Chem. Senses 34: 253–267, 2009
doi:10.1093/chemse/bjn085
Advance Access publication January 29, 2009

Characterization of Parabrachial Subnuclei in Mice with Regard to Salt Tastants: Possible Independence of Taste Relay from Visceral Processing

Koji Hashimoto[1,3], Kunihiko Obata[2,4] and Hisashi Ogawa[1,5]

[1]Department of Sensory and Cognitive Physiology, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, Honjo 1-1-1, Kumamoto 860-8556, Japan and [2]Laboratory of Neurochemistry, National Institute for Physiological Sciences, Myodaiji-cho, Okazaki 444-8585, Japan
[3]Present address: Department of Medical Laboratory Sciences, School of Health Sciences, Kumamoto University, Kuhonji 4-24-1, Kumamoto 862-0976, Japan
[4]Present address: Laboratory of Neuronal Circuit Mechanism, Brain Science Institute, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan
[5]Present address: Department of Neurology, Juryo Kumamoto Kinoh Hospital, Yamamuro 6-8-1, Kumamoto 860-8518, Japan

Correspondence to be sent to: Koji Hashimoto, Department of Medical Laboratory Sciences, School of Health Sciences, Kumamoto University, Kuhonji 4-24-1, Kumamoto 862-0976, Japan. e-mail: coji@kumamoto-u.ac.jp

Abstract

We examined whether salt taste and/or abdominal illness were dealt within different subnuclei in the parabrachial nucleus (PBN) in mice, using retrograde tracing methods and c-Fos–like immunoreactivity (FLI) detection procedures. Some PBN subnuclei have distinct functions and receive various sensory inputs from the nucleus of the solitary tract (NTS) and other areas and relay them to the higher order nuclei such as the thalamus. The afferent-dependent pattern of FLI has been investigated in the PBN. However, it is unclear in which PBN subnuclei the tastants induce c-Fos, or whether PBN subnuclei process taste inputs separately from other inputs, or integrate them. After the tracer injections into the thalamic taste relay, the retrograde labeled cells revealed the taste relay cells in the PBN at the boundary with the superior cerebellar peduncle of both the inner part of the external lateral subnucleus and the medial subnucleus and in the waist area. On the other hand, NaCl intake induced intense FLI in the dorsal lateral subnucleus, whereas LiCl intake yielded intense FLI in both the dorsal lateral subnucleus and the outer part of the external lateral subnucleus. Thus, the present findings that subnuclei relaying taste information to the thalamus do not yield FLI in response to salt taste and abdominal illness indicate that they lack FLI yielding pathways or that they are independent from the subnuclei processing salt taste and visceral information via c-Fos in mice.

Key words: c-Fos, lithium chloride, salt stimulus, visceral sensation, wheat germ agglutinin–conjugated horseradish peroxidase

Introduction

In rodents such as rats and hamsters, neural signals of taste from the oral cavity including the tongue first reach the nucleus of the solitary tract (NTS; a full list of abbreviations used in this article is available at Supplementary Material Online), the primary relay nucleus, and then ascend, mainly ipsilaterally in the brain stem, to the parabrachial nucleus (PBN) of the pons, which relays them bilaterally to the thalamic taste relay, the parvicellular part of the thalamic posteromedial ventral nucleus (VPMpc) (Norgren 1995). In the PBN, visceral (Bernard et al. 1994; Baird et al. 2001), respiratory (Dawid-Milner et al. 2003), cardiac (Jhamandas et al. 1991; Dampney 1994), and nociceptive (Menendez et al. 1996) neurons, as well as taste neurons (Norgren and Pfaffmann 1975; Ogawa et al. 1987; Halsell and Travers 1997; Miyaoka et al. 1997; Shimura et al. 2002), have been identified electrophysiologically. The PBN is a key relay station that delivers various kinds of sensory information to the thalamus, amygdala, hypothalamus, and other nuclei, although its functional features are not fully understood. As new functions are added to the PBN, studies have attempted to delineate and allocate subnuclei to these functions in rats (Fulwiler and Saper 1984; Moga et al. 1990; Chamberlin and

Saper 1992; Bernard et al. 1993, 1994, 1995; Yamamoto, Shimura, Sakai, and Ozaki 1994; Halsell and Travers 1997; Karimnamazi et al. 2002) and in hamsters (Halsell and Frank 1991; Halsell 1992). However, histological delineations in the 2 species have been inconsistent, and functional characterizations of the subnuclei are not always successful. Electrophysiological studies have located the taste relay neurons mainly at the waist area including inside the superior cerebellar peduncle (SCP), central medial subnucleus of the PBN and medial subnucleus of the PBN (MPB) (Norgren and Pfaffmann 1975; Ogawa and Akagi 1978; Halsell and Frank 1991; Halsell and Travers 1997; Miyaoka et al. 1997; Karimnamazi and Travers 1998; Karimnamazi et al. 2002; Shimura et al. 2002), and the neurons for visceral information mainly at the external lateral subnucleus of the PBN (LPBE) (Bernard et al. 1994; Baird et al. 2001). In rats and hamsters, attempts have been made to locate taste relay neurons in the PBN projecting to the VPMpc by injecting tracers into the latter (Ogawa and Akagi 1978; Fulwiler and Saper 1984; Halsell 1992; Hayama et al. 1994). However, except for a few reports (Halsell 1992; Karimnamazi and Travers 1998; Hayama and Ogawa 2001), most of the studies injected the tracer without identifying taste responsiveness in the injection sites. Moreover, no tracing study has been conducted to locate the taste relay neurons in the PBN in mice. Accordingly, the accurate location of taste relay neuron in PBN subnuclei still needs to be investigated.

Induction of c-Fos–like immunoreactivity (FLI) is useful to visually identify subnuclei responding to certain stimuli. This method has been utilized to visualize taste-activated neurons in the PBN (Yamamoto, Shimura, Sakai, and Ozaki 1994; Yamamoto and Sawa 2000; King et al. 2003). Yamamoto, Shimura, Sako, et al. (1994) found induction of FLI mainly in 2 subnuclei, the dorsal lateral subnucleus of the PBN (LPBD) and the LPBE, and they proposed that the LPBD was concerned with positive hedonics of taste whereas the LPBE was concerned with negative hedonics of taste on the basis of FLI induction after conditioned taste aversion, a form of passive aversion. However, these subnuclei were not found among the subnuclei that were retrograde traced from the VPMpc. Therefore, whether these 2 subnuclei are involved in relaying taste information to the thalamus needs to be clarified.

The present study aimed to histologically delineate the PBN subnuclei for taste relay neurons by retrograde labeling from the VPMpc and then to compare it with the distribution of FLI induced by salty tastants to investigate whether salty tastant–induced FLI represents taste relay neurons in mice.

Materials and methods

Subjects

Male mice of the ddY strain (8–15 weeks old) were used. Although the size of the brain from the anterior tip of the cortex to the posterior end of the cerebellum (measured after tissue fixation) is 11.45 ± 0.01 mm (mean ± standard error [SE], $n = 5$), similar to that of other strains, such as C57BL/J6 (Franklin and Paxinos 1997), ddY mice have a relatively large body size (body weight: 36–38 g at 8 weeks old) so that they can tolerate surgical insult. Furthermore, they are generally docile with little spontaneous locomotion activity and show a more or less stereotypical behavior in response to stimuli, such as sound, in contrast to other strains including the BALB/c and C3H/He strains (Tsuda et al. 2006).

All animal care and use procedures were approved by the Animal Care and Use Committee in our institute and were in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources 1996). Mice were housed individually in a cage (13 × 30 × 14 cm) with a metal top and sawdust bedding. The animals were housed in an air-conditioned room (23 ± 2 °C) with white light on from 7:00 to 19:00 and were allowed ad libitum access to mouse chow and water except for the days of experimental training.

Histological delineation of PBN subnuclei

Five mice (12 weeks old) were used as subjects in this part of the study. After animals were deeply anesthetized by an overdose of sodium amobarbital, they were perfused through the heart with 20–25 ml of phosphate-buffered saline (PBS, 0.9% NaCl in 20 mM phosphate buffer, pH 7.3) followed by 40–50 ml of 15% formaldehyde (formalin; Nacalai, Kyoto, Japan) dissolved in PBS. After a fixation period of longer than 30 min, the brain was then removed from the skull and soaked in 10% glycerol in PBS for at least 36 h at 4 °C. To verify PBN subdivisions, the whole brain was cut into 50-μm-thick serial coronal sections using a freezing microtome. All sections were collected, mounted on glass slides, and then stained with thionin.

Sections through the brain stem were examined using a light microscope with bright-field, polarized, or differential interference optics, and visual images were captured using a digital camera (model DMC1; Polaroid, Tokyo, Japan). Digitized images were saved on a Windows PC and were rotated and trimmed with Adobe Photoshop CS3 (Adobe Systems Inc, San Jose, CA). Delineations of the PBN were traced onto appropriate images using Adobe Illustrator CS3 (Adobe Systems Inc). Brain maps of PBN subnuclei and VPMpc were delineated referring to the atlas of the mouse brain (Franklin and Paxinos 1997) as a primary guide, supplemented with information on delineations obtained from previous studies on the rat PBN (Fulwiler and Saper 1984; Moga et al. 1990; Bernard et al. 1993, 1994, 1995; Saper 1995; Halsell and Travers 1997) and on the hamster PBN (Halsell and Frank 1991; Halsell 1992).

Electrophysiological exploration of the taste-related zone in the VPMpc

Twenty mice were used as subjects in this part of the study. The mice were anesthetized with sodium amobarbital (125 mg/kg

body weight, intraperitoneal [i.p.]), with anesthesia maintained by subsequent i.p. injections of the drug when necessary. Each mouse was mounted on a stereotaxic instrument using a pair of blunt mouse ear bars following the methods reported by Franklin and Paxinos (Franklin and Paxinos 1997). A hole was made with a drill in the left skull at 1.7–2.5 mm caudal to the bregma and 0.9–1.1 mm lateral to the midline.

Then, a glass microelectrode with a tip diameter of approximately 2 μm containing 2 M NaCl solution (the exploring electrode) was advanced vertically through the hole in the skull toward the VPMpc. The taste-responsive zones of the VPMpc were explored by applying 0.1 M NaCl solution to the anterior tongue. Multiunit neural activities recorded by the exploring electrode were led to conventional electrophysiological equipment and monitored on a cathode-ray oscilloscope and by an audio-monitor. Signals were also fed into a microcomputer to analyze the taste responsiveness online using in-house software.

Retrograde labeling study

A glass microelectrode with a tip diameter of approximately 8 μm was filled with wheat germ agglutinin–conjugated horseradish peroxidase (WGA-HRP) (5%; Toyobo, Osaka, Japan) dissolved in 0.1 M KCl solution, with the pH adjusted to 8 with 0.05 M tris-aminomethane-HCl (the WGA electrode). When a taste-responsive zone was detected by the exploring electrode, the exploring electrode was replaced with the WGA electrode. After confirming the tip of the WGA electrode in the taste-responsive zone by recording taste-evoked activities, the tracer was injected by applying positive DC pulses (10–100 V, 2 μA, 250 ms in duration, 2 Hz) to the WGA electrode for 3–30 min. When the taste responses were not successfully detected by the WGA electrode, the tracer was injected to the same position as recorded by the exploring electrode.

To obtain the site of the taste relay neuron in the PBN subnuclei, we confirmed the taste-responsive site in the VPMpc in mice to be the same as that previously reported in rats (Scott and Erickson 1971; Nomura and Ogawa 1985). Observation of retrograde labeled somata and anterograde labeled axon terminals in the ventromedial-most portion of the thalamic reticular nucleus from the VPMpc confirmed that the tracer was accurately injected in the taste relay region in the VPMpc (Hayama et al. 1994). In the present study, tracer injection was regarded as successful when both of the following conditions were met: 1) recording of taste responses with the WGA electrodes and 2) retrograde labeling of somata and anterograde labeling of axon terminals in the reticular nucleus in the thalamus.

After a survival period of 24–48 h, animals were deeply anesthetized by an overdose of sodium amobarbital and perfused through the heart with 20–25 ml of ice-cold PBS, followed by 40–50 ml of ice-cold 10% formalin diluted in PBS. The period of fixation was less than 30 min. After removing the brain from the skull, it was soaked in 10% glycerol in PBS for at least 36 h at 4 °C. The whole brain was then cut coronally into 50-μm sections with a freezing microtome, and all sections were collected in ice-cold PBS. The sections were reacted with 3,3′,5,5′-tetramethylbenzidine (Nacalai) following Mesulum's protocol (Mesulum 1978). If necessary, every second section was also lightly counterstained with thionin to identify the nuclei of the brain.

Immunohistochemical study

In total, 24 mice were used as subjects. Four mice were used for NaCl intake, 8 for LiCl intake, and 12 for control. Three control experiments were conducted: 4 mice were given distilled water (DW) intake, 4 were injected i.p. with 0.15 M LiCl (2% of body weight) to induce abdominal illness, and 4 were given no treatment.

Mice were deprived of water for approximately 24 h and trained to drink DW for 10 min daily (at around 14:00) in their individual home cages for at least 7 days to acclimatize them to experimental conditions and to avoid a reaction to a new environment or neophobia. The amount of DW intake (ca., 2.8 ml) reached a plateau within 4 days. On the experimental day, for 10 min, animals were allowed to intake a solution of 0.1 M NaCl, or 0.1 M LiCl, which tastes similar to NaCl but induces abdominal illness. The bottles were then withdrawn from the cages and the subjects left for another 20 min in their individual cages. Thirty minutes after the stimulus onset, animals were deeply anesthetized with an overdose of sodium amobarbital and perfused through the heart with 20–25 ml of PBS, followed by 40–50 ml of 15% formalin in PBS. The brains were removed and placed into the same fixative overnight at 4 °C.

After being cryoprotected in PBS containing 10% glycerol and 0.01% sodium azide for 2 days at 4 °C, brains were coronally sectioned (50-μm-thick sections) with a freezing microtome and all sections were collected in 15% formalin in PBS. The sections were then rinsed (5×, PBS, 30 min), then incubated for 30 min in 1% Triton X-100 (Nacalai) in PBS (TPBS), rinsed again (5×, PBS, 30 min), and then incubated for 30 min with 0.3% H_2O_2 in absolute methanol to quench endogenous peroxidase. Sections were then rinsed (5×, PBS, 30 min) and gently shaken with rabbit polyclonal antibody (c-Fos [4]: sc-52; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:2000 and 1.5% normal goat serum in TPBS. The polyclonal antiserum recognizes residues 3–16 of the c-Fos protein of mouse, rat, and human. After overnight incubation at 4 °C, sections were rinsed (5×, PBS, 30 min) and processed using the avidin–biotin protocol (ABC kit: Vector Laboratories, Burlingame, CA). Sections were then transferred to biotinylated anti-rabbit goat antibody in TPBS for 2 h, gently shaken at 37 °C, rinsed (10×, PBS, 60 min), transferred to avidin–biotin complex in TPBS, gently shaken for 1 h at 37 °C, rinsed again (10×, PBS, 60 min), and developed by gentle shaking for 15 min at 37 °C with

3,3′-diaminobenzidine tetrahydrochloride (DAB, Nacalai) substrate, which consisted of 0.03% DAB and 0.003% H_2O_2 in TPBS. Finally, sections were briefly rinsed (5×, PBS), mounted on gelatin-coated slides, dehydrated, and coverslipped with Malinol (Muto Pure Chemicals, Tokyo, Japan). Cells expressing FLI were examined using a light microscope, and a cell was considered FLI positive if its nucleus was completely stained, whether the staining was intense or weak.

Statistical procedures

In the retrograde labeling study, Student's *t*-test was performed to examine the possible difference in the number of labeled cells in each PBN subnuclei across the whole rostrocaudal extent between the ipsilateral and contralateral sides of the brain. In the immunohistochemical study, FLI-positive cells in 16 mice (DW intake, NaCl intake, LiCl intake, and LiCl i.p. injection, $n = 4$ each) were counted in both the LPBD and outer part of the LPBE (LPBEo) at 0 to –500 μm caudal to the end of the cuneiform nucleus (CnF) in both hemispheres of the brain. One-way analysis of variance followed by Scheffe's post hoc tests was used to assess the significant differences in induction of FLI-positive cells among the 4 groups of mice given different treatments.

Results

Cytoarchitectonic delineation of the parabrachial subnuclei

Based on the size, shape, and staining characteristics of the PBN neurons, 8 subnuclei (5 lateral and 3 medial) were identified (Figure 1). The PBN extended approximately from +200 to –600 μm to the caudal end of the CnF. Lateral subnuclei were seen in the middle third of the PBN. Among the lateral subnuclei, the internal lateral subnucleus of the PBN (LPBI) contained intensely stained, large (mean axis lengths: 16.0 × 14.9 μm; means of 20 representative cells) round cells. The ventral lateral subnucleus of the PBN (LPBV) contained lightly stained, small (9.9 × 7.9 μm) fusiform cells, covering the dorsolateral boundary of the SCP including the dorsal part of the waist area. The LPBD contained intensely stained, medium (13.7 × 5.9 μm) sized, sparsely distributed round cells and was separated from the SCP by the central lateral subnucleus of the PBN (LPBC) that contained lightly stained, medium (10.4 × 9.9 μm) round or fusiform cells. The LPBD was situated laterally at +100 to –350 μm to the caudal end of the CnF and was most condensed at the –150-μm level (Figure 1B), whereas the size of the nucleus decreased when proceeding more caudally (Figure 1C,D). The LPBE was the largest of the lateral subnuclei and was situated between the lateralmost part of the SCP and the ventral spinocerebellar tract, abutting both. Two parts were identified in the LPBE, the LPBEo and inner (LPBEi) part, which contained intensely stained, large (axis lengths: 22.4 × 8.0 μm) fusiform cells and lightly stained, large (24.1 × 6.2 μm) long fusiform cells, respectively. The LPBEo was mainly found at the middle third and was most densely packed with cells at –150 μm caudal to the end of the CnF as seen in the LPBD (Figure 1B).

Three medial subnuclei were identified along the widely spread rostrocaudal axis of the PBN. The MPB was located in the rostral two-thirds of the PBN and abutted the medial boundary of the SCP, which contained lightly stained, small (axis lengths: 10.1 × 6.8 μm) round or fusiform cells. The dorsal medial subnucleus of the PBN was located in the middle third of the PBN, capping the medial dorsal tip of the SCP, and contained intensely stained, medium (13.4 × 10.9 μm) round or oval cells. The external medial subnucleus of the PBN (MPBE) was located in the caudal two-thirds of the PBN, capping the lateral tip of the SCP, and contained a small number of large (18.3 × 9.2 μm) multipolar cells, which were sparsely distributed among the lightly stained, small (5.9 × 4.2 μm) oval or fusiform cells.

The waist area was situated at 0 to –600 μm to the caudal end of the CnF, defined as a narrow region surrounding the SCP at one-third to the dorsal end, and contained round and flat cells running parallel to the SCP as well as the internal bridge cells. The different extent of different subnuclei is shown in Figure 1E to facilitate the comparison.

Retrograde labeling study

Thalamic taste neurons showed robust spontaneous discharges and transiently responded to NaCl applied to the anterior tongue, with a low level of sustained responses (Figure 2C), as reported previously in rats (Scott and Erickson 1971; Nomura and Ogawa 1985). The center of the taste-responsive sites was located in the rostral half of the VPMpc (Figure 2A,B). Using the exploring electrodes or WGA electrodes, the averaged 3-dimensional coordinates of the location at which maximum taste responses occurred were 1.95 ± 0.04 mm (mean ± SE, $n = 20$) caudal to the bregma, 0.96 ± 0.02 mm lateral to the midline, and 3.96 ± 0.03 mm beneath the surface of the brain.

In 14 of the 20 cases, taste responses were successfully recorded with WGA electrodes. In 4 of the 14 cases, WGA-HRP injection sites were centered on the VPMpc and the injections were mostly restricted within the VPMpc. In another 9 of the 14 cases, the center of the injection sites were within 50 μm from the lateral border ($n = 4$), within 350 μm from the anterior border ($n = 3$), or within 50 μm from the dorsal or ventral border ($n = 2$) of the VPMpc. Retrograde labeled somata and anterograde labeled axon terminals were observed in the ventromedial-most portion of the thalamic reticular nucleus from the VPMpc. In the remaining mouse ($n = 1$), in which the taste responses were recorded with WGA electrodes but without labeling in the reticular nucleus, the injection center was 650 μm from the anterior border of the VPMpc.

In 3 of the remaining 6 cases in which taste responses were not recorded with WGA electrodes, the centers of the injection sites were within 50 μm from the border of the VPMpc: within the VPMpc ($n = 1$), in the parafascicular thalamic

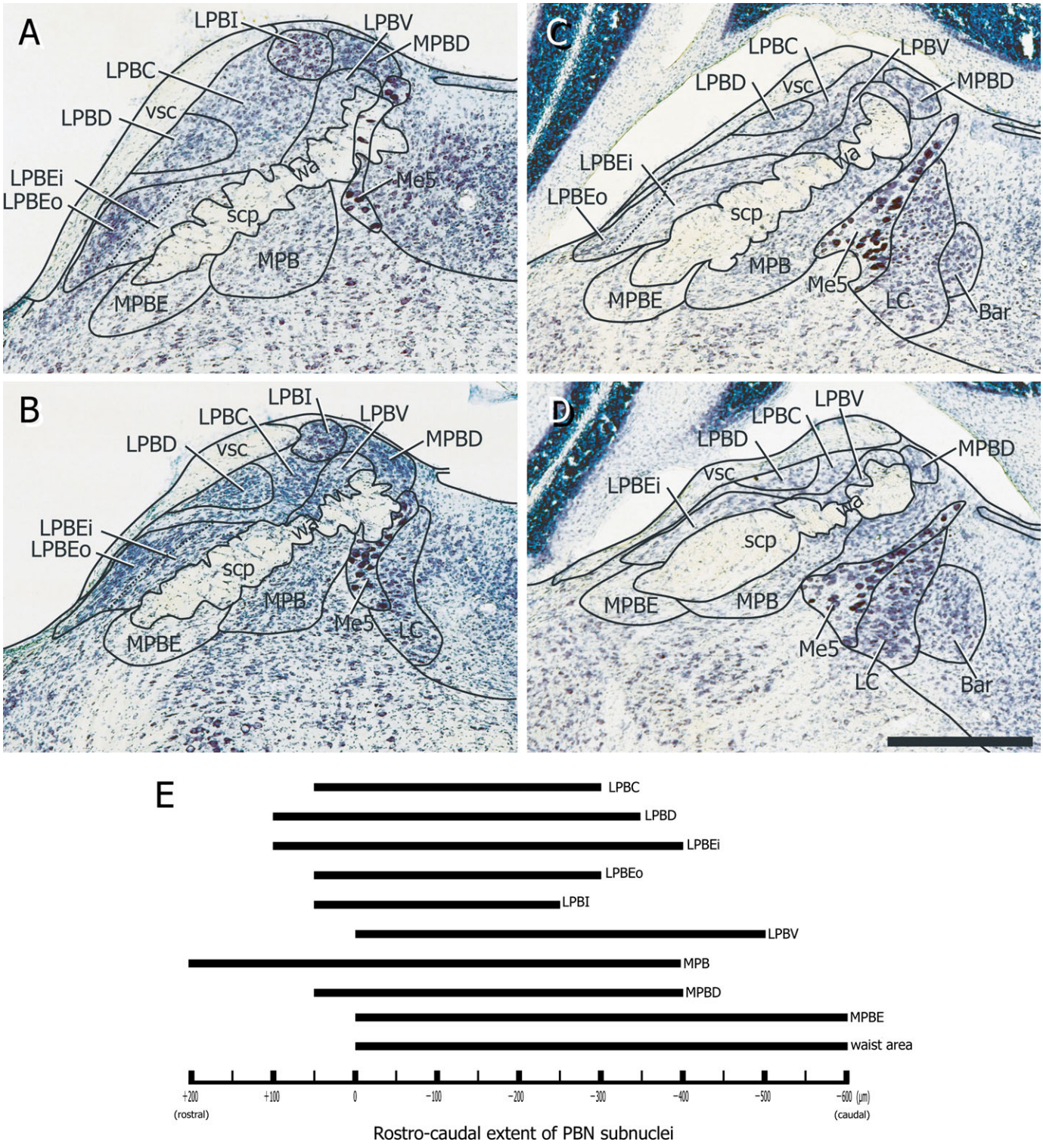


Figure 1 Low-power bright-field micrographs of coronal sections through the PBN and the rostrocaudal extent of each subnucleus **(A–D)**. Section (A) is the most rostral, and section (D) is the most caudal. Subnuclei are delineated with lines in the figure. Fifty micrometer–thick sections were stained with thionin. Sections at (A) −50 μm, (B) −150 μm, (C) −250 μm, and (D) −350 μm caudal to the caudal end of the CnF. Scale bar = 500 μm. In **(E),** the origin of the scale indicates the caudal end of the CnF.

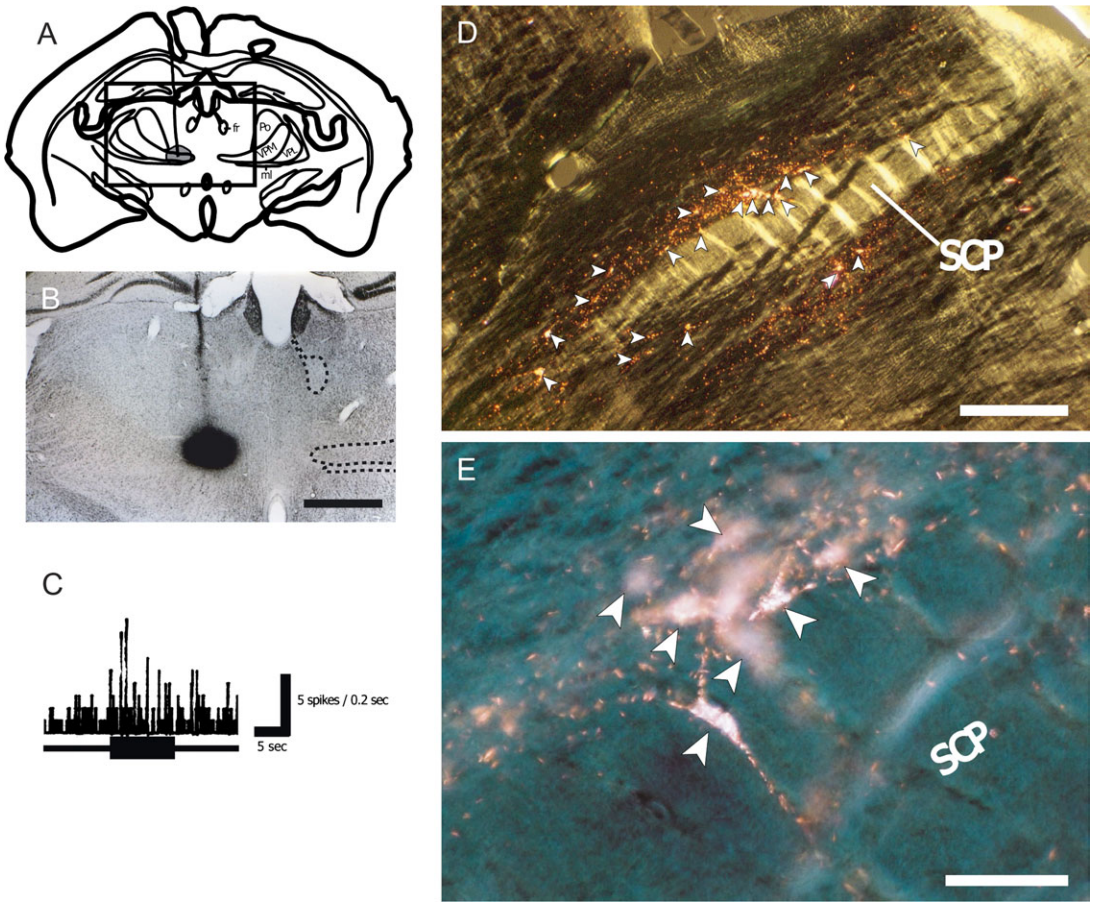


Figure 2 Location of a representative WGA-HRP injection site in the VPMpc where taste activities were recorded and distribution of retrograde labeled cells in the PBN after injection of WGA-HRP in the VPMpc in the mouse. **(A)** Schematic illustration of the injection site (shaded area). **(B)** Low-power bright-field micrograph of the field including the injection site, as indicated by the square in A. The injection site was centered at the VPMpc. **(C)** Responses of multiunit to 0.1 M NaCl recorded in the injection site. Responses are expressed in a peristimulus time histogram. Bin width, 0.2 s. The taste stimulus was applied to the anterior tongue. Thin and thick horizontal bars represent periods for DW and 0.1 M NaCl stimuli, respectively. **(D)** Dark-field micrograph of the ipsilateral PBN at −150 μm caudal to the caudal end of the CnF. The injection site is shown in A and B. **(E)** High-power micrograph of the LPBEi. Arrowheads shown in (D) and (E) indicate retrograde labeled cells including blurred labeled cells in a different plane of focus. Scale bars = 500 μm in (B), 250 μm in (D), and 50 μm in (E).

nucleus (PF, n = 1), and in the ventromedial thalamic nucleus (VM, n = 1). Retrograde labeled somata and anterograde labeled axon terminals were observed in the ventromedial-most portion of the thalamic reticular nucleus. In the other 3 cases, the injection sites were centered 450 μm from the anterior border of the VPMpc (n = 1) or 100 μm from the ventral border of the VPMpc (n = 2), that is, the VM. Retrograde labeled somata and anterograde labeled axon terminals were not observed in the ventromedial-most portion of the thalamic reticular nucleus. All these cases were also used to analyze retrograde labeling in the brain stem.

Distributions of taste relay neurons

In the 4 cases in which the WGA-HRP injection was restricted to the VPMpc (described above), neuronal somata retrogradely labeled with WGA-HRP (WGA cells) were asymmetrically distributed on the ipsilateral and contralateral sides of the PBN. However, the mean number of WGA cells did not significantly differ between the ipsilateral (123.5 ± 21.6, mean ± SE) and contralateral sides (115.5 ± 19.6) (n = 4, $P > 0.05$, Student's t-test). On the ipsilateral side, at –150 μm caudal to the caudal end of the CnF, WGA cells were observed in the LPBEi, MPB, and MPBE in the PBN and were mainly located at the boundary with the SCP (Figure 2D,E). On the other hand, at the same level on the contralateral side, WGA cells were mostly observed in the MPBE (Figure 3F). On both sides, proceeding from –150 to –350 μm caudal to the caudal end of the CnF, WGA cells shifted to the waist area including the LPBV laterally and the dorsal part of the MPB medially (Figure 3). At all these locations, WGA cells were observed at the boundary of the SCP and inside the SCP but not in the LPBD or LPBEo.

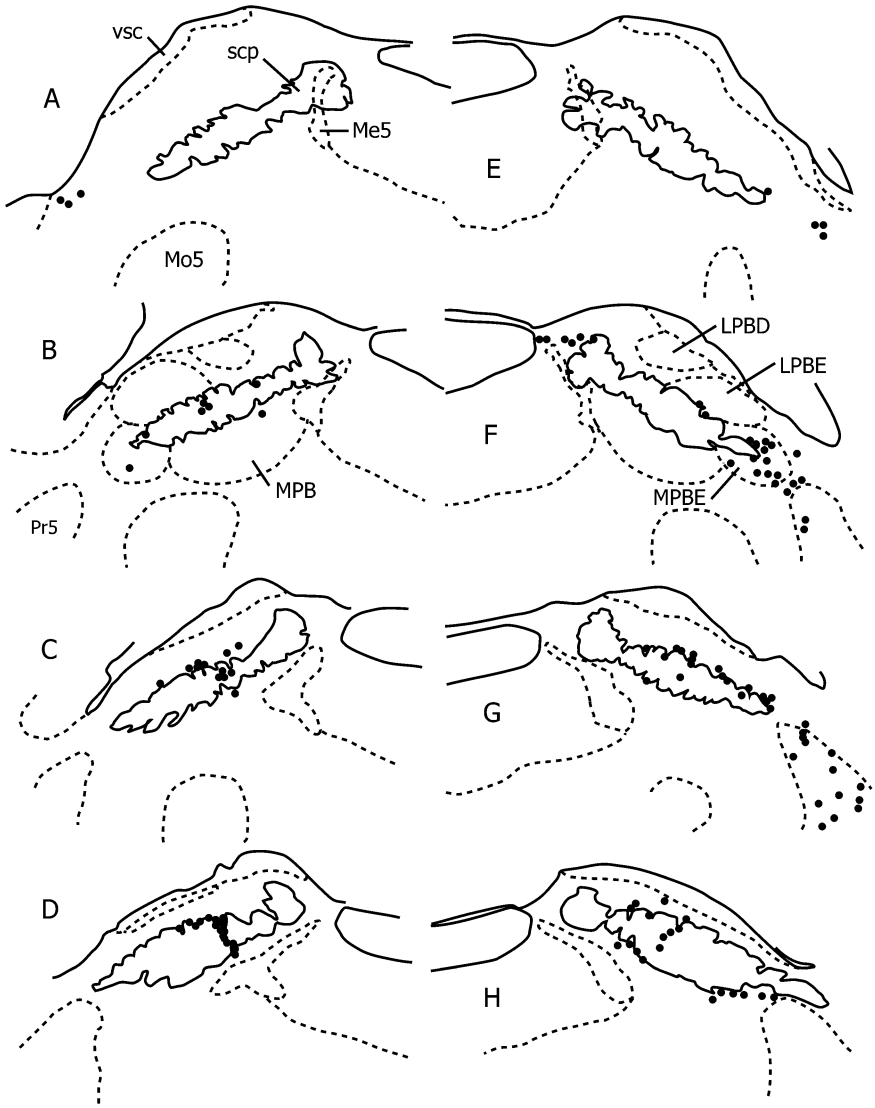


Figure 3 Schematic illustration of the distribution of retrograde labeled cells from the VPMpc in various coronal planes of the PBN in one representative case. WGA-HRP was injected into the taste-responsive region of the VPMpc. Single dots indicate single retrograde labeled cells. **(A–D)** Ipsilateral side to the injection site. **(E–H)** Contralateral side. (A and E) −50 μm, (B and F) −150 μm, (C and G) −250 μm, and (D and H) −350 μm caudal to the caudal end of the CnF.

On the contralateral side, several WGA cells were found at the boundary between the SCP and the principal sensory trigeminal nucleus (Pr5), where taste neurons have been rarely recorded except for the MPBE (Norgren and Pfaffmann 1975; Ogawa et al. 1987; Halsell and Travers 1997; Miyaoka et al. 1997; Karimnamazi et al. 2002; Shimura et al. 2002).

In 6 of the 16 cases in which WGA electrodes were not centered at the VPMpc or the injected WGA-HRP sites were not restricted within the VPMpc, injection sites were centered or 50 μm lateral from the border of the VPMpc, but a large amount of tracer was found in a broad region that included the VPMpc. WGA cells were observed in almost the same subnuclei of the PBN as for the WGA-HRP injection sites, which were restricted to the VPMpc, except for 2 cases in which the injection sites were centered dorsally (i.e., PF) or ventrally (i.e., VM). In these exceptional cases, labeled cells were also found in the LPBI or in the Pr5 and in the trigeminal motor nucleus (Mo5) and as found in the cases with dorsal or ventral injections mentioned below.

In the remaining 10 cases, WGA-HRP injections spared the VPMpc and no labels were found in the PBN except for 2 cases of WGA-HRP injection in the PF. In 2 cases in which the injection sites were 450–650 μm anterior from the anterior border of the VPMpc, no label was observed in the PBN. In 2 cases in which injections were made at 300–350 μm anterior to the VPMpc, WGA cells were mainly found in the Mo5. Injection at 100 μm posteriorly (n = 1)

resulted in WGA cells in the Pr5. Ventral injections with the VM centered (n = 3) resulted in WGA cells in the Mo5 and Pr5, and the dorsal cases with the PF centered (n = 2) showed WGA cells in the LPBI. In the cases in which the WGA cells were observed in the Mo5, there were many terminals and the cells were not typically large-sized motor neurons, but there were a few interneuron-like small multipolar cells.

Locations of FLI-positive cells

After being trained to drink DW for 10 min daily in their individual cages, mice drank 2.82 ± 0.12 ml DW (mean ± SE, n = 24) on the last training day. On the first experimental day, they drank either 3.68 ± 0.42 ml (n = 4) of 0.1 M NaCl or 3.08 ± 0.32 ml (n = 8) of 0.1 M LiCl. In the NaCl intake group, mice preferred to drink a NaCl solution continuously for the first 5 min but intermittently for the following 5 min. In contrast, although mice in the LiCl intake group also preferred to drink the LiCl solution for the first 5 min, all of them manifested uncomfortable behavioral responses, such as urination and "lying on belly" (Meachum and Bernstein 1990; Swank and Bernstein 1994), within 10 min. LiCl intake did not last as long as NaCl intake, although behavioral symptoms were continuously observed for about 30 min in all mice. Mice also manifested uncomfortable behavioral responses, such as urination and "lying on belly," shortly after receiving the LiCl i.p. injection.

In the NaCl intake group, FLI-positive cells were mainly observed in the LPBD (Figures 4A, 5A–D, and 6). A small number of FLI-positive cells were also observed in the LPBEi, MPBE, and MPB subnuclei. Figure 5A–D shows a representative map of FLI-positive cells along the rostrocaudal axis for the NaCl intake group. In this figure, the number of FLI-positive cells in the LPBD is maximal at the level B, corresponding to the level in Figure 4. In control experiments, almost no FLI-positive cells were observed in naive mice without fluid intake (n = 4, data not shown). Although there were no significant differences between NaCl intake and DW intake ($F_{3,12}$ = 2.90, P = 0.08, Scheffe's post hoc test), lightly stained FLI-positive cells were observed in the center of the LPBD in mice with DW intake compared with NaCl intake and FLI-positive cells were mainly detected at –150 μm caudal to the caudal end of the CnF in mice exposed to DW or salt solutions (Figure 4).

In contrast, in the LiCl intake group, intensely stained FLI-positive cells were observed in both the LPBD and LPBEo (Figures 4B, 5E–H, and 6). A small number of FLI-positive cells were also observed in the LPBEi, MPBE, and MPB (Figure 4B), as in the NaCl intake group. Figure 5E–H shows a representative map along the rostrocaudal axis of FLI-positive cells in the LiCl intake group. In the figure, the number of FLI-positive cells in the LPBD and LPBEo is maximal at the level of F corresponding to the level in Figure 4. Almost no FLI-positive cells were found at the boundary between the SCP and Pr5 in either group.

In the group of mice that received the LiCl i.p. injection, FLI-positive cells were found in both the LPBD and LPBEo and predominantly in the latter (Figure 6). The number of FLI-positive cells was more abundant in both the LPBD and LPBEi in the LiCl intake group than in the other groups.

Discussion

In the present study, the subnuclei of the PBN in mice were first delineated cytoarchitectonically. The subnuclei found in mice was almost the same as those found in rats and hamsters, but the LPBE was subdivided into 2 parts, LPBEo and LPBEi, although this subdivision was also suggested in a tracer study in rats (Bernard et al. 1995). The induction of FLI-positive neurons was undertaken by short exposure (10 min for a stimulus and 20 min for a poststimulus duration) to salt solutions to minimize the postingestional consequences of solution intake because a recent study (Mihály et al. 2005) showed that the induction of FLI-positive cells in the neocortex in rats was already significantly elevated at 30 min compared with that at 1 h.

Taste-related region in the PBN in mice: comparison with rats and hamsters

A summary of the retrograde labeled nuclei from the thalamus is illustrated in Figure 7. When the injection extended to the PF, the LPBI, MPB, and the waist area were bilaterally labeled (Figure 7A). When the injection extended laterally into the VPM proper, the bilateral waist area, LPBEi and MPB, and the contralateral MPBE and Pr5 were labeled (Figure 7B). When the injection site extended to the VM, the bilateral Pr5 was predominately labeled rather than the other subnuclei (Figure 7C). The possible taste relay neurons identified in mice were mainly distributed in the middle part of the PBN on both sides and they tended to localize in the LPBEi, MPB, MPBE at its rostral region, and in the waist area, including the LPBV, in the caudal region (Figure 7). The PBN subnuclei containing WGA cells are summarized in the right column of Table 1, where bold letters indicate the subnuclei that mainly contained them. The distribution of retrograde labeled cells in mice was broadly in agreement with previous findings in rats (Ogawa and Akagi 1978; Fulwiler and Saper 1984) albeit with some differences. Most WGA cells were observed in the lateral subnuclei and a few within the MPB anterior to the –250-μm level in mice, whereas a considerable number of labeled neurons were within the MPB at this level in rats. Most WGA cells in the waist area were observed at the boundary and/or inside of the SCP at the –350-μm level in mice (Figure 3), but many neurons were labeled distantly from the SCP at the corresponding level in rats.

In hamsters (Halsell 1992), retrograde labeled WGA cells from the VPMpc were distributed in all the PBN subnuclei

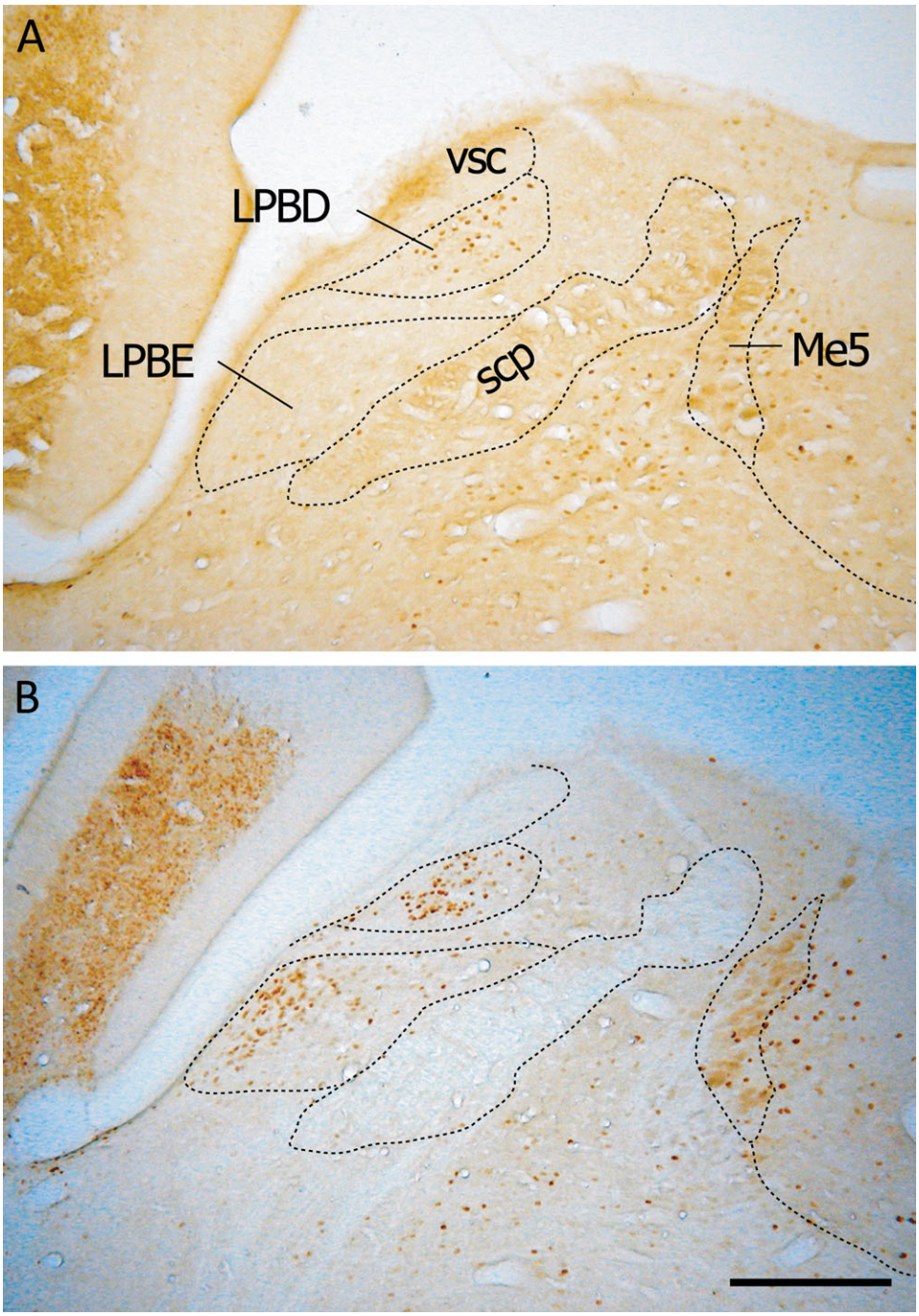


Figure 4 FLI-positive cells in the PBN, resulting from free intake of salt solution by the mouse. **(A** and **B)** Photomicrographs of representative cases. (A) After 0.1 M NaCl intake; (B) after 0.1 M LiCl intake. Sections are taken −150 μm caudal to the caudal end of the CnF, where both the LPBD and LPBEo are clearly observed. Scale bar = 500 μm.

surrounding the SCP at the rostral level (corresponding at –250-μm level in mice) and shifted to the medial subnuclei (i.e., the waist area) at a more caudal level. The distribution patterns from the lateral to medial subnuclei along the rostral to caudal level in hamsters were similar to our observations in mice. However, some differences were noticed between the 2 species. Many WGA cells were reported to be distant from the SCP in hamsters but were restricted to the waist area, MPB and LPBEi abutting to the SCP at a level more posterior to the –150-μm level in mice. This difference might have been caused by the accuracy or variation of the injection sites in the VPMpc, by injecting different amounts of the tracer or, more interestingly, by a genuine difference between the 2 species.

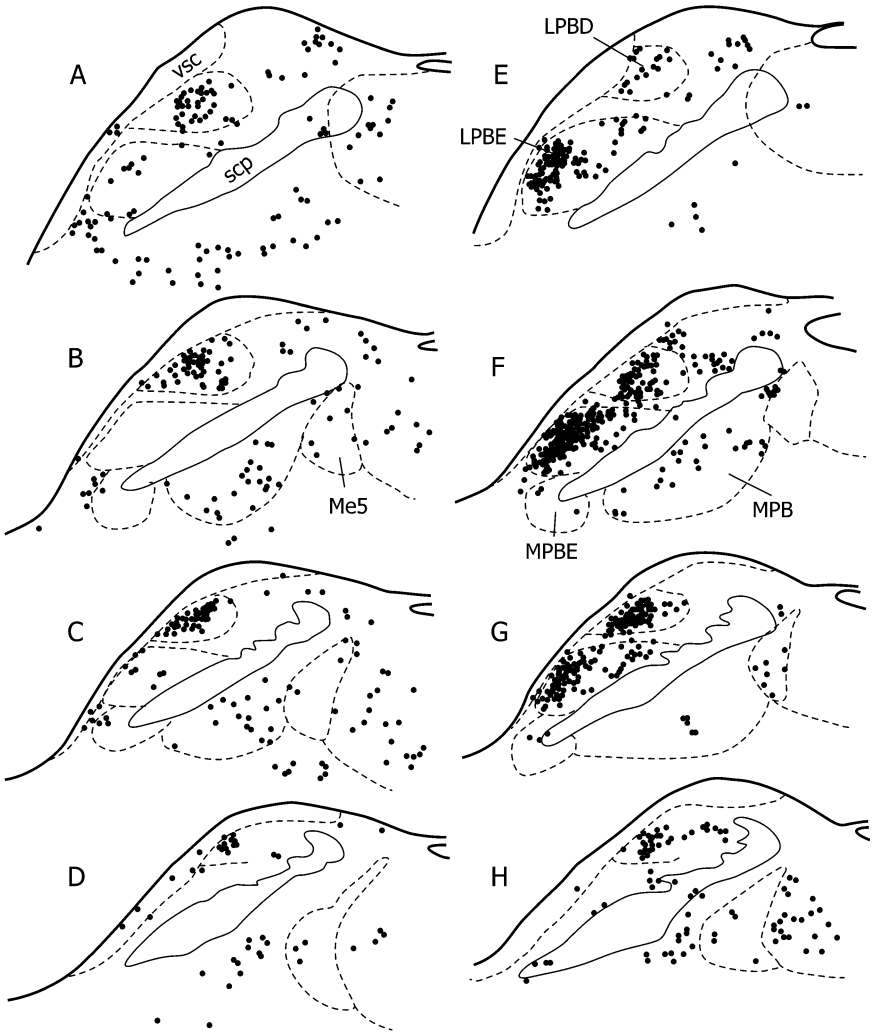


Figure 5 Schematic illustration of the distribution of FLI-positive cells in various coronal planes of the PBN in mouse after intake of one of the 2 different salt solutions. Single dots indicate single cells. **(A–D)** 0.1 M NaCl, **(E–H)** 0.1 M LiCl. Sections are at (A and E) −50 μm, (B and F) −150 μm, (C and G) −250 μm, and (D and H) −350 μm caudal to the caudal end of the CnF.

Taste-responsive neurons have been mapped electrophysiologically in the rat PBN. Taste neurons were mainly distributed in the lateral side at the rostral level (corresponding to anterior to the –150-μm level in mice) and were concentrated in the waist area at the caudal level (corresponding to posterior to the –250-μm level in mice) (Norgren and Pfaffmann 1975; Ogawa et al. 1987; Hayama et al. 1994; Miyaoka et al. 1997; Shimura et al. 2002). All previous reports have shown that most taste neurons exist in regions adjacent to the SCP but not in the LPBD and LPBEo. The electrophysiological findings in rats are consistent with a previous tracing study in rats (Fulwiler and Saper 1984) in that taste neurons were concentrated in the waist area. However, the present tracing findings in mice slightly differ from the results in rats. In the rostral PBN in rats (corresponding to anterior to the –150-μm level in mice), taste-responsive neurons were mainly detected in the waist area with a few in the MPBE; in contrast, the WGA cells in mice were observed in the MPBE, LPBEi, and MPB on the border with the SCP but not in the waist area (Figure 3B,F). However, in the caudal PBN (at the –350-μm level), WGA cells were observed in both the dorsal and ventral sides of the waist area in mice (Figure 3D,H), which is consistent with electrophysiological data in rats (Halsell and Travers 1997).

On the other hand, an earlier electrophysiological study (Halsell and Frank 1991) identified taste-responsive areas in the LPBE abutting the SCP at the rostral level (corresponding to the –250-μm level in mice) and in the MPB abutting the SCP and within the SCP at the caudal level (corresponding to the –350-μm level or more caudal in mice). The present findings in mice are consistent with the electrophysiological data in hamsters. If so, the architecture of the PBN subnuclei in mice might more closely resemble that of hamsters rather than rats. The comparable anatomical data

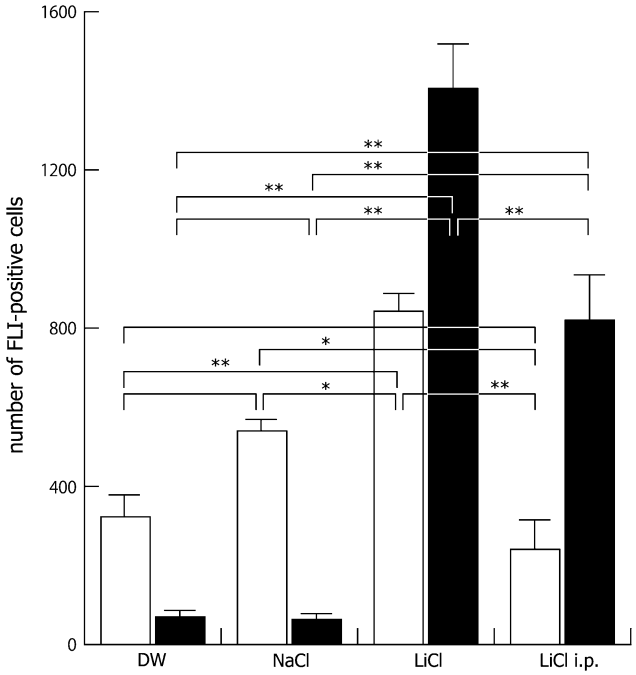


Figure 6 Mean number (±SE, $n = 4$ each) of FLI-positive cells in the LPBD (white bar) and LPBEo (black bar) after DW intake, 0.1 M NaCl intake, 0.1 M LiCl intake, or an i.p. injection of 0.15 M LiCl (2% of body weight) stimuli. FLI-positive cells were counted from 0 to −500 μm caudal to the caudal end of the CnF on both sides of the brain. $*P < 0.05$, $**P < 0.01$ (1-way analysis of variance followed by Scheffe's post hoc test).

in mice will allow us to better characterize the functional organization of taste.

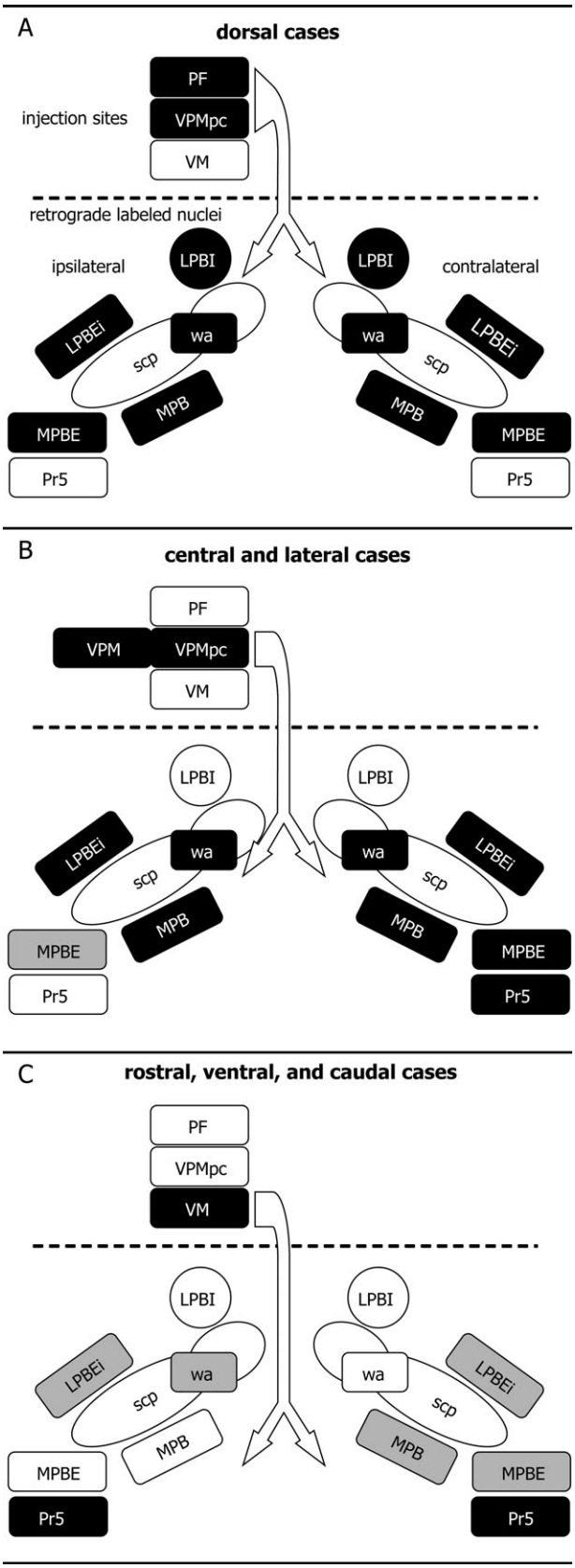
Although WGA cells were found at the boundary between the SCP and dorsal Pr5, and the Mo5, most of them were probably not taste relay neurons because taste neurons have not been recorded in these regions in rats. Therefore, the Mo5 received sparse afferent inputs from the Pr5 in rats (Waite and Tracey 1995), and the WGA cells in Mo5 might be considered to be transsynaptic transported consequences from the Pr5 in mice.

c-Fos immunohistochemical study

To investigate whether salt taste induces c-Fos expression in PBN subnuclei involved in the taste relay, we examined FLI-positive cells after intake of salt solutions, 0.1 M NaCl or 0.1 M LiCl, and compared them with those induced after DW intake and LiCl i.p. injection. The left column in Table 1 shows a summary of the distribution of FLI-positive cells in the PBN subnuclei after stimulation. We found that NaCl intake induced FLI-positive cells mainly in the LPBD (bold), whereas LiCl intake induced them in both the LPBD and LPBEo (bold), as observed in rats. However, both salts induced a few FLI-positive cells in the LPBEi, MPBE, MPB, and waist area in mice, where most of the retrograde labeled cells from the VPMpc were observed. The present findings differed from the results in rats because it has been reported that 0.2 or 0.3 M NaCl induced a cluster of FLI in a region that partly overlaps the waist area ventrally to the SCP (Yamamoto et al. 1993; Travers 2002), a region that roughly corresponds to the MPB in the present study. Moreover, 0.003 M quinine–HCl induced FLI-positive cells within the MPBE (Travers et al. 1999; King et al. 2003).

The discrepancy of our findings from those of previous studies can probably be ascribed to some or all of the following reasons; 1) functional differences of the subnuclei between species, 2) differences in the route of administration of taste stimuli to the animals, 3) differences in the concentration of solutions used, and 4) differences in the length of time for which animals were exposed to the taste stimuli. Taste neurons have been found around the SCP, including the waist area, and most sodium-sensitive neurons were observed in the MPB in rats (Ogawa et al. 1987). Accordingly, c-Fos protein was induced in the MPB by salt intake in rats (Travers 2002). However, no attempt has been made to electrophysiologically record taste neurons in the PBN in mice. We allowed the mice to drink the taste stimuli freely, but previous investigators forced the rats to drink fixed volumes of fluid through intraoral tubes (Yamamoto et al. 1993; Travers 2002). Such forced stimulation without warning might have caused the animals to breathe irregularly so that some respiration-related neurons could be activated in these subnuclei (Dawid-Milner et al. 2003). Although we used 0.1 M salt solutions, previous investigators used 0.2 or 0.3 M solutions (Yamamoto et al. 1993; Travers 2002) that were hypertonic and aversive as they induced postingestional factors (or visceral irritation) (Iwasaki and Sato 1984). In addition, mice were exposed to salt solutions for 10 min and a postingestion time of 20 min in the present study, but rats were exposed to them for 20–30 min and a postingestion time of 45 min to 2 h. Long exposure to hypertonic salt solutions and/or long postingestion time might have evoked postingestional consequences, such as visceral stress or satiety, to induce FLI-positive cells in subnuclei not related to taste sensation. In our preliminary experiments, the postingestion time for 2 h probably induced more FLI-positive cells in some PBN subnuclei than for a short postingestion time. Both the short and long postingestion times induced c-Fos protein in almost the same number of neurons in the LPBD and LPBEo subnuclei after LiCl intake stimuli, but some subnuclei were only affected by the long postingestion time. Therefore, the individual differences and the conditions of the immunohistochemical procedure likely affected the results of the number of FLI-positive cells, a finding that needs further investigation.

On the other hand, LiCl i.p. injection in mice induced FLI-positive cells, predominantly in the LPBEo (Table 1, bold), occupying the lateral two-thirds of the LPBE, as found in rats (Yamamoto, Shimura, Sakai, and Ozaki 1994; Koh et al. 2003). Therefore, the LPBEo might be the subnucleus that relays abdominal illness in mice, as in rats.



Differences between the distributions of retrograde labeled taste relay cells and FLI-positive cells

At –150 μm caudal to the caudal end of the CnF, where the LPBD, LPBEo, and LPBEi were easily delineated from each other, some of the retrograde labeled WGA cells from the taste-responsive site in the 0 were found in a few subnuclei where numerous FLI-positive cells were induced by NaCl intake. Table 1 summarizes the distribution of the FLI-positive cells and WGA cells in the PBN subnuclei. WGA cells were found in the LPBEi, MPB, and MPBE, whereas FLI-positive cells were mainly observed in the LPBD with a few in the LPBEi and MPB. Although both WGA cells and FLI-positive cells were found in the LPBEi and MPB subnuclei, their intranuclear distribution was quite different. WGA cells were localized at the boundary between the aforementioned subnuclei and the SCP, but FLI-positive cells were diffusely distributed in the subnuclei. In contrast, at –350 μm caudal to the caudal end of the CnF, WGA cells and FLI-positive cells were differentially distributed. WGA cells were located in the waist area, but FLI-positive cells occupied the LPBD and LPBEo, the same subnuclei as found at –150 μm. A large number of FLI-positive cells were induced in the LPBD.

Table 1 Summary of the distribution of the FLI-positive cells and the WGA cells in the PBN subnuclei[a]

	FLI-positive cells	WGA cells	
Stimuli	Both rostral and caudal	Rostrally	Caudally
NaCl intake	**LPBD**	**LPBEi**	**Waist area**
	LPBEi	**MPB**	
	MPB	**MPBE**	
LiCl intake	**LPBD**		
	LPBEo		
	LPBEi		
	MPB		
LiCl i.p.	LPBD		
	LPBEo		

[a]Bold letters indicate the main induction sites of the FLI-positive cells and the subnuclei where retrograde labeled WGA cells (taste relay cells) were most frequently observed.

Figure 7 Schemes summarizing the innervations between the injection sites and the retrograde labeled nuclei around the SCP. Each nucleus is collectively shown along the rostrocaudal axis. **(A)** Dorsal injection cases (n = 3), **(B)** Central and lateral injection cases (n = 9), **(C)** Rostral, ventral, and caudal injection cases (n = 8). The possibility of innervations between the injection sites and retrograde labeled nuclei are indicated as high (≥50% of the cases, black), middle (16–49% of the cases, gray), or low (≤15% of the cases, white).

FLI-positive cells induced by LiCl intake were distributed differently from WGA cells and even from those induced by NaCl intake. At both –150 and –350 μm caudal to the caudal end of the CnF, the former were mainly located in both the LPBD and LPBEo with some cells in the LPBEi and MPB, whereas no WGA cells were found in the LPBD and LPBEo.

Thus, except for a few cells abutting the SCP in the LPBEi, the distribution pattern of WGA cells relaying taste signals greatly differed from that of FLI-positive cells evoked by both salt stimuli. Based on our criteria for successful tracer injection, we feel that both the LPBD and LPBEo are not involved in the taste relay and that PBN cells yielding c-Fos protein respond to those stimuli evoked secondarily by taste stimulation, such as abdominal information but not salt stimuli themselves. In other words, the cells involved in conveying signals secondary to taste stimulation and/or pure abdominal irritation caused by activation of the vagal nerve innervating visceral organs or activation of the area postrema via vascular routes probably do not carry taste information to the thalamic taste relay. This hypothesis remains to be proved in future studies by double labeling taste-induced FLI-positive PBN neurons with WGA in the VPMpc. It has been reported that transection of both the glossopharyngeal and chorda tympani nerves did not significantly attenuate quinine-stimulated FLI in the external subdivision (King et al. 2003) and that neurons in the area postrema are excited by gastric administration of LiCl (Tsukamoto and Adachi 1994).

The present study cytoarchitecturally divided the LPBE into 2 parts, the LPBEi and the LPBEo. The LPBEi contained taste relay neurons that were retrograde labeled from the VPMpc and a few FLI-positive cells in response to NaCl intake or LiCl intake, although their intranuclear distributions were different. In contrast, the LPBEo did not contain any taste relay neurons but contained numerous FLI-positive cells in response to LiCl intake and LiCl i.p. injection. These findings suggest that the LPBEi is involved in both taste relay and visceral information whereas the LPBEo is involved only in visceral information, consistent with previous studies in rats (Krout et al. 1998; Karimnamazi et al. 2002), in which the LPBEo was reported to receive inputs from the caudal nucleus of the NTS (visceral) and that the LPBEi receives inputs from both the caudal and rostral (taste-responsive) NTS.

In the present study, we identified 2 separate groups of subnuclei in the mouse PBN: 1) subnuclei containing cells that were retrograde labeled from the thalamic taste relay nucleus (i.e., the LPBEi, MPB, and waist area) and 2) subnuclei that mainly displayed FLI-positive cells in response to salt intake (i.e., the LPBD and LPBEo). Moreover, the LPBEi and LPBEo were cytoarchitectonically distinct in mice, and FLI-positive cells were mainly located in the LPBEo. Although some taste relay neurons and FLI-positive cells were found in the LPBEi and MPB, their intranuclear distribution was apparently different. Thus, c-Fos protein was not induced in most of the taste relay neurons in the PBN projecting to the thalamic taste relay nucleus. Moreover, FLI-positive cells in the LPBEo were induced by LiCl irrespective of the administration protocol but not by NaCl. Thus, they did not represent taste signals but rather signals that were secondarily induced by taste stimuli, for example, visceral signals themselves, or that might be involved in taste aversion learning or memory. We consider that these issues might be resolved in future studies by using various strains of mice or transgenic mice.

Funding

Japanese Ministry of Education, Science and Culture (research grant 06680789).

Acknowledgements

We thank Dr Tomio Hayama for advice on electrophysiological and anatomical techniques, Dr Tamio Nakamura for writing a spike-analyzing program based on LabVIEW (National Instruments Co, Austin, TX), and Dr Tatsuya Tajima for invaluable suggestions.

Abbreviations

Bar	Barrington's nucleus
CnF	cuneiform nucleus
fr	fasciculus retroflexus
LC	locus coeruleus
LPBC	central lateral subnucleus of the parabrachial nucleus
LPBD	dorsal lateral subnucleus of the parabrachial nucleus
LPBE	external lateral subnucleus of the parabrachial nucleus
LPBEi	inner part of the external lateral subnucleus of the parabrachial nucleus
LPBEo	outer part of the external lateral subnucleus of the parabrachial nucleus
LPBI	internal lateral subnucleus of the parabrachial nucleus
LPBV	ventral lateral subnucleus of the parabrachial nucleus
Me5	mesencephalic trigeminal nucleus
ml	medial lemniscus
Mo5	trigeminal motor nucleus
MPB	medial subnucleus of the parabrachial nucleus
MPBC	central medial subnucleus of the parabrachial nucleus
MPBD	dorsal medial subnucleus of the parabrachial nucleus
MPBE	external medial subnucleus of the parabrachial nucleus
NTS	solitary tract
PBN	parabrachial nucleus
PF	parafascicular thalamic nucleus
Po	posterior thalamic nuclear group
Pr5	the principal sensory trigeminal nucleus
scp	superior cerebellar peduncle
VM	ventromedial thalamic nucleus
VPL	ventral posterolateral nucleus in the thalamus
VPM	ventral posteromedial nucleus in the thalamus
VPMpc	parvicellular part of the ventral posteromedial nucleus in the thalamus
vsc	ventral spinocerebellar tract
wa	waist area

References

Baird JB, Travers SP, Travers JB. 2001. Integration of gastric distension and gustatory responses in the parabrachial nucleus. Am J Physiol Regul Integr Comp Physiol. 281:R1581–R1593.

Bernard JF, Alden M, Besson JM. 1993. The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: a *Phaseolus vulgaris* leucoagglutinin (PHA-L) study in the rat. J Comp Neurol. 329:201–229.

Bernard JF, Dallel R, Raboisson P, Villanueva L, Bars DL. 1995. Organization of the efferent projections from the spinal cervical enlargement to the parabrachial area and periaqueductal gray: a PHA-L study in the rat. J Comp Neurol. 353:480–505.

Bernard JF, Huang GF, Besson JM. 1994. The parabrachial area: electrophysiological evidence for an involvement in visceral nociceptive processes. J Neurophysiol. 71:1646–1660.

Chamberlin NL, Saper CB. 1992. Topographic organization of cardiovascular responses to electrical and glutamate microstimulation of the parabrachial nucleus in the rat. J Comp Neurol. 326:245–262.

Dampney RAL. 1994. Functional organization of central pathways regulating the cardiovascular system. Physiol Rev. 74:323–364.

Dawid-Milner MS, Lara JP, López de Miguel MP, López-González MV, Spyer KM, González-Barón S. 2003. A5 region modulation of the cardiorespiratory responses evoked from parabrachial cell bodies in the anaesthetized rat. Brain Res. 982:108–118.

Franklin KBJ, Paxinos G. 1997. The mouse brain in stereotaxic coordinates. San Diego (CA): Academic Press Inc.

Fulwiler CE, Saper CB. 1984. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res Rev. 7:229–259.

Halsell CB. 1992. Organization of the parabrachial nucleus efferents to the thalamus and amygdala in the golden hamster. J Comp Neurol. 317:57–78.

Halsell CB, Frank ME. 1991. Mapping study of the parabrachial taste-responsive area for the anterior tongue in the golden hamster. J Comp Neurol. 306:708–722.

Halsell CB, Travers SP. 1997. Anterior and posterior oral cavity responsive neurons are differentially distributed among parabrachial subnuclei in rat. J Neurophysiol. 78:920–938.

Hayama T, Hashimoto K, Ogawa H. 1994. Anatomical location of a taste-related region in the thalamic reticular nucleus in rats. Neurosci Res. 18:291–299.

Hayama T, Ogawa H. 2001. Two loci of the insular cortex project to the taste zone of the nucleus of the solitary tract in rats. Neurosci Lett. 303:49–52.

Institute of Laboratory Animal Resources. 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press.

Iwasaki K, Sato M. 1984. Neural and behavioral responses to taste stimuli in the mouse. Physiol Behav. 32:803–807.

Jhamandas JH, Aippersbach SE, Harris KH. 1991. Cardiovascular influences on rat parabrachial nucleus: an electrophysiological study. Am J Physiol. 260:R225–R231.

Karimnamazi H, Travers JB. 1998. Differential projections from gustatory responsive regions of the parabrachial nucleus to the medulla and forebrain. Brain Res. 813:283–302.

Karimnamazi H, Travers SP, Travers JB. 2002. Oral and gastric input to the parabrachial nucleus of the rat. Brain Res. 957:193–206.

King CT, Deyrup LD, Dodson SE, Galvin KE, Garcea M, Spector AC. 2003. Effects of gustatory nerve transection and regeneration on quinine-stimulated Fos-like immunoreactivity in the parabrachial nucleus of the rat. J Comp Neurol. 465:296–308.

Koh MT, Clarke SN, Spray KJ, Thiele TE, Bernstein IL. 2003. Conditioned taste aversion memory and c-Fos induction are disrupted in RIIβ-protein kinase A mutant mice. Behav Brain Res. 143:57–63.

Krout KE, Jansen ASP, Loewy AD. 1998. Periaqueductal gray matter projection to the parabrachial nucleus in rat. J Comp Neurol. 401:437–454.

Meachum CL, Bernstein IL. 1990. Conditioned responses to a taste conditioned stimulus paired with lithium chloride administration. Behav Neurosci. 104:711–715.

Menendez L, Bester H, Besson JM, Bernard JF. 1996. Parabrachial area: electrophysiological evidence for an involvement in cold nociception. J Neurophysiol. 75:2099–2116.

Mesulum MM. 1978. Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J Histochem Cytochem. 26:106–117.

Mihály A, Borbély S, Világi I, Détári L, Weiczner R, Zádor Z, Krisztin-Péva B, Bagosi A, Kopniczky Z, Zádor E. 2005. Neocortical *c-fos* mRNA transcription in repeated, brief, acute seizures: is c-fos a coincidence detector? Int J Mol Med. 15:481–486.

Miyaoka Y, Shingai T, Takahashi Y, Yamada Y. 1997. Responses of parabrachial nucleus neurons to chemical stimulation of posterior tongue in chorda tympani-sectioned rats. Neurosci Res. 28:201–207.

Moga MM, Herbert H, Hurley KM, Yasui Y, Gray TS, Saper CB. 1990. Organization of cortical, basal forebrain, and hypothalamic afferents to the parabrachial nucleus in the rat. J Comp Neurol. 295:624–661.

Nomura T, Ogawa H. 1985. The taste and mechanical response properties of neurons in the parvicellular part of the thalamic posteromedial ventral nucleus of the rat. Neurosci Res. 3:91–105.

Norgren R. 1995. Gustatory system. In: Paxinos G, editor. The rat nervous system. 2nd ed. San Diego (CA): Academic Press, Inc. p. 751–771.

Norgren R, Pfaffmann C. 1975. The pontine taste area in the rat. Brain Res. 91:99–117.

Ogawa H, Akagi T. 1978. Afferent connections to the posteromedial ventral nucleus from the pons and the rostra medulla in the rat. Kumamoto Med J. 31:54–62.

Ogawa H, Hayama T, Ito S. 1987. Response properties of the parabrachio-thalamic taste and mechanoreceptive neurons in rats. Exp Brain Res. 68:449–457.

Saper CB. 1995. The spinoparabrachial pathway: shedding new light on an old path. J Comp Neurol. 353:477–479.

Scott TR Jr, Erickson RP. 1971. Synaptic processing of taste-quality information in thalamus of the rat. J Neurophysiol. 34:868–883.

Shimura T, Tokita K, Yamamoto T. 2002. Parabrachial unit activities after the acquisition of conditioned taste aversion to a non-preferred HCl solution in rats. Chem Senses. 27:153–158.

Swank MW, Bernstein IL. 1994. c-Fos induction in response to a conditioned stimulus after single trial taste aversion learning. Brain Res. 636:202–208.

Travers JB, Urbanek K, Grill HJ. 1999. Fos-like immunoreactivity in the brainstem following oral quinine stimulation in decerebrate rats. Am J Physiol. 277:R384–R394.

Travers SP. 2002. Quinine and citric acid elicit distinctive Fos-like immunoreactivity in the rat nucleus of the solitary tract. Am J Physiol Regul Integr Comp Physiol. 282:R1798–R1810.

Tsuda N, Tohmi M, Mizuno M, Nawa H. 2006. Strain-dependent behavioral alterations induced by peripheral interleukin-1 challenge in neonatal mice. Behav Brain Res. 166:19–31.

Tsukamoto G, Adachi A. 1994. Neural responses of rat area postrema to stimuli producing nausea. J Auton Nerv Syst. 49:55–60.

Waite PM, Tracey DJ. 1995. Trigeminal sensory system. In: Paxinos G, editor. The rat nervous system. 2nd ed. San Diego (CA): Academic Press, Inc. p. 705–724.

Yamamoto T, Sawa K. 2000. c-Fos-like immunoreactivity in the brainstem following gastric loads of various chemical solutions in rats. Brain Res. 866:135–143.

Yamamoto T, Shimura T, Sakai N, Ozaki N. 1994. Representation of hedonics and quality of taste stimuli in the parabrachial nucleus of the rat. Physiol Behav. 56:1197–1202.

Yamamoto T, Shimura T, Sako N, Sakai N, Tanimizu T, Wakisaka S. 1993. c-Fos expression in the parabrachial nucleus after ingestion of sodium chloride in the rat. Neuroreport. 4:1223–1226.

Yamamoto T, Shimura T, Sako N, Yasoshima Y, Sakai N. 1994. Neural substrates for conditioned taste aversion in the rat. Behav Brain Res. 65:123–137.

Accepted December 24, 2008