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Dietary NaCl Influences the Organization of Chorda Tympani Neurons Projecting to the Nucleus of the Solitary Tract in Rats

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Abstract

Prior research has shown that maintained exposure to either a low or high NaCl diet from conception to adulthood is associated with changes in NaCl solution intake and neural responses of the chorda tympani (CT) nerve. The present study examined the influence of maintained exposure to a low or high NaCl diet on the central organization of CT neurons projecting to the nucleus of the solitary tract (NST). Three groups of rats were reared and maintained on regular chow containing either basal 0.1%, intermediate 1.0% or high 6% NaCl from conception to adulthood. The fluorescent marker DiI was applied to the CT for characterization of afferent terminations and efferent cell body labeling in the brainstem. The total NST area occupied by CT afferent fibers was the same for all three dietary groups. However, the pattern of CT innervation differed such that there was an enlarged dorsal terminal field in the high group. There were no group differences in body and brain weight, or in efferent labeled neurons. Thus, DiI has been demonstrated to be an effective transport marker of the gustatory system and the parameters of dietary NaCl exposure that influence the pattern of the CT fibers projecting to the NST have been further clarified.

Introduction

Sensory experience plays a critical role in the functional and structural development of the visual (Wiesel and Hubel, 1965; Blakemore and Cooper, 1970; Hubel and Wiesel, 1970; Aslin *et al*., 1981) and auditory (Gottlieb, 1975; Kerr *et al*., 1979; Deitch and Rubel, 1984; Renehan *et al*., 1989) sensory systems. Sensory deprivation (Wiesel and Hubel, 1965; Hubel and Wiesel, 1970; Gottlieb, 1975; Deitch and Rubel, 1984), as well as selective modifications of sensory experience can have profound effects on sensory system development (Blakemore and Cooper, 1970; Cynader and Chernenko, 1976; Kerr *et al*., 1979; Aslin *et al*., 1981; Brunjes and Frazier, 1986). While much is known about the visual and auditory systems, relatively little is known about the parameters of sensory experience necessary for the proper functional and morphological development of the gustatory system.

In taste, research has focused on the chorda tympani (CT) nerve innervating the taste receptors on the anterior tongue, and the nucleus of the solitary tract (NST), the first central synapse in the taste pathway. Lasiter and his colleagues (Lasiter *et al*., 1989; Lasiter and Kachele, 1990; Lasiter, 1991, 1995; Lasiter and Diaz, 1992) have conducted a series of studies showing that early sensory deprivation, through intragastric feeding or damage to anterior tongue receptors, selectively altered the migration and ramification of CT afferents into the NST. Hill and his colleagues have narrowed the scope of sensory deprivation and focused on salt and the influence of dietary NaCl restriction during early development. They have shown that dietary NaCl restriction during the critical period of taste development from embryonic day 3 to at least postnatal day (PD) 28 reduced peripheral CT nerve responses to NaCl compared to rats raised on normal NaCl (Hill *et al*., 1986; Hill, 1987; Hill and Przekop, 1988; Przekop *et al*., 1990; Ye *et al*., 1993; Stewart and Hill, 1996). This was a temporary effect of NaCl restriction, because after placing NaCl-restricted animals on a normal NaCl-containing diet, neural responsivity returned to normal. However, NaCl restriction during prenatal and early postnatal development led to permanent morphological (King and Hill, 1991) and electrophysiological changes (Vogt and Hill, 1993) in central gustatory neurons of the NST. In particular, it has been demonstrated that dietary NaCl restriction (0.03% NaCl) was associated with an enlarged terminal area for CT afferents in the dorsal aspect of the NST (King and Hill, 1991).

We have taken a similar approach to Hill and his colleagues of manipulating dietary NaCl early in development, but with two exceptions. First, we increased dietary NaCl from a restricted level of 0.03% to a basal level of 0.1% that supports normal pregnancy without compromising the body weight of the offspring. Secondly, we have examined the influence of NaCl excess (6%, high), while

using the same control diet of 1% NaCl found in commercial chow for rats. The research shows that animals reared on basal NaCl consumed less saline (Moe, 1987; Contreras and Ryan, 1990) and those on high NaCl more saline under certain circumstances compared to animals reared on intermediate NaCl (Contreras, 1999). Recently, we discovered that the CT nerve responses to NaCl of adult rats reared and maintained on basal NaCl were reduced by ~60% compared to responses from control animals (Pittman and Contreras, 2001). In contrast, there was an increased amiloride-sensitive portion of the CT nerve response to NaCl in adult rats reared on a high NaCl diet compared to responses from control animals.

The present study extends our prior research examining the influence of basal 0.1% NaCl and high 6% NaCl early in development on the gustatory system. Our objective was to determine whether basal or high dietary NaCl altered the central organization of CT neurons projecting to the NST in adult rats. Previous workers (King and Hill, 1991) used horseradish peroxidase (HRP) histochemistry to reveal that NaCl restriction led to an enlarged terminal area for CT afferents in the dorsal aspect of the NST. Although we have used HRP (Contreras *et al*., 1980) and autoradiographic (Contreras *et al*., 1982) tract-tracing methods to characterize gustatory afferents into the NST, in the present study we elected to use DiI to trace CT afferents into the NST. DiI has proven to be a reliable anterograde and retrograde label in living tissue (Honig, 1993). Furthermore, DiI is by far a simpler and more efficient means of tracing fiber pathways than techniques requiring significant histochemical processing. The results from the present study confirmed the usefulness of DiI in tracing neuronal pathways and provided evidence linking dietary NaCl with a significant alteration in the gustatory NST.

Materials and methods

Subjects

Twenty-seven nulliparous female rats [Sprague–Dawley, CrL:CD(SD)BR, Charles River Breeding Laboratories], 66 days old and non-littermates, were housed doubly in clear plastic cages in a temperature-controlled room with a 14:10 light–dark cycle. Each rat was given access to deionized water and a standard pelleted test diet (Harlan Teklad, modifications of Sodium Deficient Diet TD 90228) consisting of either 0.1% (basal), 1% (intermediate), or 6% (high) NaCl. These levels are within the recommended levels of NaCl exposure necessary for normal rodent development set forth in 'Nutrient Requirements of Laboratory Animals' [National Research Council (US), Subcommittee on Laboratory Animal Nutrition, 1995]. Furthermore, each dietary NaCl level corresponds to typical salt intake levels found in subpopulations of humans. The basal condition approximates the recommended salt intake of persons under treatment for high blood pressure (0.5 g/2500 kcal). The intermediate level, at 5 g/2500 kcal, approximates the recommended average daily intake of salt of (United States Department of Health and Human Services and United States Department of Agriculture (2000). The high condition approximates the amount of salt consumed by persons with high daily salt intake (30 g/2500 kcal). The research reported herein fully conforms to the current 'Guiding Principles for Research Involving Animals' published by the American Physiological Society.

The females were adapted to their respective NaCl diet for 14 days and then each female was housed with a single adult male. The breeding pairs were not littermates. The male was removed after 14 days and the females were housed singly thereafter. The females remained on their respective NaCl diets throughout pregnancy and lactation until weaning PD 21. Pups were born as early as 21 days after the initial pairing of males and females. Approximately 24 h after birth, litters were culled to eight pups, retaining as many males as possible per litter.

The pups began consuming pelleted chow and deionized water at ~PD 15. At PD 21, pups were weaned and given *ad libitum* access to deionized water and the same NaCl diet as their mothers. Pups remained on their respective NaCl diets for 9 days following weaning. At PD 30, male pups were separated into cages of two males per cage and were maintained on their respective basal, intermediate, or high NaCl pelleted chow through adulthood and the remainder of the experiment. Female offspring were killed at PD 30. Between 30 and 45 days of age, offspring were identified with a tail tattoo to indicate dietary group and litter number.

Terminal field labeling

Only one male from each litter was used, resulting in nine animals per dietary group. Each rat was anesthetized with sodium pentobarbital (50 mg/kg body wt), with supplemental injections administered as needed to maintain a deep level of anesthesia. After placement in a nontraumatic head holder, the right-side chorda tympani branch of the facial nerve was isolated through a middle ear approach. The ear canal was retracted to reveal the tympanic membrane. Following removal of the tympanic membrane, the ossicle bones were removed using No. 5 fine forceps with care to prevent injury to the CT nerve that lies directly beneath the ossicles. Once the ossicles were removed, the CT nerve was visible, suspended from the rostral wall to the caudal wall of the ear canal. The CT nerve was transected at the distalmost visible point and crystals of the fluorescent labeling chemical DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; Molecular Probes, Eugene, OR) were liberally applied to the proximal cut end of the CT nerve. Crystals were applied to the central end of the nerve without incidental application to nearby tissue. The CT nerve immediately absorbed the DiI crystals, but the animal was maintained in the head holder for an additional 10 min to ensure uninterrupted uptake of the fluorescent label.

Following surgery, the rats were returned to their home cages where they showed no signs of discomfort; there were no obvious changes in food and water intake, body weight or grooming. There were also no unusual displays of head-tilting or circling behavior. After 48 h, the rats were anesthetized deeply with sodium pentobarbital and perfused transcardially with physiological saline followed by 9% formalin. Pilot experiments using 1–3 days of survival indicated that 48 h was optimal for maximal DiI transport from the CT to the NST without degradation.

The whole brain including the olfactory bulbs and an upper portion of the spinal cord was extracted. Following removal of the olfactory bulbs and transection of the spinal cord at CNIII, the brains were weighed. Next, a brainstem block was obtained by making a perpendicular transection through the transverse sinus between the cortex and the cerebellum and a horizontal transection through the cerebellar peduncles to remove the cerebellum. The spinal cord at CNIII determined the caudal boundary of the brainstem block. The brainstem blocks were weighed and embedded in agar. The brainstems were then sectioned horizontally (the blade was parallel to the floor of the fourth ventricle) at 50 μm intervals using a Leica vibratome. This plane permitted visualization of the full CT terminal field in its medial–lateral and rostral–caudal extent. Consecutive serial sections were stored in individual wells containing saline and kept refrigerated until microscopic analysis of the CT terminal field and NST volume. The analysis was completed within 3 weeks of sectioning to minimize decay in the expression of DiI fluorescence.

Quantification

All microscopy examinations utilized a conventional epi-fluorescent microscope (Nikon Optiphot) equipped with an excitation filter (510–560 nm) and an Optronics video camera system. The horizontal brainstem sections were wet-mounted on a slide allowing 4–40× visualization of the NST under brightfield illumination and the CT terminal field under fluorescent illumination. In addition to afferent sensory axons, the CT also contains efferent axonal projections from the brainstem to the sublingual and submaxillary salivary glands. Thus, in addition to the anterograde labeled afferent gustatory axons, the cell bodies of motor neurons projecting to the salivary glands also contained a retrograde DiI label in the brainstem. The anterograde transport of DiI produced a clearly defined boundary of the CT terminal field. The Optronics video system allowed digitizing of images for analysis by NIH Image software in which the perimeter of the labeled terminal field in each 50 μm section was outlined and the area calculated. The sum of all area measurements from each 50 μm section containing the labeled CT terminal field was multiplied by the number of labeled sections times 50 μm to compute the total volume of the CT terminal field.

Following measurement of the fluorescent-labeled CT terminal field, the unilateral NST was measured using brightfield microscopy in the same brainstem sections that contain the CT terminal field. After outlining the perimeter of the unilateral NST using the midline as a medial boundary when necessary, NIH Image software calculated the area. The sum of all NST area measurements was multiplied by the number of labeled sections times 50 μm to compute the total volume of the unilateral NST that contained the labeled CT terminal field.

Additionally, under fluorescent illumination the number of labeled efferent cell bodies was quantified as a control measure to ensure similar uptake and labeling by DiI across preparations. Following quantification of efferent cell bodies and measurement of both the CT terminal field and NST volume, the sections were stained with thionin and dry mounted for preservation.

Statistical analyses

An analysis of variance statistical test (ANOVA) was used to identify any effect of the perinatal dietary conditions on the dependent measurements. Statistically significant ANOVA tests were followed with *post hoc* analysis utilizing Tukey HSD tests. Statistical results with an alpha level of $P < 0.05$ were reported as significant.

Results

Effectiveness of the DiI labeling technique in the CT nerve

Figure 1 shows a typical horizontal section through the NST under both brightfield (A) and fluorescence illumination (B). In the brightfield image (Figure 1A), the rostral tip of the NST is evident in the lower left corner. The same section was viewed under fluorescent illumination (Figure 1B), revealing the afferent fiber tract of the CT nerve and its typical oval-shaped terminal field. In the upper left area of Figure 1B, clusters of efferent neuronal cell bodies were identified. Through digital imaging techniques, it was possible to overlay the fluorescent DiI label on top of the brightfield image to create a composite image (Figure 1C) illustrating the proportion of the NST innervated by the CT terminal field. At higher magnification (20×) axonal and dendritic processes of the efferent neurons were clearly visible (Figure 1D).

Measurement of the CT terminal field volume

The labeled CT terminal field was first visualized ~300–400 μm below the dorsal boundary of the NST. The CT terminal field could then be visualized ventrally in consecutive 50 μm sections for an average of 7.7 (±0.54) sections and an average thickness of 375 ± 27 μm. The number of sections containing the labeled terminal projections did not vary significantly between dietary conditions [$F(2,26) = 0.670$, $P = 0.521$]. Across all preparations, the CT terminal field was contained in no fewer than five sections and no more than 10 sections (Figure 2). The average

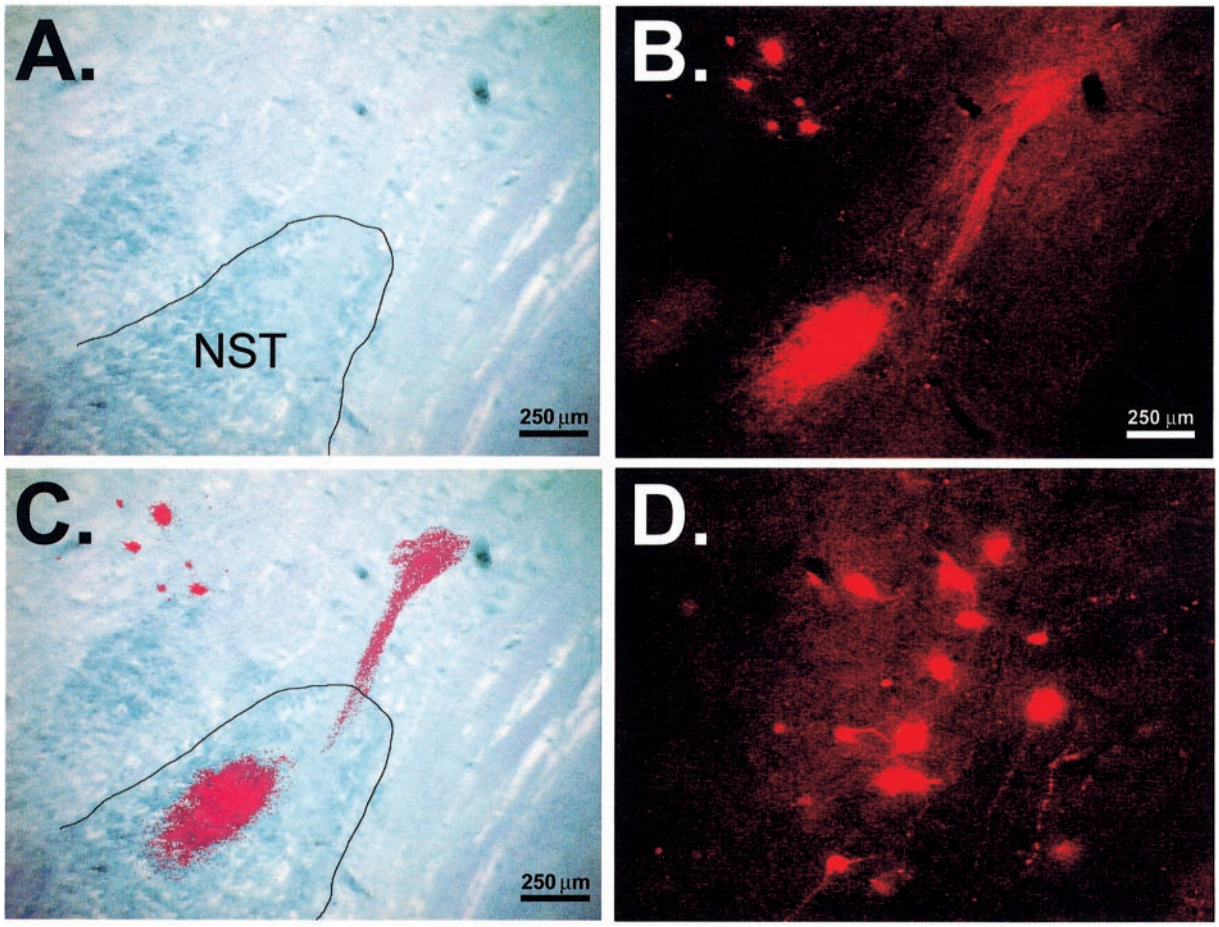

Figure 1 **(A)** Horizontal section of the rostral NST viewed under brightfield illumination. **(B)** Same NST section as in panel (A) showing the distribution of fluorescent DiI label under dark-field illumination. **(C)** Composite image of the fluorescent DiI label in panel (B) superimposed over the bright-field image in panel (A). Note the labeled cell somas of efferent neurons in the upper left and the incoming fiber tract in the upper right. **(D)** DiI-labeled efferent neurons in the medullary reticular formation at 20×.

labeled area per section was 3.98×10^4 µm^2 ($\pm 2.9 \times 10^3$) for the basal group, 4.33×10^4 µm^2 ($\pm 3.8 \times 10^3$) for intermediate group and 4.86×10^4 µm^2 ($\pm 2.9 \times 10^3$) for the high group. In general, the entire CT terminal field was egg-shaped, expanding in a medial to lateral and rostral to caudal direction as the sections were viewed consecutively from dorsal to ventral. The average total volume of CT label in the NST was similar for the three groups, being $1.63 \times 10^7 \pm 1.83 \times 10^6$ µm^3 for the basal salt group, $1.61 \times 10^7 \pm 1.50 \times 10^6$ µm^3 for the intermediate group and $1.75 \times 10^7 \pm 2.05 \times 10^6$ µm^3 for the high salt group (Figure 3A).

Guided by prior work (King and Hill, 1991; Krimm and Hill, 1997), the egg-shaped zone of labeled CT terminals was divided into three equally proportionate divisions (dorsal, central and ventral). The dorsal division contained the upper one-third, the central division the middle one-third and the ventral division the bottom one-third of sections containing labeled fibers. Consistent with a general egg shape, the volume in the central division was greater than that in the dorsal and ventral divisions [$F(2,26) = 7.21$, $P <$

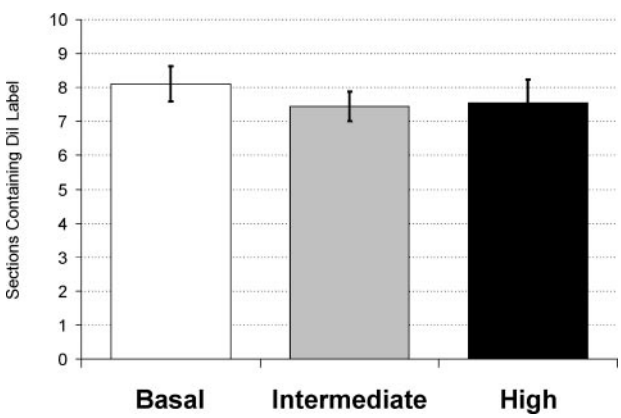

Figure 2 The mean (± SEM) number of 50 µm thick sections containing labeled CT nerve afferents in the three salt groups.

0.01] for all three groups (Figure 3B). Although the average volume of the CT terminal field was similar for all three groups, there was a disproportionate enlargement of the dorsal division in the high group. The volume of label in the

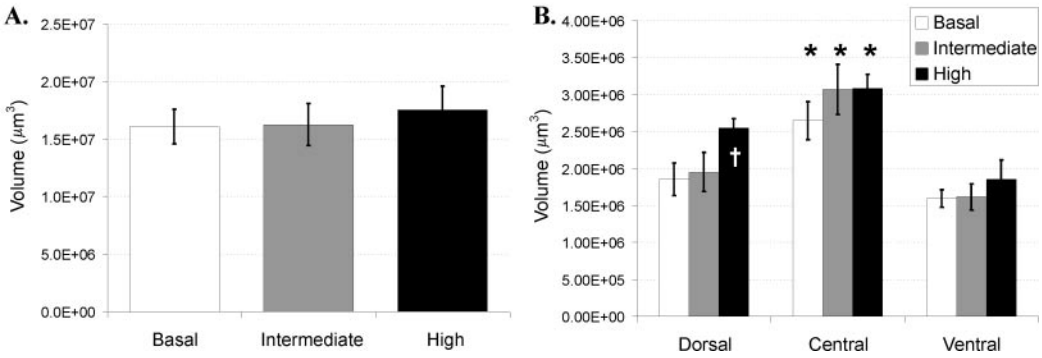

Figure 3 **(A)** The mean (± SEM) volume of the CT terminal field in the three salt groups. **(B)** The mean volume (± SEM) of the CT terminal field separated into three equal divisions: dorsal, central and ventral. The asterisks indicate that for all three salt groups, the central division volume was significantly ($P <$ 0.01) greater than that of the other two divisions, which were not different from each other. The white cross indicates that the dorsal division volume in the high group was significantly ($P < 0.05$) greater than that of the intermediate and basal groups, which were not different from each other.

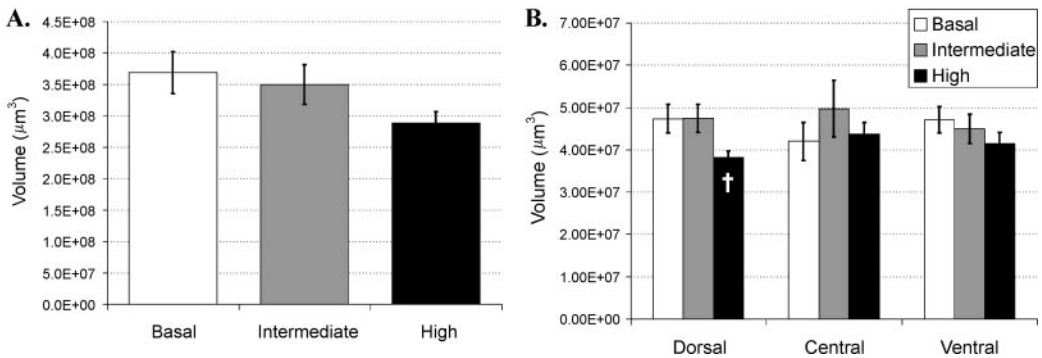

Figure 4 **(A)** The mean (± SEM) volume of the NST containing DiI labeled terminals. **(B)** Mean (±SEM) volume of the NST separated into three equal divisions containing the dorsal, central and ventral portions of the DiI-labeled field in basal (open bars), intermediate (gray bars) and high (black bars) salt groups. The white cross indicates that the volume of dorsal NST in the high group was significantly ($P < 0.05$) smaller than that of the intermediate and basal groups, which were not different from each other.

dorsal division was ~25% larger in the high group compared to the intermediate and basal salt groups [$F(2,26) = 3.835$, $P < 0.05$; Tukey HSD *post hoc* tests, $P < 0.05$].

Measurement of the NST volume

The overall shape of the unilateral NST is similar to a horizontal cylinder, with the DiI label located within its rostral core. For each subject, we computed the volume of the unilateral NST that contained labeled CT terminals. The average area of the NST sections (mean = 7.7, see Figure 2) that contained label was $7.38 \times 10^6 \pm 6.65 \times 10^5$ μm^2 for the basal salt group, $7.00 \times 10^6 \pm 6.32 \times 10^5$ μm^2 for the intermediate group, and $5.76 \times 10^6 \pm 3.65 \times 10^5$ μm^2 for the high group. The total volume of the NST was $3.69 \times 10^8 \pm 3.33 \times 10^7$ μm^3 for the basal group, $3.50 \times 10^8 \pm 3.16 \times 10^7$ μm^3 for the intermediate group and $2.88 \times 10^8 \pm 1.82 \times 10^7$ μm^3 for the high group. The area and volume measures of the unilateral NST containing labeled CT terminals were similar for the three salt groups (see Figure 4A).

Despite its overall cylindrical shape, the portion of the unilateral NST containing the dorsal, central and ventral components of the label was similar to three long and narrow rectangular segments stacked one on top of the other. Breaking the NST containing the label into dorsal, central and ventral segments revealed that, despite similar total volumes (Figure 4A), the dorsal NST segment in the high group was significantly smaller as compared with the dorsal segments of the other two groups [$F(2,26) = 3.826$, $P < 0.05$] as shown in Figure 4B.

Proportion of the NST innervated by the CT

Figure 5 shows the mean volume of the DiI-labeled field as a percentage of the NST volume in which the label occurred. Due to the ovoid shape of the terminal field, the label in the central division occupied a greater percentage of the NST than the labels in either the dorsal or ventral divisions [$F(2,26) = 11.887$, $P < 0.01$], as depicted in Figure 5B. As shown in Figure 5A, the percentage volume of the entire terminal field in the high group (6.0 ± 0.5%) tended to be greater than that in the intermediate (4.9 ± 0.7%) and basal NaCl (4.7 ± 0.6%) groups. Analysis of the CT terminal field by division revealed that the dorsal division was the source

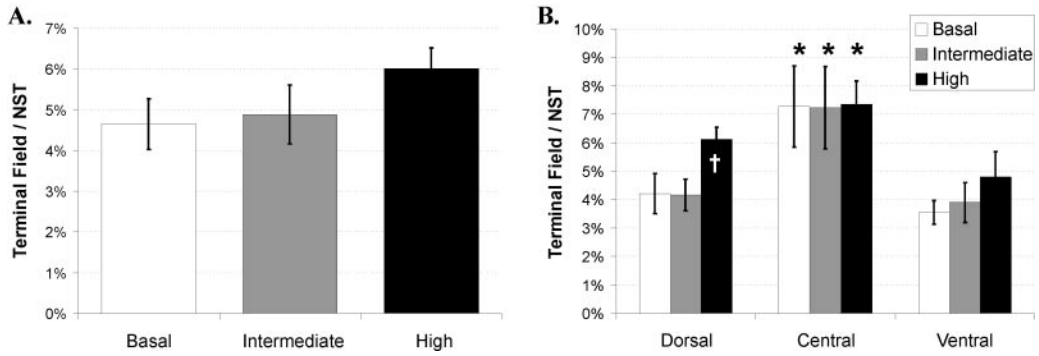

Figure 5 **(A)** The mean (± SEM) volume of the entire DiI-labeled field as a percentage of the NST volume in which the label occurred. **(B)** The mean (± SEM) volume of the dorsal, central and ventral portions of the labeled field as a percentage of the NST volume in which it occurred. The asterisks indicate that for all three salt groups, the percentage central division volume was significantly ($P < 0.01$) greater than that of the other two divisions, which were not different from each other. The white cross indicates that the percentage dorsal division volume in the high group was significantly ($P < 0.05$) greater than that of the intermediate and basal groups, which were not different from each other.

Table 1 The average (± SEM) control measures from subjects raised and maintained on a normal chow diet containing basal 0.1%, intermediate 1% or high 6% NaCl

Measures	Basal	Intermediate	High
Birth weight (g)	5.1 ± 0.2	7.3 ± 0.4	6.1 ± 0.1
Adult weight (g)	597.5 ± 34.0	606.7 ± 20.4	614.0 ± 16.3
Brain weight (g)	2.50 ± 0.07	2.48 ± 0.06	2.62 ± 0.08
Brainstem weight (g)	0.29 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
Labeled efferent neurons (*n*)	51.4 ± 8.2	69.3 ± 18.9	60.0 ± 14.7

of the group difference. The high group had a larger relative volume of label in the dorsal division than the intermediate and basal groups [$F(2,26) = 4.431$, $P < 0.05$]. The label occupied 6.1% of the dorsal division in the high group, 4.1% in the intermediate group and 4.2% in the basal group. There were no group differences in percentage label in the central and ventral divisions of the unilateral NST.

Control measures

DiI uptake was similar across preparations and diet conditions, as demonstrated by a comparable number of labeled efferent cell bodies [$F(2,26) = 1.118$, $P = 0.343$] among the three dietary NaCl groups. The average number of labeled efferent neurons was 51.4 ± 8.2 in the basal group, 69.3 ± 18.9 in the intermediate group and 60.0 ± 14.7 in the high group. In addition, the average birth weight [$F(2,26) = 2.744$, $P = 0.084$], adult body weight [$F(2,26) = 0.152$, $P = 0.859$], total brain weight [$F(2,26) = 0.642$, $P = 0.535$] and brainstem weight [$F(2,26) = 0.435$, $P = 0.652$] were similar for the three dietary NaCl groups (see Table 1).

Discussion

DiI proved to be an excellent agent for characterizing the terminal field of the CT nerve in the rat NST. As a lipophilic compound, DiI was readily absorbed by the transected nerve and transported quickly within axons to label the afferent fibers into the NST and the cell soma of salivatory neurons in the medullary reticular formation. Robust and reliable labeling was obtained in an efficient and effective manner without the encumbrance of significant histological processing required by other tract-tracing methods. Importantly, the present results approximate the findings from other studies. For example, the volume of the DiI-labeled field of 1.63×10^7 μm^3 reported in the current study is similar to volume reported previously: 1.2×10^7 μm^3 (Lasiter and Kachele, 1990); 1.225 10^7 μm^3 (King and Hill, 1991); and 2.14×10^7 μm^3 (Krimm and Hill, 1997). These prior studies used HRP rather than DiI to trace CT nerve terminals into the NST from horizontal brain sections, as was done in the present study. Additionally, the present results are also consistent with those of previous studies using HRP (Hamilton and Norgren, 1984) and amino acid autoradiography (Contreras *et al.*, 1982), but characterized the label from coronal brain sections instead of horizontal. Thus, the DiI method of tracing a neuronal pathway proved relatively easy to perform and yielded reliable results comparable to findings from previous studies using different methods.

The present study showed that a reduction in dietary NaCl from a standard level of 1% found in commercial chow to a basal level of 0.1% was without effect on the volume of the CT nerve projection in the NST. Adult rats,

reared by mothers fed basal NaCl throughout pregnancy and lactation, were maintained on basal NaCl throughout their life until testing. We have recently shown that rearing rats on basal NaCl led to a 60% reduction in the CT nerve response to NaCl stimulation of the tongue (Pittman and Contreras, 2001). Furthermore, animals reared on basal NaCl consumed less saline (Moe, 1987; Contreras and Ryan, 1990; Contreras, 1999) compared to animals reared on intermediate 1% NaCl. Previous research conducted in offspring maintained on a deprived 0.03% NaCl diet also found a reduction in CT nerve response to NaCl (Hill *et al*., 1986; Hill, 1987; Hill and Przekop, 1988; Przekop *et al*., 1990; Ye *et al*., 1993; Stewart and Hill, 1996), as well as an enlargement in the dorsal region of the CT terminal field in the NST (King and Hill, 1991; Krimm and Hill, 1997). Presumably, a dietary NaCl level of 0.1% can alter CT nerve responsiveness without a permanent change in the CT terminal field within the NST.

This is the first study to explore the possible influence of dietary NaCl excess on the volume of the CT nerve projection in the NST. Rats were reared on a 6-fold higher NaCl level (6%) than fed to animals in the intermediate, control NaCl group and a 60-fold higher level than fed to basal NaCl animals. We discovered that the total NST area occupied by CT afferent fibers was the same for animals reared for a lifetime either on basal, intermediate, or high dietary NaCl. However, the pattern differed such that there was an enlarged dorsal segment in rats reared and maintained on high 6% NaCl compared to animals reared and maintained on basal and intermediate levels of dietary NaCl. There were no group differences in body and brain weight, or in the number of labeled efferent neurons. Thus, the NaCl levels used in the present study spanned a broad range, yet seemed to be within a reasonable physiological limit that did not compromise the health and growth of the offspring. Furthermore, the fact that the number of labeled efferent neurons was the same across groups negates the possibility that differential DiI uptake accounts for the results. Thus, the present observations suggest that a relatively benign dietary NaCl level of 6% led to a relatively specific alteration in the first central synapse of the taste pathway.

We have previously demonstrated that adult rats reared on high dietary NaCl consumed more saline than rats reared on intermediate or basal NaCl (Contreras and Kosten, 1983; Contreras and Ryan, 1990; Contreras, 1999). Recently, we have shown that there was an increased amiloride-sensitive portion of the CT nerve response to NaCl in adult rats reared on high NaCl compared to responses from control animals (Pittman and Contreras, 2001). The amiloride-sensitive portion of the CT response is thought to be the receptor mechanism underlying NaCl detection by the peripheral gustatory system (Contreras and Lundy, 2000). Indeed, when amiloride was used in behavioral experiments, it disrupted taste-mediated saline intake in rodents. For example, in rats amiloride disrupted a conditioned aversion to NaCl (Hill *et al*., 1990), the expression of a depletion-induced NaCl appetite (Bernstein and Hennessy, 1987; McCutcheon, 1991), NaCl–KCl discrimination (Spector *et al*., 1996) and decreased the unconditioned licking response to NaCl (Contreras and Studley, 1994). It may be that exposure to high dietary NaCl influences CT nerve input during a critical period of gustatory development, which in turn alters the central organization of CT afferents in the NST. Although it is unclear how, the central reorganization of CT afferents in the NST may be related to long-term changes in NaCl solution intake due to dietary NaCl excess early in development.

A less interesting possibility is that the larger CT nerve input into the dorsal segment of the NST in high NaCl animals is unrelated to their elevated NaCl solution intake. In this respect, it is a curious coincidence that dietary NaCl excess led to the same enlarged dorsal portion of the labeled zone in the NST as was reported following NaCl restriction (King and Hill, 1991; Krimm and Hill, 1997). Of course, it is unknown if this is just a superficial similarity between the two dietary NaCl treatments, accounted for by a significantly different profile of terminal endings. This, notwithstanding, raises the possibility that dietary NaCl, at either an extreme lower or higher level, leads to an alteration in a particularly susceptible region of the gustatory NST. As noted previously, dietary NaCl restriction has the disadvantage of adversely compromising the body weight of the offspring, thus making it difficult to attribute the alteration in the NST to a specific effect of NaCl on the development of the central gustatory system. However, dietary NaCl restriction altered CT nerve responses specifically to NaCl stimulation, as the neural responses to other non-sodium salt stimuli were unaffected (Hill *et al*., 1986; Hill, 1987; Hill and Przekop, 1988; Przekop *et al*., 1990; Ye *et al*., 1993; Stewart and Hill, 1996). Furthermore, dietary NaCl excess did not alter birth number or offspring body weight and there were no gross changes in the brain to indicate a generally negative effect of the diet. Taken all together, the weight of the evidence suggests that dietary NaCl restriction and excess led to specific alterations in the gustatory system, that were not secondary to a general debilitating effect of too little or too much NaCl.

Why and how then might dietary NaCl restriction and NaCl excess cause a similar alteration in the NST? The most obvious explanation is that the dorsal segment of the CT nerve terminal field may be an area critically involved in salt taste processing and salt ingestion. Thus, any manipulation that modifies salt taste or salt ingestion mechanisms, such as dietary NaCl restriction or NaCl excess, would have a consequence in this region of the brain. The present and prior studies using tract-tracing methods merely define the area of importance, suggesting a chemotopic organization in the gustatory NST with NaCl being more strongly represented in the dorsal portion. Future studies using other directed methods would help determine any differences that

dietary NaCl restriction and NaCl excess might have on the neurons in this region.

There are sensory and nutritional explanations as to how NaCl restriction and NaCl excess modified the gustatory NST. With respect to a sensory explanation, we hypothesize that the amount of NaCl stimulation directly contacting the anterior tongue taste receptors during a critical period of development is a determining factor influencing CT nerve terminals in the brain. Further, the neurons so affected are NaCl-sensitive fibers in synaptic contact with taste receptor cells pre-ordained to utilize amiloride-sensitive proteins to detect sodium. Reduced or exaggerated NaCl input through this peripheral salt-sensitive pathway is responsible for the enlarged terminal field in the NST. An equally plausible alternative hypothesis is that dietary NaCl acts indirectly on gustatory development through changes in the hormones that regulate water and electrolyte balance. For example, dietary NaCl restriction elevates plasma levels of angiotensin II and aldosterone, and dietary NaCl excess has the opposite effect (Contreras, 1999). These hormones may influence the development of amiloride-sensitive proteins on taste cells (Lundy, 1998) or modify angiotensin and aldosterone receptors in brain regions, including the NST, known to be involved in controlling salt intake (Epstein, 1986).

In conclusion, rats were reared and maintained either on basal, intermediate, or high dietary NaCl that spanned a broad 60-fold concentration range, yet permitted normal gross development. The specific NaCl levels were selected on the basis of prior work demonstrating their influence in altering saline intake and CT nerve responsiveness to NaCl stimulation. In the present study, we demonstrated that DiI was an excellent agent for characterizing the terminal field of the CT nerve in the rat NST. Using DiI, we showed that there was a critical association between dietary NaCl level and morphological development of the central taste pathway. The mechanism(s) by which dietary NaCl leads to an alteration in the CT terminal field and its role in taste-mediated behavior remain to be elucidated.

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