for the adult rat chorda tympani nerve, early sodium restriction results in a dramatic loss of stimulus-elicited

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afferent activity transmitted to the brain. This result suggests that normal taste response development may be dependent on proper amounts of gustatory stimulation with sodium salts.

Interestingly, sodium taste transduction is only affected by sodium restriction when the dietary manipulation begins on or before E8 (Hill and Przekop, 1988). This dietary-induced effect is especially intriguing when placed within the time course of normal gustatory development. Taste buds first appear in rat fungiform papillae at birth (E21; Farbman, 1965; Mistretta, 1972), and amiloride-sensitive sodium taste responses begin at postnatal day 11 (Hill and Bour, 1985). Thus, the end of the sensitive period is 13 days before the appearance of the first taste buds and 24 days before expression of functional amiloride-sensitive sodium channels, the transduction element primarily involved in rodent sodium taste (Avenet and Lindemann, 1988; Brand et al., 1985; DeSimone and Ferrell, 1985; Formaker and Hill, 1988; Ye et al., 1993a) and the element affected most by early dietary sodium restriction (Hill, 1987; Ye et al., 1993b). Even though the onset of this period of susceptibility must occur very early in fetal development, there is no clear offset of the period. Namely, normal peripheral gustatory system function in early sodium-restricted rats can be restored at any time during development and even in adulthood (Hill, 1987; Stewart and Hill, 1995). These findings suggest that events very early in development can alter the normal development of peripheral sensory function that emerges as much as 3 weeks later.

Given the striking effects of dietary sodium restriction on the functional development of the peripheral gustatory system, the altered afferent input may have profound effects on central gustatory development. Indeed, dietary sodium restriction during pre- and postnatal development produces both abnormally distributed and irregularly shaped chorda tympani terminal fields in the nucleus of the solitary tract (NST; King and Hill, 1991). It is critical to note that the central anatomical effects are limited to the chorda tympani field; the size and topography of the projections from another gustatory nerve, the lingual-tonsillar branch of the glossopharyngeal, are unaffected by dietary manipulations (King and Hill, 1991). Furthermore, the central morphological consequences are not reversible. Institution of a sodium-replete diet in developmentally sodium-restricted rats exaggerates the effect instead of eliminating it (King and Hill, 1991).

While it is clear that sodium restriction begun early in development and continued throughout pre- and postnatal periods has widespread, permanent effects on central gustatory development, it is not clear what defines the period of susceptibility for the dietary-induced effects. Identification of this period may be useful in understanding the events that occur normally in directing the development of the chorda tympani terminal field. For example, similar to peripheral functional development, events very early in development may have a major influence in shaping later morphological development of central gustatory structures. Alternatively, later events that coincide with onset of taste function may be especially important, suggesting that primary afferent activity shapes central morphological features. Therefore, to begin elucidating the period(s) that determines central gustatory development, terminal fields of the chorda tympani nerve in the NST were studied after systematically manipulating the period and timing of dietary sodium restriction.

MATERIALS AND METHODS

Animals

Terminal fields of the chorda tympani nerve in rats with different developmental histories of dietary sodium restriction were studied with anterograde transport of horseradish peroxidase (HRP). Sodium restriction was accomplished by feeding pregnant Sprague-Dawley rats a sodium-deficient diet (0.03% NaCl) from embryonic (E) day 3 through E9, E12, E15, the day of birth, or postnatal (PN) days 5 or 28 of the offspring. Upon completion of the restricted dietary schedule, animals were fed the standard, sodium-replete diet (1.0% NaCl), and were maintained on that diet until they were at least 60 days of age. Control groups consisted of rats maintained on the sodium-replete diet throughout development, and of rats fed the sodium-restricted diet for at least 50 days, but only during adulthood. The period of sodium restriction in adult-restricted controls exceeded that of all the developmentally restricted rats.

Surgical preparations

Rats were anesthetized with sodium Brevital (60 mg/kg, i.p.). Access to the left chorda tympani nerve was accomplished by exposing the nerve through the neck at the point of bifurcation with the lingual branch of the trigeminal nerve. The chorda tympani nerve was transected, the central end placed on a small piece of parafilm, and treated briefly with dimethyl sulfoxide. HRP crystals (Type IV; Boehringer Mannheim) were then placed on the nerve for 20–30 minutes before closing wounds with surgical silk.

The optimal time for transport of HRP to the NST was determined in pilot work to be 24 hours (also see King and Hill, 1991). Thus, approximately 24 hours after labeling the nerve with HRP, animals were sacrificed with an overdose of urethane, perfused through the heart with Krebs solution, and fixed with 2% glutaraldehyde followed by a mixture of 2% paraformaldehyde/2.5% glutaraldehyde. All fixatives were dissolved in 0.1 M phosphate buffer (pH = 7.4). Brainstems were sectioned in the horizontal plane at 50 μ m with a Vibratome to allow visualization of the entire extent of the terminal fields in both the rostral-caudal and medial-lateral planes. Sectioning in the horizontal plane was chosen because of the horizontal orientation of incoming chorda tympani fibers and cellular processes within the NST (Davis, 1988; Lasiter et al., 1989; Whitehead, 1986), and because the largest dietary-induced effects seen earlier in chorda terminal field development occurred in these planes (King and Hill, 1991). Sectioning only in this plane precluded a precise determination of the subnuclei in the NST most affected. Additionally, precise localization of the field within specific subnuclei was prevented because the tissue could not be counterstained with the appropriate array of stains while retaining the fidelity of HRP label. Tissue was processed according to a modified tetramethylbenzidine (TMB) histochemical technique (Mesulam, 1978). In order to maximize visualization of the chorda tympani terminal field, the TMB reaction proceeded for up to 45 minutes. These reactions led to an easily visualized HRP reaction product (see Fig. 1).

Quantification

Terminal fields of the chorda tympani nerve were observed under darkfield microscopy (Fig. 1). Since variabil-

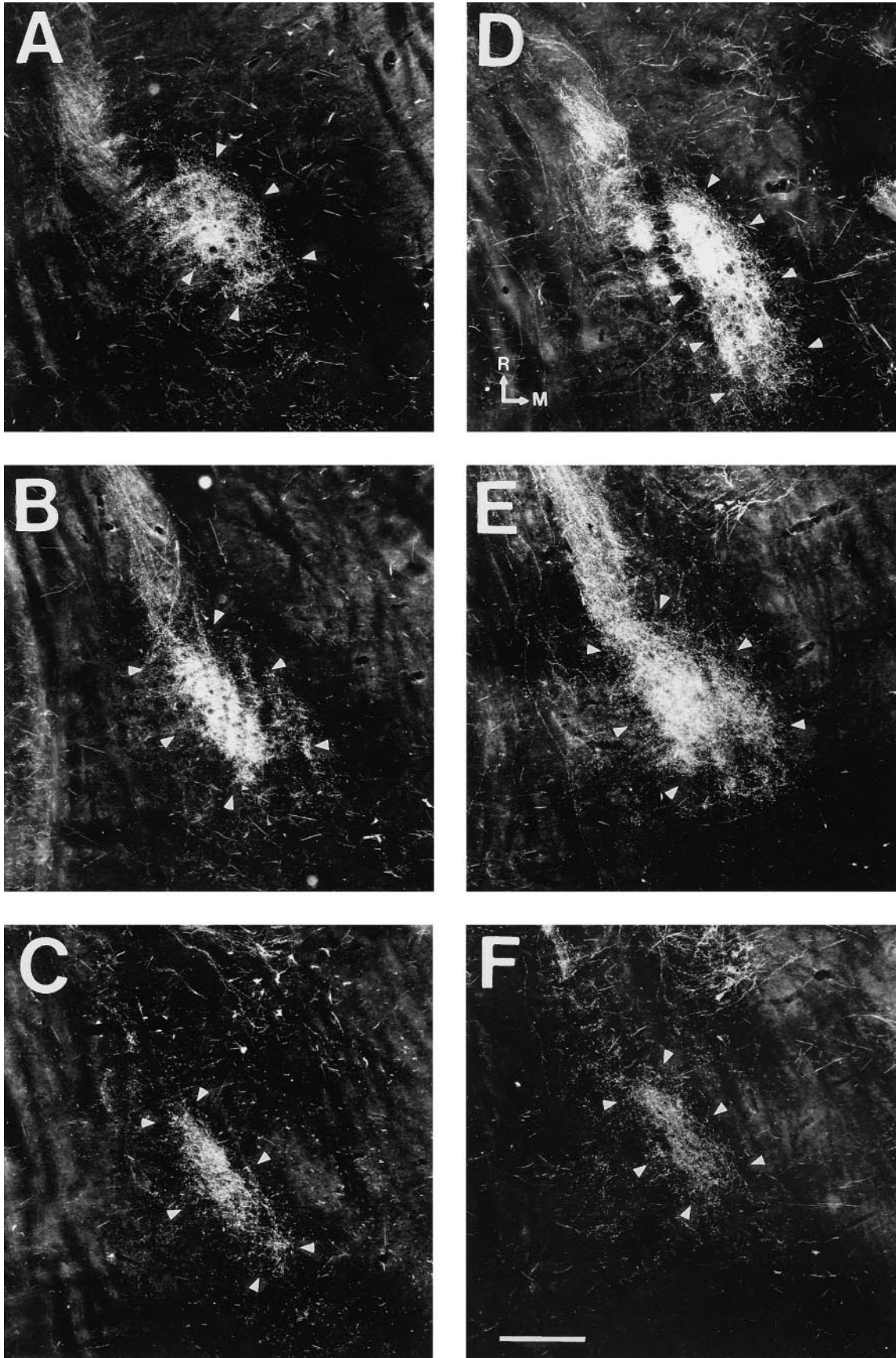


Figure 1

ity in the "intensity" of the HRP labeling was apparent within and between groups, the volume of the label within the NST, rather than the density of the label, was measured. In order to obtain volumes, the perimeter encompassing the anterograde label in each 50- μm section was outlined and the corresponding area was computed by an Olympus Cue-2 image analysis system. Only areas in which fine puncta labeling occurred were measured; this corresponds to terminations of afferents and not labeled fibers (e.g., Lasiter, 1992). The areas from all sections for each animal were summed and multiplied by 50 μm to derive an estimate of the total volume of the chorda tympani terminal fields. These techniques are similar to those used by us and others (Davis and Jang, 1986; King and Hill, 1991; Lasiter et al., 1989). To allow for comparisons with our earlier work (King and Hill, 1991), the terminal field in each rat was divided into three contiguous dorsal-ventral zones, termed here as dorsal, intermediate, and ventral. Each terminal field zone comprised the label found in two consecutive 50- μm sections. Typically, the dorsal zone was distinguished by the presence of the solitary tract at the lateral edge of the nucleus and by the dorsalmost portion of the hypoglossal nucleus. The intermediate zone, which is the next 100 μm of tissue, was just below the level of the solitary tract. The ventral zone of the field was designated as the label in the next 100 μm of tissue. Sections containing the ventral zone of the terminal field typically contained cells of the superior salivatory nucleus.

Additionally, the rostral to caudal and medial to lateral distances were measured for the dorsal, intermediate, and ventral regions of the terminal field. This was accomplished by measuring the longest rostral to caudal distance of the terminal field parallel to the medial edge of the NST, and then measuring the longest distance of the field perpendicular to the rostral-caudal measurement to obtain the largest medial-lateral terminal field distance.

Statistical analysis

The total volume occupied by the chorda tympani terminal field in the NST was analyzed for 53 rats (see Results for n/group). Analyses of variance (ANOVA) were used to determine the effect of the dietary manipulation on the total volume, volumes within each of the three dorsal to ventral regions, and the rostral-caudal and medial-lateral distances within each of the three regions. Following a significant ANOVA, posttests were accomplished with a

one-tailed Dunnett's test for comparisons involving a control mean.

RESULTS

The portion of the NST receiving chorda tympani nerve terminations began approximately 200–250 μm below the dorsal boundary of the NST in each group and extended approximately 300 μm ventrally. This is similar to results reported by King and Hill (1991). Only data from rats that had intense labeling of the incoming fibers and terminal fields throughout this extent were used for subsequent data analyses (see Fig. 1). In all cases, this represented labeling in six contiguous horizontal sections, thereby meeting the same criterion as used by King and Hill (1991). Terminal fields of the chorda tympani nerve were heavily labeled in the following number of rats within each group: controls ($n = 7$), adult sodium-restricted ($n = 6$), sodium-restricted from embryonic days 3 through 9 (E3–E9; $n = 5$), from E3–E12 ($n = 6$), from E3–E15 ($n = 8$), from E3–Birth ($n = 6$), from E3–PN5 ($n = 4$), from E3–PN28 ($n = 6$), and from E0–E9 ($n = 5$).

Total terminal field volume

In control rats, the mean total volume of the chorda tympani terminal field was $21.4 (\pm 1.4) \times 10^6 \mu\text{m}^3$. As reported previously by King and Hill (1991), most of the field was contained in the intermediate zone, and the least in the ventral zone. Measurements from this group were used as the standard to which all comparisons were made (see Statistical Analysis section).

Unexpectedly, the total size of the terminal field is susceptible to dietary sodium restriction during a brief period of early embryonic development. Adult rats fed the sodium-deficient diet via their mothers when they were aged E3 to E9 had terminal fields only 5% larger than controls ($P > 0.10$; Figs. 1 and 2). However, rats placed on the sodium-deficient diet when they were E3 and returned to the sodium-replete diet at E12 had terminal field sizes 43% greater than controls ($F_{(8,44)} = 4.2$, $P = 0.0009$; post-test $P < 0.01$; Figs. 1 and 2). Therefore, extending the duration of sodium restriction by only 3 days during early prenatal development (i.e., from E9 to E12) produced an extensive, permanent alteration in the chorda tympani terminal field. Further increases in the total terminal field were not apparent when restoration of the sodium-replete diet occurred any time after E12 (Fig. 2). For example, sodium restricting mothers from E3 to birth of their offspring resulted in an increase of 9% above that observed in rats born to mothers restricted from E3 to E12 and 52% greater than controls ($P < 0.01$; Fig. 2). Thus, the effect of the dietary-induced effect was established during a 9-day period from E3 to E12.

While an early embryonic, 9-day period appears necessary for the anatomical alteration of the central gustatory system, it is possible that this period of sodium restriction has similar effects when instituted any time during development. That is, it may be that the most important variable in producing the dietary-induced effects relates to the duration of sodium restriction, independent of the animal's age. If so, 9 days of sodium restriction begun at E0 would be expected to produce the enlarged terminal fields similar to those produced by sodium restriction from E3 to E12. However, as seen in Figure 2, terminal field sizes of rats sodium-restricted from E0 to E9 ($n = 5$) were not

Fig. 1. Darkfield photomicrographs comparing the chorda tympani nerve terminal fields (outlined by arrowheads) in 50- μm horizontal sections in a rat whose mother was sodium-restricted from embryonic day (E) 3 to E9 (A–C), and in a rat whose mother was restricted from E3 to E12 (D–F). Fibers of the chorda tympani nerve can be seen coursing from the rostral-lateral portion of the brainstem into the rostral pole of the nucleus of the solitary tract (NST) where they form terminal fields. Note that the dorsal zone (A, D) of the chorda tympani terminal field extends caudally for more than twice the distance in rats whose mothers were sodium-restricted from E3 to E12 (D) compared to rats whose mothers were placed on a normal diet just three days earlier (A). The intermediate zone (B, E) appears slightly larger in the rats whose mothers were restricted 3 days longer (E); however, there is not a reliable difference between the intermediate zone for the two groups depicted. The ventral zone (C, F) of the chorda tympani terminal field is similar between groups. The arrows in D refer to rostral (R) and medial (M). Scale bar = 200 μm .

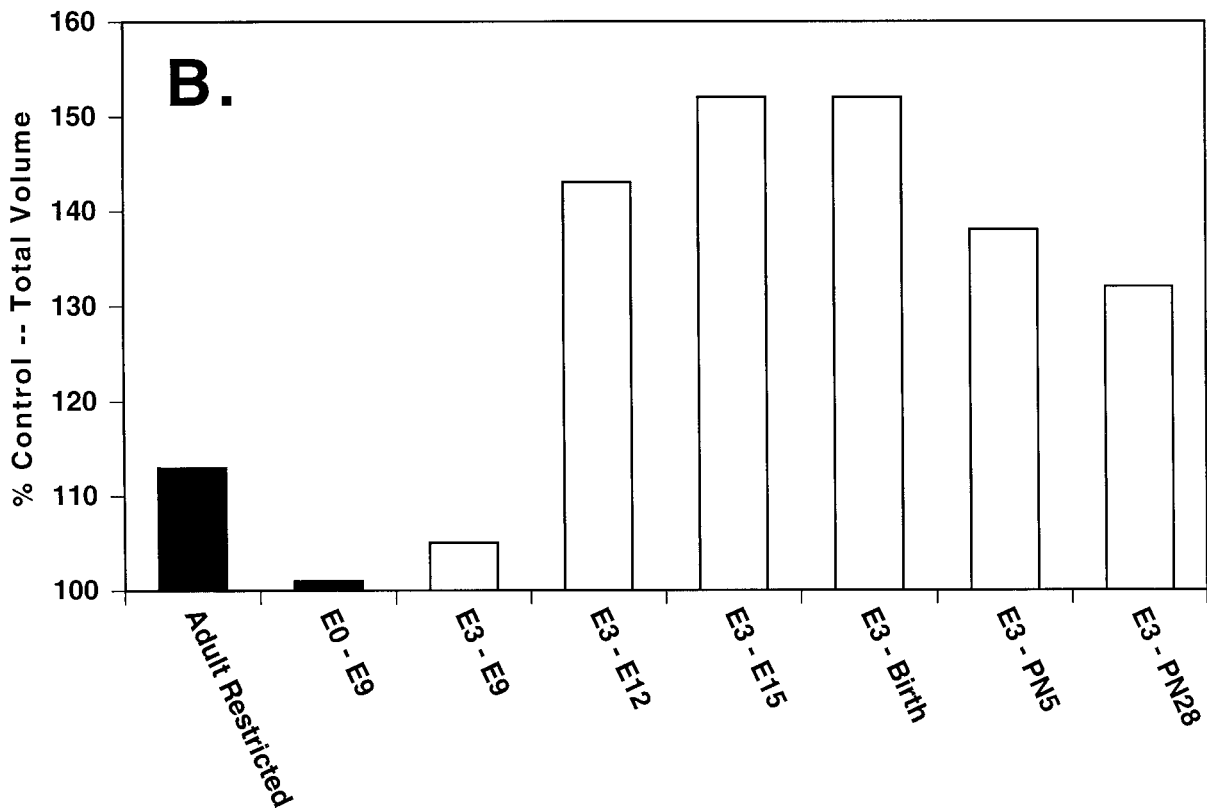
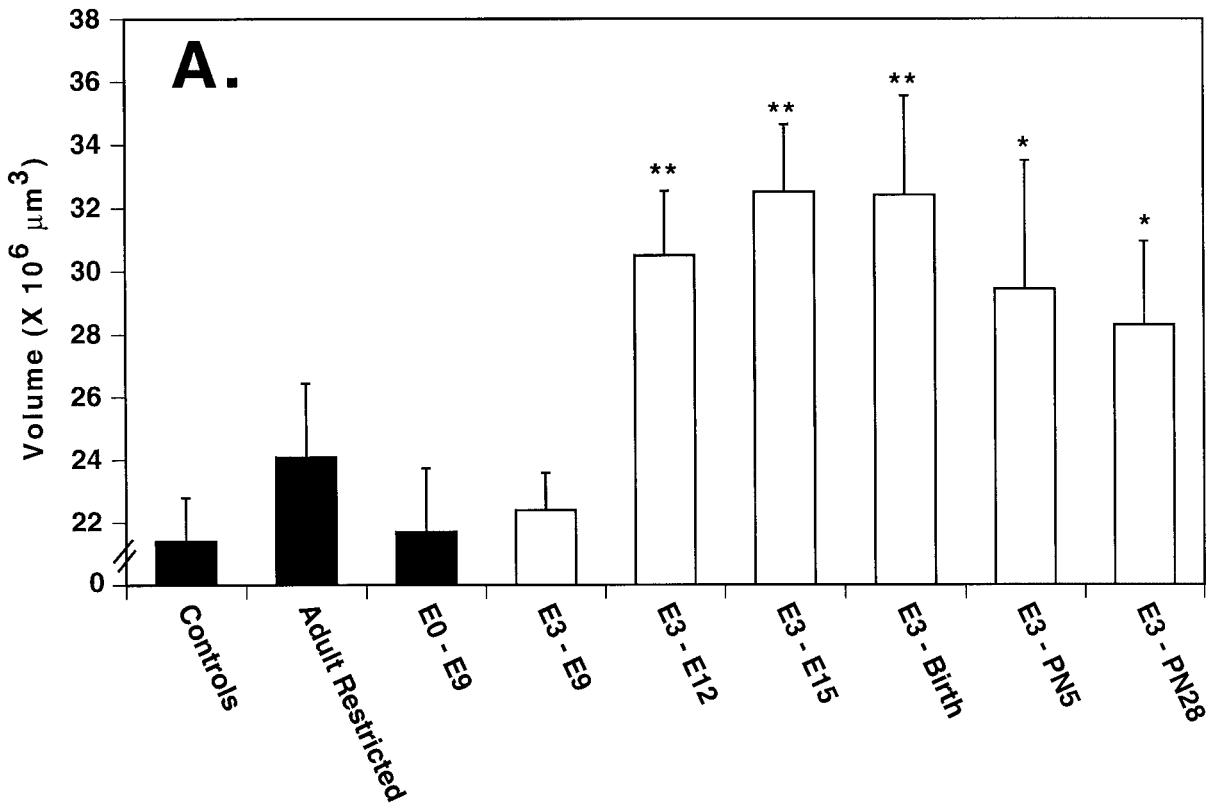


Figure 2

significantly different from controls ($P > 0.10$). Likewise, dietary sodium restriction begun during adulthood had little effect on terminal fields in the NST. Rats placed on a sodium-restricted diet during adulthood and maintained on the diet for a minimum of 50 days had terminal field sizes similar to controls ($P > 0.10$). Thus, from these results, it is clear that the period during gestation coincident with sodium restriction is a more important variable than the duration of dietary restriction.

Terminal field volume within dorsal to ventral zones

Earlier experiments that examined the influence of early dietary sodium restriction on chorda tympani terminal field development demonstrated that not only was the total terminal field volume affected, but that there were dramatic and selective changes in specific zones of the terminal field (King and Hill, 1991). Specifically, the dorsal zone of the terminal field was much greater in restricted rats than in control rats. The effects on intermediate and ventral zones were less pronounced than observed for the differences in the dorsal zone. To examine whether similar changes occur when the dietary restriction is limited to earlier developmental periods, the terminal field volumes were analyzed for the dorsal, intermediate, and ventral zones of the terminal field.

As seen in our earlier study, the dorsal zone of the terminal field is most susceptible to early dietary influences. Compared to the dorsal zone in controls ($7.99 (\pm 0.53) \times 10^6 \mu\text{m}^3$), the terminal field volume in rats that were sodium-restricted from E3 through E12 was approximately 75% greater ($F(8,44) = 4.1, P = 0.0009$; posttest $P < 0.05$; Fig. 3). This trend continued in rats with longer periods of sodium restriction throughout development ($P < 0.05$), with the exception of sodium-restricted rats from E3 to PN28 ($P > 0.05$; Fig. 3). The terminal field in the dorsal zone of adult-restricted rats and rats with sodium restriction from E0 to E9, or from E3 to E9 was no more than 26% above controls ($P > 0.05$). Thus, as noted in analyses of total field volume, the effects of the dietary manipulation initially occurred when rats were fed the sodium-restricted diet from E3 to E12. By comparison, there were no differences between control terminal field volumes and any of the experimental groups for both the intermediate and ventral zones ($P > 0.10$; Fig. 3).

Rostral-caudal and medial-lateral distances

While there are age-dependent changes in total chorda tympani field volumes, most notably in the dorsal zone, it is possible that the shape of the field is relatively unaffected by early dietary manipulations. That is, even though the size of the field is susceptible to early sodium restriction, the shape of the field may not be affected. Thus, there

Fig. 2. **A:** Mean total volume (\pm S.E.M.) of terminal field label in rats whose mothers were placed on the sodium-restricted diet beginning on embryonic day (E) 3 (open bars) and were removed from the diet on E9, E12, E15, birth, postnatal day 5 (PN5), or PN28. Solid bars denote mean total volume (\pm S.E.M.) of terminal field label in control groups that are comprised of rats fed a NaCl-replete diet throughout development (Controls), rats sodium-restricted only at adulthood (Adult Restricted) and rats fed the NaCl-deficient diet from E0 to E9. **Denotes means significantly different from the controls at $P < 0.01$, and *denotes $P < 0.05$. **B:** Data from A expressed as percentage of control total volume.

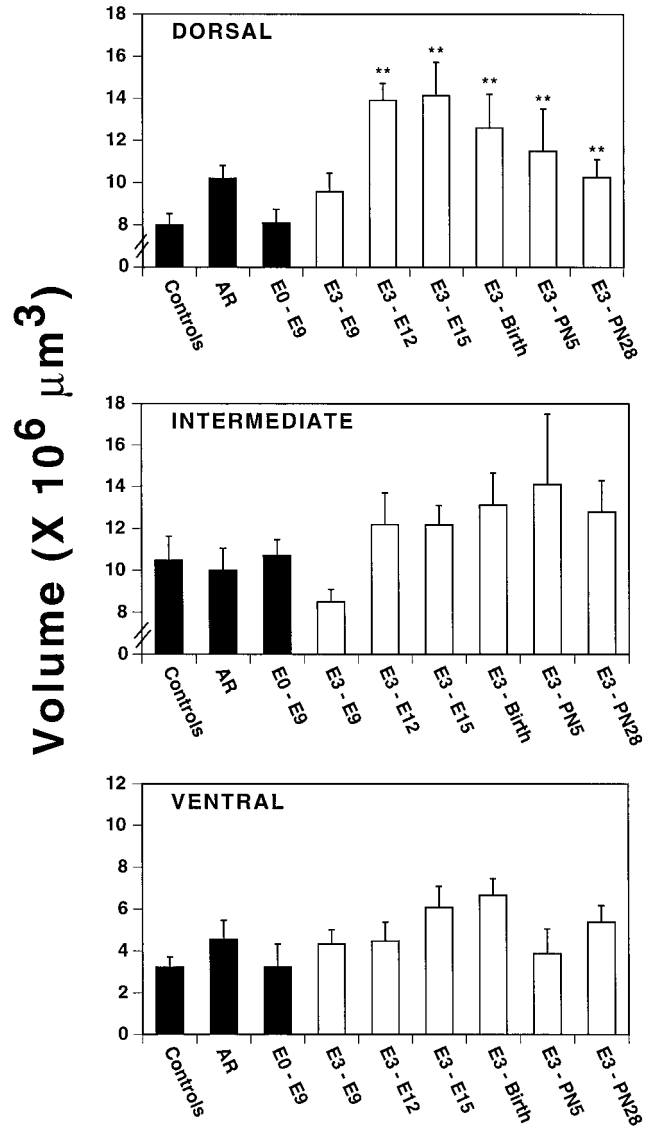


Fig. 3. Mean total volume (\pm S.E.M.) of terminal field label in the dorsal zone (top), intermediate zone (middle), and ventral zone (bottom) in the NST. The groups and significance levels are the same as noted in Figure 2 with the exception that AR denotes adult restricted rats. Note the change in the range of the total volume shown for the ventral zone compared to the other two.

would be a proportional expansion along all axes in rats sodium-restricted from E3 to at least E12. Conversely, the experimentally induced expansion may occur primarily along one dimension and not others.

The number of horizontal sections with terminal field did not differ among groups ($P > 0.10$); therefore, it appears as though the dorsal to ventral distances were not affected by the dietary manipulation. In contrast, however, the rostral to caudal distance was longer in rats that were sodium-restricted from E3 to E12 compared to controls ($557.4 \pm 43.5 \mu\text{m}$; Fig. 3). This increase in rostral to caudal length was specific for the dorsal region of the chorda tympani field ($P < 0.05$). That is, there were no differences in terminal field distances for either the intermediate or ventral regions of the field. For example, the rostral to

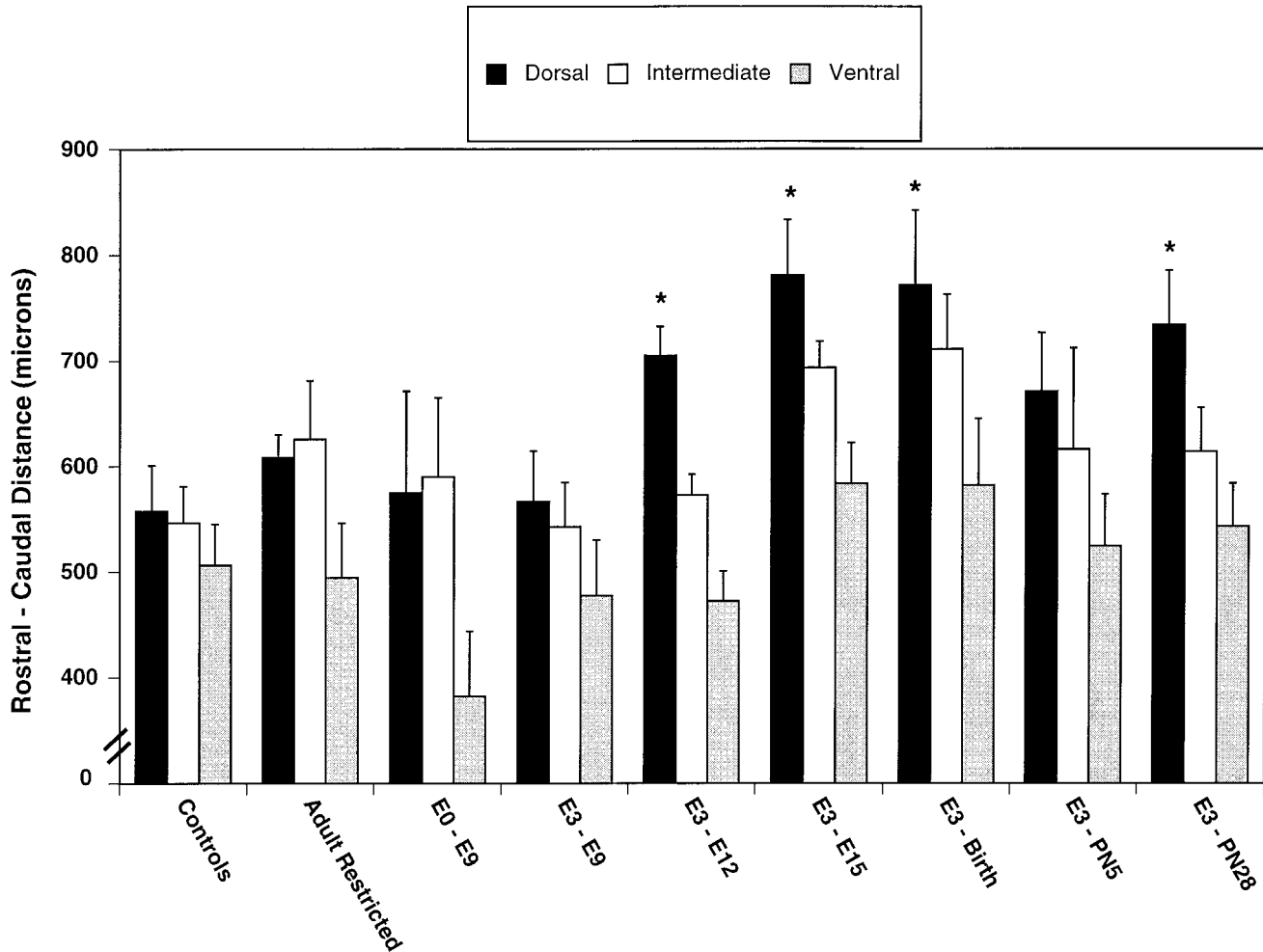


Fig. 4. Mean rostral-caudal distances (\pm S.E.M.) of terminal field label in the dorsal (solid bars), intermediate (open bars) and ventral (hatched bars) zones of the NST. *Denotes significant differences ($P < 0.05$) compared to the respective control mean.

caudal distance of the terminal field in the dorsal zone of rats sodium-restricted from E3 to E12 was $147 \mu\text{m}$ more than that in controls ($P < 0.05$; Fig. 4). By comparison, the rostral to caudal distance of the terminal field in the intermediate and ventral zones was $26 \mu\text{m}$ greater than and $34 \mu\text{m}$ less than the respective rostral-caudal control distances ($P > 0.10$; Fig. 4). With the exception of rats sodium-restricted from E3 to PN5, the trend of longer rostral to caudal distances in the dorsal zone compared to controls continued in rats with longer periods of sodium restriction throughout development ($P < 0.05$; Fig. 4). No differences in length of the field occurred among groups for the medial to lateral distances ($P > 0.10$; Fig. 5). It is apparent, therefore, that the increase in the total terminal field volume can be accounted for by a dramatic, yet localized, expansion of the dorsal terminal field along the rostral to caudal plane.

DISCUSSION

The current study revealed that the central gustatory system is susceptible to dietary influences during an early, restricted period of development. Merely restricting di-

etary sodium from embryonic day 3 (E3) through E12 resulted in a 43% increase in the total volume of the terminal field compared to rats fed a sodium-replete diet throughout development. Moreover, institution of the dietary manipulation for a shorter period (E3 through E9), for the same period but earlier in development (E0 through E9), or only during adulthood resulted in terminal field volumes like controls. Thus, there is a rapid transitional period where development of the chorda tympani terminal field is not susceptible to dietary manipulations (from E3 to E9) to where there is a complete effect (from E3 to E12). Furthermore, the dietary-induced changes seem to be restricted to the dorsal zone of the terminal field. Increases in terminal field volume and rostral-caudal distances were most apparent in this zone, with little changes occurring in the intermediate and ventral zones.

These results compliment earlier findings that demonstrated that dietary sodium restriction from E3 to PN28 resulted in changes in chorda tympani nerve terminal fields, most notably in the dorsal zone (King and Hill, 1991). In addition to replicating the earlier findings, the current study further demonstrates that the effects can be induced at about the time when geniculate ganglion cells,

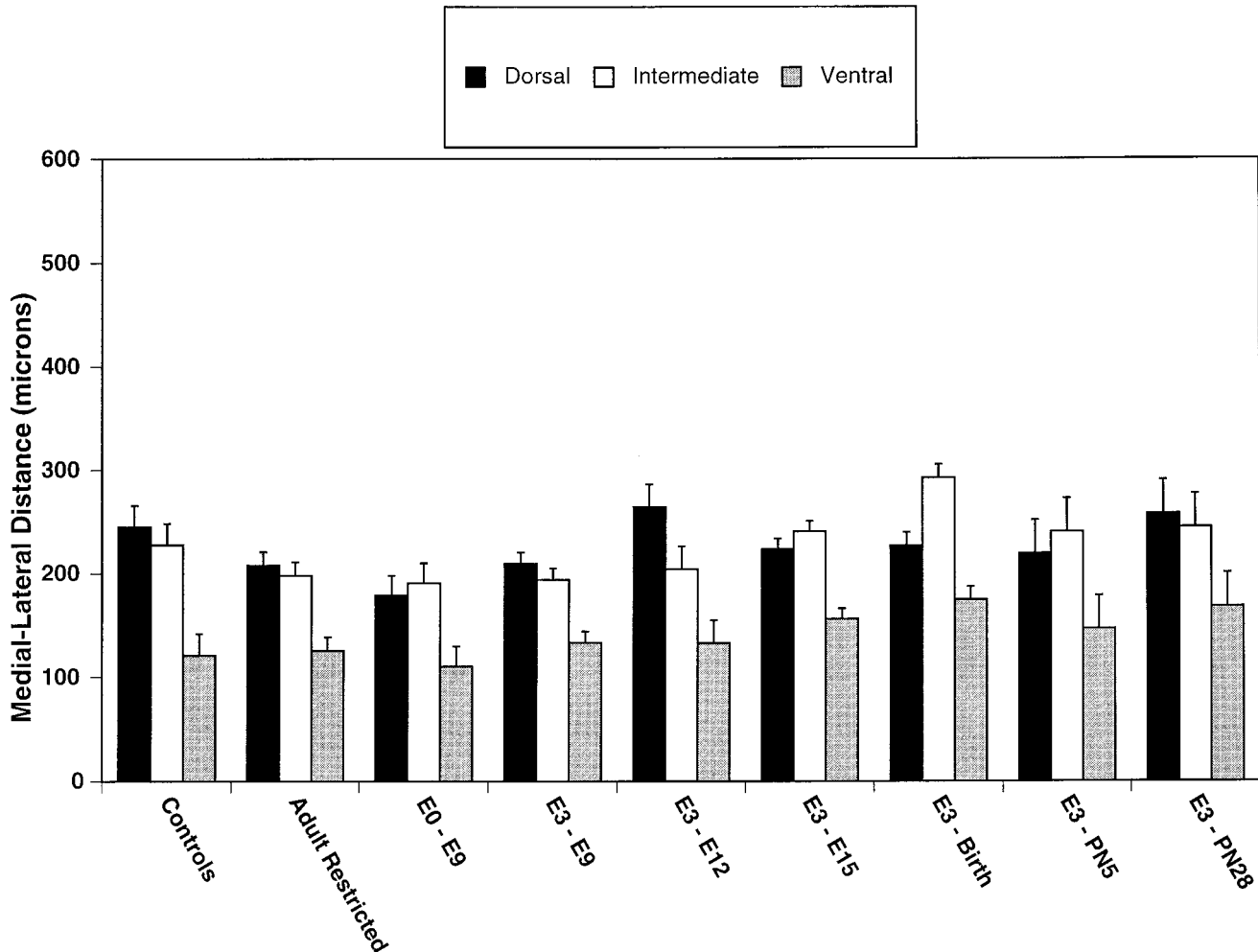


Fig. 5. Mean medial-lateral distances (\pm S.E.M.) of terminal field label in the dorsal (solid bars), intermediate (open bars) and ventral (hatched bars) zones of the NST. There were no significant differences between means.

the cell bodies of the chorda tympani nerve, arise. Geniculate ganglion cells are born between E11 and E14, with the peak proliferation occurring at E12 (Altman and Bayer, 1982). Therefore, the effects shown here can be induced long before the chorda tympani nerve makes connections with taste receptors and with the NST. Specifically, in normal rats, chorda tympani fibers first innervate the apex of fungiform papillae at E17 peripherally (Farbman and Mbiene, 1991), and likely make their initial central projections to the NST just before birth (E21; Lasiter et al., 1989). Most of the terminal field expansion in the NST occurs during the first two postnatal weeks (Lasiter et al., 1989). Thus, the dietary manipulation can be completed (E12) approximately 2 weeks before the greatest expansion of the field occurs and still result in an expanded field.

Interestingly, the early period of susceptibility to the dietary manipulation found for terminal formation of the chorda tympani nerve is similar to that found for the dietary effects on the functional development of the nerve (Hill and Przekop, 1986). Both periods occur within the first 2 weeks postconception, long before the gustatory system becomes functional. Therefore, there are now two

instances in gustatory development in which events that occur very early in development determine later, postnatal outcomes.

Given the early period of susceptibility shown here, it is difficult to attribute activity-dependent mechanisms to the shaping of gustatory terminal field development in the NST. If taste-elicited activity played a role in chorda tympani terminal field formation, sodium restriction would be expected to produce effects only after the first postnatal week. Significant chorda tympani taste responses initially occur around the first postnatal week, and gradually mature to adult levels at about 30 days postnatal (Ferrell et al., 1981; Hill and Almlı, 1980; Yamada, 1980). During this period, early prenatal sodium restriction is first expressed functionally (Hill et al., 1986). However, the period is long after the end of the period identified here (E12). Therefore, it is not possible that differences in stimulus-related activity between control and sodium-restricted animals could account primarily for the increase in terminal field size. Similarly, it is difficult to argue that spontaneous activity has a role in determining central terminal fields, as documented for the visual system

(Sretavan et al., 1988), because geniculate ganglion cells do not arise until the end of the period of susceptibility. Thus, functional peripheral and central connections of the chorda tympani nerve would not be present until after the dietary manipulation was terminated. While it is clear that activity plays a major role in developing sensory systems (e.g., Antonini and Stryker, 1993; Casagrande and Condo, 1988; Guthrie et al., 1990; Shatz, 1996; Shatz and Stryker, 1988), there are instances in other sensory systems (e.g., somatosensory; Chiaia et al., 1992; Henderson et al., 1992) in addition to the gustatory system where nonactivity dependent factors are involved.

The apparent lack of neuronal activity in determining terminal field size here is in contrast to that advanced by Lasiter and colleagues (Lasiter, 1995; Lasiter and Diaz, 1992; Lasiter and Kachele, 1990). Taste receptor destruction in the anterior tongue of postnatal rats aged 2 days results in a decreased chorda tympani field in the NST, whereas similar destruction after this age fails to influence the size of the field (Lasiter and Kachele, 1990). Since taste responses initially occur at about this age (Hill and Almlı, 1980), this result suggests that the onset of function is important in determining terminal field characteristics. Similarly, lack of lingual stimulation with salts in intact rats during early postnatal development results in decreased field size (Lasiter, 1995). Such observations are in concordance with those of other sensory systems in which activity plays a major role in forming central structures (e.g., Antonini and Stryker, 1993; Casagrande and Condo, 1988; Guthrie et al., 1990; Shatz, 1996; Shatz and Stryker, 1988). The results from Lasiter (1995) are different from those reported here. With receptor destruction and limited postnatal gustatory experience, terminal field size is decreased. With early dietary sodium restriction, the terminal field size is increased.

It is likely that the different outcomes reflect different mechanisms. Neural activity may be crucial in shaping the chorda tympani terminal field once the proper numbers of geniculate ganglion neurons are present and once the initial central contacts are made. In fact, early receptor cell destruction does not alter geniculate ganglion cell numbers (Lasiter and Kachele, 1990). However, determination of proper ganglion cell numbers and initial central contacts may be under the control of much earlier events. Specifically, early dietary manipulations employed here may have consequences on the survival of geniculate ganglion cells, perhaps by way of altered neurotrophic levels. Indeed, nutritional manipulations during early developmental periods can result in altered amounts of neurotrophic factors (Diamond et al., 1991). It is possible that such an alteration in the gustatory system due to early sodium restriction may result in increased numbers of geniculate ganglion cells surviving throughout early development. That is, there may not be the normal amount of ganglion cell death which is characteristic of developing peripheral sensory neurons (e.g., Lam et al., 1982). Accordingly, the increased numbers of central processes from increased numbers of geniculate ganglion cells may spread beyond the normal terminal field borders, resulting in an enlarged terminal field. Such an effect of increased sensory ganglion cell survival and the resultant central morphological alteration has been shown in the trigeminal system. Increased numbers of trigeminal ganglion cells resulting

from increased levels of exogenous NGF during prenatal development disrupted whisker-related patterns in the trigeminal brainstem complex postnatally (Henderson et al., 1994). Therefore, similar to what may have occurred in the present study, increased survival of projecting neurons into the brainstem resulted in expanded terminal fields. Experiments that examine geniculate ganglion cell numbers and pattern of terminal field development in normal and in dietary-restricted rats will be important in reconciling these experimental differences.

Although activity may contribute to chorda tympani field development, there is converging evidence that systemic factors may also contribute significantly to gustatory development, namely: (1) sodium taste response development in taste receptor cells is coincident with the expression of many hormonal or growth factors; (2) dietary sodium restriction has to be implemented long before the appearance of functional responses but during times when many hormonal and growth factor systems are forming; (3) recovery of sodium sensitivity requires postingestional absorption of sodium and not direct taste stimulation with the ion; and (4) results from studies of sodium channel immunoreactivity indicate a systemic-factor dependence (Bondy, 1991; Millan et al., 1989; Nielsen et al., 1991; Pons et al., 1991; Stewart and Hill, 1995; Tribollet et al., 1991). It is reasonable to hypothesize that circulating or local factors may also be important in defining the structural development of the chorda tympani nerve. Indeed, the developmental timing of the period identified here is consistent with such a hypothesis.

Regardless of the actual mechanisms (i.e., neuronal activity or systemic factors), there are several potential anatomical bases that account for the increased size of the terminal field. These would include arbor expansion of individual chorda tympani neurons into regions not normally occupied and/or displacement of normal-shaped arbors of neurons outside the typical terminal field boundary. Such an expansion or displacement would occur along the rostral to caudal axis, which is the same axis by which normal development of the field proceeds. Accordingly, the aberrant projection of the chorda tympani nerve would likely invade territory normally occupied only by the glossopharyngeal nerve (Hamilton and Norgren, 1984). Therefore, processes that determine the caudal boundaries of the field may be altered in sodium-restricted rats (e.g., extracellular matrix molecules). As such, the functional and structural implications of chorda tympani neurons invading the "new" territory are numerous. For example, chorda tympani fibers may instruct the aberrant dendritic expansion of postsynaptic cells during development that occurs in developmentally sodium-restricted rats (King and Hill, 1993). In this example, the morphological changes, perhaps directed by the abnormal chorda tympani nerve development, would result in functional changes such as differential convergence of taste information from the anterior and posterior tongue, and the formation of new gustatory coding strategies. Indeed, persistent functional changes are apparent in NST neurons of developmentally sodium restricted rats (Vogt and Hill, 1993).

In summary, these results show that the first synaptic relay of the gustatory system is susceptible to dietary manipulations during a remarkably brief period of very

early embryonic development. The effects are profound and permanent. Correspondingly, these findings have major implications concerning the role of maternal diet on central neural development and behavior.

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