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Afferent connections of the parabrachial nucleus in C57BL/6J mice

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Abstract

Although the mouse is an experimental model with an increasing importance in various fields of Neuroscience, the characteristics of its central gustatory pathways have not yet been well documented. Recent electrophysiological studies using the rat and hamster have revealed that taste processing in the brainstem gustatory relays is under the strong influence of inputs from forebrain gustatory structures. In the present study, we investigated the organization of afferent projections to the mouse parabrachial nucleus (PbN), which is located at a key site between the brainstem and gustatory, viscerosensory and autonomic centers in the forebrain. We made injections of the retrograde tracer Fluorogold centered around the “waist” area of the PbN, whose neurons are known to be highly responsive to taste stimuli. Retrogradely labeled neurons were found in the infralimbic, dysgranular and agranular insular cortex as well as the claustrum; the bed nucleus of the stria terminalis and the substantia innominata; the central nucleus of the amygdala; the lateral and medial preoptic areas, the paraventricular, the dorsomedial, the ventromedial, the arcuate, and the lateral hypothalamic areas; the periaqueductal gray, the substantia nigra pars compacta, and the ventral tegmental area; the supratrigeminal nucleus, rostral and caudal nucleus of the solitary tract; the parvocellular intermediate and gigantocellular reticular nucleus; the caudal and interpolar divisions of the spinal trigeminal nucleus, dorsomedial spinal trigeminal nucleus, and the area postrema. Numbers of labeled neurons in the main components of the gustatory system including the insular cortex, bed nucleus of the stria terminalis, central nucleus of the amygdala, lateral hypothalamus, and rostral nucleus of the solitary tract were quantified. These results are basically consistent with those of the previous rat and hamster studies, but some species differences were found. Functional implications of these afferent inputs are discussed with an emphasis on their role in taste.

Keywords

taste system; cortex; amygdala; hypothalamus; bed nucleus of the stria terminalis; nucleus of the solitary tract

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Introduction

A number of previous studies have elucidated the basic neuroanatomical characteristics of the central taste system in the rat and hamster, traditional model species in the field of gustatory neurobiology. In these model organisms, afferent fibers of the VIIth, IXth, and Xth cranial nerves convey gustatory information to the rostral nucleus of the solitary tract (NST) (Brining and Smith, 1996; Contreras et al., 1982; Hamilton and Norgren, 1984; Hayakawa et al., 2001; Torvik, 1956; Whitehead and Frank, 1983; Whitehead, 1986). From the NST, ascending fibers project to the pontine parabrachial nucleus (PbN) (Halsell et al., 1996; Herbert et al., 1990; Norgren, 1978; Norgren and Leonard, 1971; Travers, 1988; Whitehead, 1990). The PbN projects to the gustatory cortex via the ventroposteromedial nucleus of the thalamus (VPMpc), and to various limbic structures including the central nucleus of the amygdala (CeA), the bed nucleus of the stria terminalis (BST), and the lateral hypothalamus (LH) (Fulwiler and Saper, 1984; Halsell, 1992; Karimnamazi and Travers, 1998; Norgren and Leonard, 1973; Norgren, 1974; Norgren and Wolf 1976; Saper and Loewy, 1980). These same forebrain areas, excepting the VPMpc, project back to the NST and PbN (Allen et al., 1991; Halsell, 1992; Kang and Lundy, 2009; Krettek and Price, 1978; Moga et al., 1989, 1990a,b; Saggi and Lundy, 2008; Shipley, 1982; Shipley and Sanders, 1982; van der Kooy et al., 1984; Veening et al., 1978; Whitehead et al., 2000). Electrophysiological studies demonstrate that these descending projections play a role in modulating gustatory-evoked activity in brainstem nuclei (Cho et al., 2003; Di Lorenzo, 1990; Di Lorenzo and Monroe, 1992, 1995; Huang et al., 2003; Kang et al., 2004; Li et al., 2002, 2005; Li and Cho, 2006; Lundy and Norgren, 2001, 2004; Matsuo et al., 1984; Tokita et al., 2004; Smith et al., 2005).

The mouse also has become one of the most important model organisms for the study of the taste system, yielding a wealth of information about the genetic basis of gustatory and ingestive behavior (see, for review, Boughter and Bachmanov, 2007). Work stemming from strain comparisons and from gene-targeted models has enabled the recent advancement of molecular biological insights into the structure, development, and function of both peripheral and central taste systems (i.e. Chandrashekar et al., 2006).

Although previous reports concerning the gustatory abilities of mice are more or less confined to behavior and the peripheral gustatory nervous system, recent neurophysiological and *c-fos* immunohistochemical studies, some of them utilizing knockout or transgenic mice, have focused on the characterization of responses of taste neurons in the mouse NST and PbN (Hashimoto et al., 2009; McCaughey, 2007; Lemon and Margolskee, 2009; Travers et al., 2007). Moreover, a recent study using genetic tracing techniques demonstrated functionally segregated pathways for sweet and bitter receptor input in the mouse central nervous system (Sugita and Shiba, 2005).

The current state of knowledge of the neuroanatomical organization of the gustatory system in mice is limited as compared to traditional models such as rat or hamster. However there is evidence of intriguing species differences: A recent study has shown that mouse fungiform taste buds are innervated by only four or five geniculate ganglion neurons whose fibers converge on a single taste bud, whereas in rats and hamsters branching afferent nerve fibers innervate several buds on the anterior tongue (Zafini and Whitehead, 2006; Fish et al., 1944; Whiteside, 1927). Centrally, the distribution pattern of terminals of peripheral taste nerves in the mouse NST appears similar to that of rat and hamster although only a few studies have been conducted in the mouse so far (Åstrom, 1953; Ganchrow et al., 2007). Zaidi et al. (2008) injected transsynaptic anterograde pseudorabies virus into fungiform taste papillae in order to describe the pattern of synaptically connected pathways from the geniculate ganglion to the first two brainstem taste relays, i.e. the NST and PbN. They found that different geniculate ganglion cells are engaged in either the ascending taste pathway involving the gustatory

subdivision of the NST and PbN (lemniscal pathway), or in local oromotor circuits involving the NST and reticular formation. Finally, Hashimoto et al. (2009) have demonstrated the possibility of a species difference in mouse parabrachial taste neuron responsiveness, and subnuclear projection pattern to the VPMpc, in comparison to rats and hamsters. Using both anatomical (WGA-HRP) and functional (c-fos immunohistochemistry) methods, they demonstrated that VPMpc-projection neurons in the mouse PbN were located more closely to the superior cerebellar peduncle than in the hamster, and that oral NaCl stimulation induced only weak c-fos expression in the waist area where NaCl induces both robust c-fos expression and electrophysiological taste responses in the rat (Shimura et al., 2002; Tokita et al., 2004, 2007; Travers, 2002).

Although these studies demonstrate that knowledge about the mouse central gustatory system is beginning to accumulate, the organization of afferent inputs to the PbN, a significant source of modulation of gustatory activity in the rat and hamster, has not yet been elucidated. In the present study, we investigated the afferent connection patterns of the mouse PbN, a key interface between brainstem and forebrain gustatory and visceral areas. We analyzed projections to the PbN both from the brainstem and forebrain areas by injection of a retrograde tracer into the whole PbN. Thus, the aim of this study is to offer anatomical data in the mouse that will help in understanding the comparative basis of taste behavior, and physiology.

Experimental Procedures

Subjects

A total of 12 male and female C57BL/6J mice (19 – 28 g) were used. The animals were maintained in a temperature- and humidity-controlled colony room on a 12 h light/12 h dark cycle (lights on at 0700 h, off at 1900 h), and were given *ad libitum* access to normal dry pellet (22/5 rodent diet, Harlan Teklad, Madison, WI) and water. This study was approved by the Animal Care and Use Committee at UTHSC, and all experiments were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996.

Surgery

Animals were anesthetized with Ketamine/Xylazine (100/10 ml/kg) and positioned in a stereotaxic frame (Stoelting, Wood Dale, IL). The scalp was opened with a midline incision, and the skull was leveled between bregma and lambda by adjusting the bite bar. The body temperature was maintained at 35 °C using a heating pad (Elenco electronics, Wheeling, IL). A glass micropipette (20–25 µm tip diameter) filled with 5% Fluorogold (FG) was lowered into the PbN (5.4 mm caudal, 1.2 mm lateral, 3.55 mm ventral to bregma) with a micromanipulator (SM-191, Narishige, Tokyo, Japan). FG was injected into the unilateral PBN either by iontophoresis (2 µA, 5 s on/off for 15 min; n = 7) or via a Picospritzer II (General Valve Corp., Fairfield, NJ) pressure injector system (60 nl, n = 5). The injection pipette was left in place for 10 min both before and after the injection was made. Although injections were not physiologically guided, we have recorded *in vivo* both single-unit and multi-unit taste activity at these same coordinates in similarly sized C57BL/6J male and female mice (data not shown). Supplemental anesthetic was administered as necessary throughout the surgery to maintain the animals under deep anesthesia. After recovery from surgery, none of the mice displayed abnormal behavior or locomotion.

Tissue preparation

After a 4-day survival period, mice were perfused transcardially with phosphate-buffered saline and 4% paraformaldehyde. The brains were removed and placed in 10% formalin and then transferred to a 30% buffered sucrose solution and stored at 4°C for at least 1 week. Coronal

sections (40 μ m) were cut serially using a freezing microtome and divided into two adjacent series. One series was stained with cresyl violet to reveal cytoarchitecture, and the adjacent series was used for observation of fluorescent tracer. Both series were mounted and coverslipped on silane-coated slides (Scientific Device Laboratory, Des Plaines, IL) with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI).

Microscopic analysis of sections

Fluorescent labeling in all sections was imaged and analyzed using a Leica (DMRXA2, Leica Microsystems, Bannockburn, IL) episcopic-fluorescence microscope equipped with a digital camera (Hamamatsu ORCA-ER) and imaging software (SimplePCI). The distribution of retrogradely labeled neurons by FG was established by plotting labeled neurons onto representative atlas sections (e.g. Figure 3). In select regions of the gustatory axis, including IC, BST, CeA, LH, and rNST, retrogradely labeled neurons were counted in each animal. The rostral – caudal extent of each area in each animal was determined, and fluorescent cell profiles were counted within each nucleus in every other section along the rostral – caudal axis.

In all regions quantified, numbers of labeled neurons were compared with respect to ipsilateral–contralateral location (relative to injection site) with *t*-tests. Numbers of labeled neurons across gustatory regions, and NST subnuclei, were compared with a repeated measures ANOVA and Post hoc analysis where appropriate (Tukey's test). The statistical rejection criterion (i.e., α) was set at 0.05.

Results

PbN injection site

Microscopic observation revealed that in all of the 12 animals used in this study injection sites encompassed both medial and lateral parts of the PbN including the “waist area” which is known to be highly responsive to taste stimuli in the rat and hamster. FG spread to some extent to the dorsal edge of the supratrigeminal nucleus, but there was no invasion into the locus coeruleus, cuneiform nucleus, or cerebellum. Examples of typical injections, and plots of the maximum extent of all injection sites are shown in Figure 1. Generally, injections made via pressure ($n = 5$) produced slightly larger injection volumes, and therefore a greater number of cells were labeled in projection sites. However, injections made via iontophoresis ($n = 7$), while somewhat smaller, produced an overall pattern of labeling that was identical to those made via pressure.

Projections to PbN

Neurons retrogradely labeled with Fluorogold were found in both forebrain and brainstem. Examples of labeled neurons in several structures along the gustatory axis are shown in Figure 2. Labeled neurons were found in the dysgranular and agranular insular cortex (DI and AI) as well as the claustrum (Cl) (Fig. 2A); the BST (Fig. 2B); the CeA (Fig. 2C); the LH (Fig. 2D); and the NST (Fig. 2F).

Cortex

Retrogradely labeled neurons were found in layer V of the insular cortex along its whole rostrocaudal axis, preferentially in the ipsilateral dysgranular and agranular parts (Fig. 3A–G). Labeled neurons in the contralateral side were sparse. Some neurons were located in the somatosensory cortex, infralimbic and dorsal peduncular cortex (Fig. 3A–G). There was also dense labeling in the claustrum which is located medial to the insular cortex (Fig. 3A–G).

Ventral forebrain

Strong labeling was seen in various areas in the ventral forebrain such as the bed nucleus of the stria terminalis (BST), substantia innominata (SI), central nucleus of the amygdala (CeA) and hypothalamus. The BST as well as the CeA and LH were the forebrain regions where the most robust labeling was observed. Multiple BST subnuclei, especially the LD, LP, TLP and TLI, contained numerous labeled neurons (Fig. 3D–E). These neurons extended ventrally into the SI, lateral preoptic area, medial preoptic area, and medial sublentiform external amygdala (Fig. 3E–F). Within the caudal CeA, labeled neurons were most numerous in the lateral division, but significant labeling appeared in the medial subdivision in the rostral CeA. Numbers of labeled neurons in the lateral division (115.25 ± 6.43) was significantly higher than in the medial subdivision (79.7 ± 4.80) at the rostrocaudal level of Fig. 3H (*t*-test, $p < 0.05$). No labeling was found in the lateral or basolateral amygdaloid subnuclei (Fig. 3G–H). Retrogradely labeled neurons in the BST and CeA were almost exclusively found ipsilateral to the injection site. Relatively heavy labeling was present in the paraventricular nucleus of the hypothalamus at the rostrocaudal level of the rostral edge of the ventromedial hypothalamic nucleus (Fig. 3G). There were dense labeled neurons in the lateral hypothalamus (LH) throughout its rostrocaudal extent (Fig. 3F–J). Labeling in the dorsomedial, ventromedial, arcuate, and median preoptic nucleus was weak (Fig. 3D–I).

Midbrain and pons

There was moderate labeling in the bilateral ventral tegmental area (Fig. 3K). In the periaqueductal gray (PAG), retrogradely labeled neurons were particularly numerous in the ipsilateral dorsolateral PAG (Fig. 3J–L). Ventral to the PAG, a few labeled neurons were found in the dorsal raphe nucleus (Fig. 3L). The lateral part of the compact zone of the substantia nigra contained many labeled neurons (Fig. 3K). In the contralateral PbN, there were some labeled neurons in the lateral aspect. Only moderate or weak labeling was found in the taste-responsive area in the PbN (Fig. 3M). Ventral to the PbN, the supratrigeminal nucleus contained many labeled cells on both sides (Fig. 3M).

Medulla

Numerous retrogradely labeled neurons were present throughout the rostrocaudal axis of the NST. The most extensive labeling was found in the rostral part of the NST. Ipsilateral dominance of labeling was greater in the rostral than in the caudal NST (Fig. 3N–R). Labeled neurons in the NST extended ventrally to the parvicellular, intermediate, and gigantocellular reticular nuclei and spinal trigeminal nucleus. Labeling in the reticular medullary formation was widespread, and became denser more rostrally. Strong labeling was seen in the caudal part of the area postrema, but it became very weak with slight ipsilateral dominance as it extended rostrally (Fig. 3Q). Many labeled neurons were found with a very slight ipsilateral dominance in the entire medullary reticular formation along the rostrocaudal axis (Fig. 3N–R). Labeling was most evident in the rostral parvicellular reticular nucleus (Fig. 3N–O). Numerous labeled neurons were located in the spinal trigeminal nucleus with moderate ipsilateral dominance. In the caudal spinal trigeminal nucleus, labeled cells were found preferentially in the outer laminae (Fig. 3R). Rostrally, labeling was widely seen in the spinal trigeminal nucleus as well as dorsomedial part of it (Fig. 3N–Q). Labeling was also found in cell bodies in the spinal trigeminal tract (Fig. 3P–Q).

Quantification of labeled neurons

Number of labeled neurons in the GC, BST, CeA, LH, and rostral NST (rNST) are summarized in Table 1. In all regions quantified, numbers of labeled neurons on the ipsilateral side were significantly higher than those of the contralateral side to the injection site ($p < 0.01$). Numbers of neurons per section in these areas are shown in Figure 4. Repeated measures ANOVA

revealed a significant main effect of region ($F[4,44] = 33.13, p < 0.01$). Post hoc analysis showed that numbers of labeled neurons in the CeA were significantly higher than all other areas ($p < 0.01$).

The rNST was further divided into 4 subdivisions (M, RC, RL and V) in accordance with previous reports using rat, hamster, and mouse (Fig. 4C and D) (Ganchrow, 2007; Gill et al., 1999; Halsell et al., 1996; Harrer and Travers, 1996; Whitehead, 1988, 1990; Whitehead et al., 1993; Zaidi et al., 2008) and numbers of labeled neurons in these regions were quantified. Repeated measures ANOVA revealed a significant main effect of region ($F[3,33] = 57.69, p < 0.01$). Post hoc analysis (Tukey's test) demonstrated that numbers of labeled neurons in the RC were significantly higher than all other areas ($p < 0.01$). Neurons with different morphological characteristics such as elongate and stellate cells (Fig. 4A and B), which have been described in the rat, hamster and mouse rNST, were also observed in the present study (Halsell et al., 1996; King and Bradley, 1994; Whitehead, 1988, 1990; Whitehead et al., 1993; Zaidi et al., 2008).

Discussion

The anatomical organization of the mouse central taste system has been described by only a limited numbers of studies (Åstrom, 1953; Ganchrow et al., 2007; Hashimoto et al., 2009; Shipley, 1982; Shipley and Geinisman, 1982; Zaidi et al., 2008). The results of the present experiments were qualitatively similar to those of previous studies using other species of rodents (Allen et al., 1991; Halsell, 1992; Herbert et al., 1990; Kang and Lundy, 2009; Krettek and Price, 1978; Moga et al., 1989, 1990a,b; Ricardo and Koh, 1978; Saggu and Lundy, 2008; Saper, 1982a,b; Shipley, 1982; Shipley and Sanders, 1982; Travers, 1998; van der Kooy et al., 1984; Veening et al., 1978; Whitehead, 1990; Whitehead et al., 2000) by demonstrating that the mouse PbN receives afferent fibers from a variety of gustatory, visceral, autonomic, and oromotor regions in the brainstem and forebrain. This report offers the beginnings of a central anatomical basis for physiological and behavioral approaches in this important species.

Recent electrophysiological studies in the rat and hamster have focused on understanding the functional roles of descending projections from the higher gustatory centers to the PbN (Di Lorenzo, 1990; Di Lorenzo and Monroe, 1992; Li and Cho, 2006; Li et al., 2005; Lundy and Norgren, 2001, 2004; Matsuo et al., 1984; Tokita et al., 2004). These studies clearly indicate that parabrachial gustatory activity is under strong centrifugal influence. Although such experiments have not yet been conducted in the mouse, our results suggest that similar mechanisms also exist in this species. Because our injections targeted the taste-responsive waist area of the PbN, we focus predominantly on the role of these projections in taste processing; however, our tracer injections spread throughout other areas of the PbN, and it is important recognize that many of these afferent fibers will also play a role in non-taste sensory and visceral function.

Projections from the forebrain

Consistent with previous studies in the rat, we showed that the mouse infralimbic and insular cortex also have descending projections to the PbN (Moga et al., 1990a; Saper, 1982a,b; van der Kooy, 1984). Insular cortical neurons that send axons to the PbN were mainly located in the dysgranular and agranular regions, similar to previous studies (Allen et al., 1991; Kang and Lundy, 2009; Moga et al., 1990a; Saggu and Lundy, 2008; Saper, 1982a,b; Shipley and Sanders, 1982). These regions correspond to electrophysiologically identified taste-responsive insular cortex in the rat (Kosar et al., 1986), which is also known to send efferent fibers back to the rNST and medial PbN where gustatory information is processed (Whitehead et al., 2000).

Prominent labeling was found in both the CeA and the BST, as has been shown in the rat and hamster (Dong et al., 2001; Dong and Swanson, 2003; Halsell, 1992; Kang and Lundy, 2009; Kretek and Price, 1978; Moga et al., 1989, 1990a; Saggi and Lundy, 2008; van der Kooy et al., 2004; Veening et al., 1984). Several independent findings showed that descending inputs from the CeA to the PbN can modulate taste activities (Huang et al., 2003; Kang et al., 2004; Li et al., 2005; Lundy and Norgren, 2001, 2004; Tokita et al., 2004). Veening et al. (1984) reported that by far the majority of retrogradely labeled neurons were observed in the medial subdivision of the CeA after injection of the retrograde tracers True Blue or bisbenzimidazole in the rat. In contrast, Halsell (1992) observed much denser labeling in the lateral subdivision than in the medial subdivision of the CeA after injection of WGA-HRP in the hamster. An intermediate result using rats was reported by Moga et al. (1990a), where they found that FG injections into medial PbN resulted in dense labeling in the medial division of the CeA. However, many neurons were also labeled following injections into the ventrolateral part of the PbN. As our FG injections encompassed almost the whole PbN it is impossible to show topographic differences in the projection patterns from various PbN subnuclei. However, we observed strong labeling in both medial and lateral divisions of the CeA. Caudally, labeling was strong in the lateral division and rostrally, labeling was strong in the medial division. The gustatory function of PbN-projecting neurons in the BST is unclear, but Li and Cho (2006) recently demonstrated that electrical stimulation of BST produced exclusively inhibitory effects on taste neurons in the hamster PbN.

It has been demonstrated that the PbN receives descending projections from the lateral hypothalamus (LH) in the rat (Berk and Finkelstein 1982; Moga et al., 1990a,b; Saper et al., 1979; Veening et al., 1987). In agreement with these studies, we showed numerous labeled neurons extending from the regions dorsal and lateral to the fornix to the ventrolateral part of the LH. These PbN-projecting neurons are known to contain various neuropeptides (Moga et al., 1990b). Recent studies have shown that the rat PbN-projecting neurons in the LH contain feeding-related neuropeptides such as corticotropin-releasing hormone, melanin-concentrating hormone and orexin (Kelly and Watts, 1998; Peyron et al., 1998; Touzani et al., 1993). This anatomical evidence suggests that the taste system and the LH “feeding center” have a close interaction (Yamamoto, 2008).

Moga et al. (1990a) reported that numerous cells in the median preoptic nucleus (MnPO) were labeled following FG injection into the rat PbN. Considering large FG injections in the present study, it is noteworthy that only a few scattered neurons in the median preoptic nucleus were retrogradely labeled. This slight discrepancy could be due to species differences between rat and mouse although its functional implication is not clear. PbN-MnPO connections are reported to be reciprocal and have been implicated in various physiological functions such as autonomic responses to noxious or thermal stimuli in the rat (Hermanson et al., 1998; Moga et al., 1990a; Nakamura and Morrison, 2008).

Projections from the pons and medulla

Retrogradely-labeled neurons in the PbN contralateral to the injection side were mainly located in the dorsal-half of the rostral PbN which is not highly responsive to taste stimuli (Halsell and Travers, 1997; Shimura et al., 2002; Tokita, 2004). In the typical taste-responsive zone in the PbN, the “waist” region, there were only a few labeled cells. These results were consistent with those of rats (Moga et al., 1990a) but not of hamster where no single labeled neurons were found in the contralateral PbN even after large HRP injection (Whitehead, 1990). It is suggested that interactions of gustatory information between the PbN on both sides are not frequent even though there seem to exist some species differences as to the strength of mutual connections.

Although FG injection into the PbN was too large to investigate subnuclear projection patterns, our results are generally consistent with previous reports showing that the PbN receives a

massive projection from both rostral and caudal nucleus of the solitary tract (Cho et al., 2002; Cho and Li, 2008; Halsell et al., 2006; Herbert et al., 1990; Karimnamazi et al., 2002; Norgren, 1978; Whitehead, 1990; Williams et al., 1996). Projection from the NST was bilateral, but much stronger from the ipsilateral side to the injection than from the contralateral side as has been reported for the rat (Herbert et al., 1990; Karimnamazi et al., 2002; Norgren, 1978; Williams et al., 1996). However, hamster NST neurons are known to project almost exclusively to the ipsilateral PbN (Cho and Li, 2008; Travers, 1988; Whitehead, 1990). The percentage of PbN-projection neurons in each subnucleus in the rNST were very similar to those reported in the rat and hamster (Gill et al., 1999; Halsell et al., 1996). In the present study, 53.1 % of PbN-projection neurons were located in the RC, where there exists the heaviest synaptic endings of taste primary afferent axons (Ganchrow et al., 2007; May and Hill, 2006; Whitehead, 1988; Whitehead and Frank, 1983; Zaidi et al., 2008), and where taste-responsive neurons are located (Harrer and Travers, 1996; McPeeters et al., 1990; Travers and Norgren, 1995). Consistent with the previous reports using mouse (Zaidi et al., 2008), NST neurons with similar morphological characteristics (i.e. elongate and stellate cells) described in the rat and hamster were also found in the present study (Halsell et al., 1996; King and Bradley, 1994; Whitehead, 1988, 1990; Whitehead et al., 1993). Our results, generally similar to those of previous reports in the rat and hamster (Herbert et al., 1990; Whitehead, 1990), also showed that numerous cells are retrogradely labeled in the medullary reticular formation after FG injections into the PbN; this area is thought to contain pre-motor neurons controlling oromotor behaviors (DiNardo and Travers, 1997). This projection may be related to circuitry coordinating oromotor rejection or ingestive responses; trigeminal premotor neurons involved in mastication have been localized in the lateral part of the PbN (Inoue et al., 1992).

Reciprocal connections between the forebrain structures and PbN

It is noteworthy to indicate that the forebrain structures which send descending fibers to the PbN in the present study corresponds well to those which receive efferent inputs from the PbN in the rat and hamster (Fulwiler and Saper, 1984; Halsell, 1992; Saper and Loewy, 1980). Furthermore, the density of afferent and efferent connections is strongly correlated, i.e., many of the structures with dense inputs from the PbN tend to send dense fibers back to the PbN. Although a detailed study about efferent connections of the mouse PbN has not yet been done, findings from other rodent species strongly suggest that gustatory information is processed in a highly mutual fashion between the PbN and forebrain. Future studies may investigate these reciprocal connections in gene-targeted mouse models in pursuit of understanding the link between taste and motivational processes such as feeding or reward.

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Abbreviations

ac	anterior commissure
AcbC	nucleus accumbens core
AcbSh	nucleus accumbens shell
AI	agranular insular cortex

AID	agranular insular cortex, dorsal
AIV	agranular insular cortex, ventral
AP	area postrema
Arc	arcuate hypothalamic nucleus
BST	bed nucleus of the stria terminalis
MPI	medial division, posterointermediate part
MV	medial division, ventral part
LD	lateral division, dorsal part
LP	lateral division, posterior part
LTI	lateral division, intermediate part
LV	lateral division, ventral part
BLA	basolateral nucleus of the amygdala
cc	corpus callosum
CeA	central nucleus of the amygdala
CeC	central nucleus of the amygdala, capsular division
CeL	central nucleus of the amygdala, lateral division
CeM	central nucleus of the amygdala, medial division
CM	central medial thalamic nucleus
Cl	claustrum
CPu	caudate putamen

Cu	cuneate nucleus
DI	dysgranular insular cortex
DM	dorsomedial spinal trigeminal nucleus
DMH	dorsomedial nucleus of the hypothalamus
DP	dorsal peduncular cortex
DRD	dorsal raphe nucleus, dorsal part
DRV	dorsal raphe nucleus, ventral part
DRVl	dorsal raphe nucleus, ventrolateral part
Ect	ectorrhinal cortex
ECu	external cuneate nucleus
En	endopiriform nucleus
f	fornix
fr	fasciculus retroflexus
GI	granular insular cortex
Gi	gigantocellular reticular nucleus
IC	insular cortex
ic	internal capsule
icp	inferior cerebellar peduncle
IL	infralimbic cortex
IP	interpeduncular nucleus

IRt	intermediate reticular nucleus
LA	lateral nucleus of the amygdala
LH	lateral hypothalamus
LPO	lateral preoptic area
LRt	lateral reticular nucleus
LPAG	lateral periaqueductal gray
MD	mediodorsal thalamic nucleus
mlf	medial longitudinal fasciculus
MnPO	median preoptic nucleus
Mo5	motor trigeminal nucleus
MPA	medial preoptic area
MVe	medial vestibular nucleus
NST	nucleus of the solitary tract
M	medial subnucleus of the NST
RC	rostral central subnucleus of the NST
RL	rostral lateral subnucleus of the NST
rNST	rostral nucleus of the solitary tract
V	ventral subnucleus of the NST
PAG	periaqueductal gray
PbN	parabrachial nucleus

PbNm	medial parabrachial nucleus
PbNI	lateral parabrachial nucleus
CL	central lateral subnucleus
CM	central medial subnucleus
DL	dorsal lateral subnucleus
EL	external lateral subnucleus
EM	external medial subnucleus
VL	ventral lateral subnucleus
PCRt	parvicellular reticular nucleus
PCRtA	parvicellular reticular nucleus, alpha
Pir	piriform cortex
PF	parafascicular thalamic nucleus
PH	posterior hypothalamic area
PMnR	paramedian raphe nucleus
PnO	pontine reticular nucleus, oral part
PRh	perirhinal cortex
Pr5DM	principal sensory trigeminal nucleus, dorsomedial part
Pr5VL	principal sensory trigeminal nucleus, ventrolateral part
PS	parastrial nucleus
PV	paraventricular thalamic nucleus

PVA	paraventricular thalamic nucleus, anterior
PVH	paraventricular nucleus of the hypothalamus
py	pyramidal tract
RCh	retrochiasmatic area
RN	red nucleus
SCol	superior colliculus
scp	superior cerebellar peduncle
SI	substantia innominata
SLEAM	sublentic external amygdala, medial
SNC	substantia nigra pars compacta
SpVe	spinal vestibular nucleus
S1	primary somatosensory cortex
S1BF	primary somatosensory cortex, barrel field
S2	secondary somatosensory cortex
sol	solitary tract
sp5	spinal trigeminal tract
SP5C	spinal trigeminal nucleus, caudal division
SP5I	spinal trigeminal nucleus, interpolar division
STh	subthalamic nucleus
SuM	supramammillary nucleus

Su5	supratrigeminal nucleus
VL	ventrolateral thalamic nucleus
VLPAG	ventrolateral periaqueductal gray
VMH	ventromedial nucleus of the hypothalamus
VP	ventral pallidum
VPM	ventral posteromedial nucleus of the thalamus
VPMpc	ventral posteromedial nucleus of the thalamus, parvicellular part
VTa	ventral tegmental area
ZI	zona incerta
12N	hypoglossal nucleus

References

- Allen GV, Saper CB, Hurley KM, Cechetto DF. Organization of visceral and limbic connections in the insular cortex of the rat. *J Comp Neurol* 1991;311:1–16. [PubMed: 1719041]
- Åström KE. On the central course of afferent fibers in the trigeminal, facial, glossopharyngeal, and vagal nerves and their nuclei in the mouse. *Acta Physiol Scand Suppl* 1953;106:209–320.
- Bartel DL, Whitehead MC, Finger TE. Immunohistochemical map of the nucleus of the solitary tract (NTS) in mice. *Chem Senses* 2007;32:A110.
- Berk ML, Finkelstein JA. Efferent connections of the lateral hypothalamic area of the rat: an autoradiographic investigation. *Brain Res Bull* 1982;8:511–526. [PubMed: 6811106]
- Boughter JD Jr, Bachmanov AA. C57BL/6J and DBA/2J mice vary in lick rate and ingestive microstructure. *BMC Neurosci* 2007;18:S3. [PubMed: 17903279]
- Brining SK, Smith DV. Distribution and synaptology of glossopharyngeal afferent nerve terminals in the nucleus of the solitary tract of the hamster. *J Comp Neurol* 1996;365:556–574. [PubMed: 8742302]
- Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. *Nature* 2006;444:288–294. [PubMed: 17108952]
- Cho YK, Li CS, Smith DV. Gustatory projections from the nucleus of the solitary tract to the parabrachial nuclei in the hamster. *Chem Senses* 2002;27:81–90. [PubMed: 11751472]
- Cho YK, Li CS, Smith DV. Descending influences from the lateral hypothalamus and amygdala converge onto medullary taste neurons. *Chem Senses* 2003;28:155–171. [PubMed: 12588737]
- Cho YK, Li CS. Gustatory neural circuitry in the hamster brain stem. *J Neurophysiol* 2008;100:1007–1019. [PubMed: 18525019]
- Contreras RJ, Beckstead RM, Norgren R. The central projections of the trigeminal, facial, glossopharyngeal and vagus nerves: an autoradiographic study in the rat. *J Auton Nerv Syst* 1982;6:303–322. [PubMed: 7169500]

- Di Lorenzo PM. Corticofugal influence on taste responses in the parabrachial pons of the rat. *Brain Res* 1990;530:73–84. [PubMed: 2271954]
- Di Lorenzo PM, Monroe S. Corticofugal input to taste-responsive units in the parabrachial pons. *Brain Res Bull* 1992;29:925–930. [PubMed: 1473025]
- Di Lorenzo PM, Monroe S. Corticofugal influence on taste responses in the nucleus of the solitary tract in the rat. *J Neurophysiol* 1995;74:258–272. [PubMed: 7472329]
- DiNardo LA, Travers JB. Distribution of fos-like immunoreactivity in the medullary reticular formation of the rat after gustatory elicited ingestion and rejection behaviors. *J Neurosci* 1997;17:3826–3839. [PubMed: 9133401]
- Dong HW, Petrovich GD, Watts AG, Swanson LW. Basic organization of projections from the oval and fusiform nuclei of the bed nuclei of the stria terminalis in adult rat brain. *J Comp Neurol* 2001;436(4):430–455. [PubMed: 11447588]
- Dong HW, Swanson LW. Projections from the rhomboid nucleus of the bed nuclei of the stria terminalis: implications for cerebral hemisphere regulation of ingestive behaviors. *J Comp Neurol* 2003;463(4):434–472. [PubMed: 12836178]
- Fish H, Malone PD, Richter CP. The anatomy of the tongue of the domestic Norway rat. I. The skin of the tongue; the various papillae; their number and distribution. *Anat Rec* 1944;89:429–440.
- Ganchrow G, Ganchrow J, Warner N, Whitehead M. The mouse NST: a cytoarchitectonic atlas. *Chem Senses* 2007;32:A110.
- Gill CF, Madden JM, Roberts BP, Evans LD, King MS. A subpopulation of neurons in the rat rostral nucleus of the solitary tract that project to the parabrachial nucleus express glutamate-like immunoreactivity. *Brain Res* 1999;821:251–262. [PubMed: 10064811]
- Halsell CB. Organization of parabrachial nucleus efferents to the thalamus and amygdala in the golden hamster. *J Comp Neurol* 1992;317:57–78. [PubMed: 1374087]
- Halsell CB, Travers SP, Travers JB. Ascending and descending projections from the rostral nucleus of the solitary tract originate from separate neuronal populations. *Neuroscience* 1996;72:185–197. [PubMed: 8730716]
- Halsell CB, Travers SP. Anterior and posterior oral cavity responsive neurons are differentially distributed among parabrachial subnuclei in rat. *J Neurophysiol* 1997;78:920–938. [PubMed: 9307125]
- Hamilton RB, Norgren R. Central projections of gustatory nerves in the rat. *J Comp Neurol* 1984;222:560–577. [PubMed: 6199385]
- Harrer MI, Travers SP. Topographic organization of Fos-like immunoreactivity in the rostral nucleus of the solitary tract evoked by gustatory stimulation with sucrose and quinine. *Brain Res* 1996;711:125–137. [PubMed: 8680855]
- Hashimoto K, Obata K, Ogawa H. Characterization of Parabrachial Subnuclei in Mice with Regard to Salt Tastants: Possible Independence of Taste Relay from Visceral Processing. *Chem Senses* 2009;34:253–267. [PubMed: 19179538]
- Hayakawa T, Takanaga A, Maeda S, Seki M, Yajima Y. Subnuclear distribution of afferents from the oral, pharyngeal and laryngeal regions in the nucleus tractus solitarii of the rat: a study using transganglionic transport of cholera toxin. *Neurosci Res* 2001;39:221–232. [PubMed: 11223468]
- Herbert H, Moga MM, Saper CB. Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. *J Comp Neurol* 1989;283:315–332. [PubMed: 2568370]
- Herbert H, Moga MM, Saper CB. Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. *J Comp Neurol* 1990;293:540–580. [PubMed: 1691748]
- Hermanson O, Telkov M, Geijer T, Hallbeck M, Blomqvist A. Preprodynorphin mRNA-expressing neurones in the rat parabrachial nucleus: subnuclear localization, hypothalamic projections and colocalization with noxious-evoked fos-like immunoreactivity. *Eur J Neurosci* 1998;10:358–367. [PubMed: 9753144]
- Huang T, Yan J, Kang Y. Role of the central amygdaloid nucleus in shaping the discharge of gustatory neurons in the rat parabrachial nucleus. *Brain Res Bull* 2003;61:443–52. [PubMed: 12909288]

- Inoue T, Masuda Y, Nagashima T, Yoshikawa K, Morimoto T. Properties of rhythmically active reticular neurons around the trigeminal motor nucleus during fictive mastication in the rat. *Neurosci Res* 1992;14:275–294. [PubMed: 1334246]
- Kang Y, Yan J, Huang T. Microinjection of bicuculline into the central nucleus of the amygdala alters gustatory responses of the rat parabrachial nucleus. *Brain Res* 2004;1028:39–47. [PubMed: 15518640]
- Kang Y, Lundy RF Jr. Terminal field specificity of forebrain efferent axons to brainstem gustatory nuclei. *Brain Res* 2009;1428:76–85. [PubMed: 19028464]
- Karimnamazi H, Travers JB. Differential projections from gustatory responsive regions of the parabrachial nucleus to the medulla and forebrain. *Brain Res* 1998;813:283–302. [PubMed: 9838165]
- Karimnamazi H, Travers SP, Travers JB. Oral and gastric input to the parabrachial nucleus of the rat. *Brain Res* 2002;957:193–206. [PubMed: 12445962]
- Kelly AB, Watts AG. The region of the pontine parabrachial nucleus is a major target of dehydration-sensitive CRH neurons in the rat lateral hypothalamic area. *J Comp Neurol* 1998;394:48–63. [PubMed: 9550142]
- King MS, Bradley RM. Relationship between structure and function of neurons in the rat rostral nucleus tractus solitarii. *J Comp Neurol* 1994;344:50–64. [PubMed: 8063955]
- Kosar E, Grill HJ, Norgren R. Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. *Brain Res* 1986;379:329–341. [PubMed: 3742225]
- Krettek JE, Price JL. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J Comp Neurol* 1978;178:225–254. [PubMed: 627625]
- Lemon CH, Margolskee. Contribution of the T1r3 taste receptor to the response properties of central gustatory neurons. *J Neurophysiol*. 2009in press
- Li CS, Cho YK, Smith DV. Modulation of parabrachial taste neurons by electrical and chemical stimulation of the lateral hypothalamus and amygdala. *J Neurophysiol* 2005;93:1183–1196. [PubMed: 15483060]
- Li CS, Cho YK. Efferent projection from the bed nucleus of the stria terminalis suppresses activity of taste-responsive neurons in the hamster parabrachial nuclei. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R914–926. [PubMed: 16966389]
- Lundy RF Jr, Norgren R. Pontine gustatory activity is altered by electrical stimulation in the central nucleus of the amygdala. *J Neurophysiol* 2001;85:770–783. [PubMed: 11160511]
- Lundy RF Jr, Norgren R. Activity in the hypothalamus, amygdala, and cortex generates bilateral and convergent modulation of pontine gustatory neurons. *J Neurophysiol* 2004;9:1143–1157. [PubMed: 14627662]
- Matsuo R, Shimizu N, Kusano K. Lateral hypothalamic modulation of oral sensory afferent activity in nucleus tractus solitarius neurons of rats. *J Neurosci* 1984;4:1201–1207. [PubMed: 6726325]
- May OL, Hill DL. Gustatory terminal field organization and developmental plasticity in the nucleus of the solitary tract revealed through triple-fluorescence labeling. *J Comp Neurol* 2006;497:658–669. [PubMed: 16739199]
- McCaughey SA. Taste-evoked responses to sweeteners in the nucleus of the solitary tract differ between C57BL/6ByJ and 129P3/J mice. *J Neurosci* 2007;27:35–45. [PubMed: 17202470]
- McPheeters M, Hettinger TP, Nuding SC, Savoy LD, Whitehead MC, Frank ME. Taste-responsive neurons and their locations in the solitary nucleus of the hamster. *Neuroscience* 1990;34:745–758. [PubMed: 2352650]
- Moga MM, Saper CB, Gray TS. Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. *J Comp Neurol* 1989;283:315–332. [PubMed: 2568370]
- Moga MM, Herbert H, Hurley KM, Yasui Y, Gray TS, Saper CB. Organization of cortical, basal forebrain, and hypothalamic afferents to the parabrachial nucleus in the rat. *J Comp Neurol* 1990a;295:624–661. [PubMed: 1694187]
- Moga MM, Saper CB, Gray TS. Neuropeptide organization of the hypothalamic projection to the parabrachial nucleus in the rat. *J Comp Neurol* 1990b;295:662–682. [PubMed: 1972710]
- Nakamura K, Morrison SF. A thermosensory pathway that controls body temperature. *Nat Neurosci* 2008;11:62–71. [PubMed: 18084288]

- Norgren R, Leonard CM. Taste pathways in rat brainstem. *Science* 1971;173:1136–1139. [PubMed: 4329178]
- Norgren R, Leonard CM. Ascending central gustatory pathways. *J Comp Neurol* 1973;150:217–237. [PubMed: 4723066]
- Norgren R. Gustatory afferents to ventral forebrain. *Brain Res* 1974;81:285–295. [PubMed: 4434198]
- Norgren R, Wolf G. Taste pathways to hypothalamus and amygdala. *J Comp Neurol* 1976;166:17–30. [PubMed: 1262547]
- Norgren R. Projections from the nucleus of the solitary tract in the rat. *Neuroscience* 1978;3:207–218. [PubMed: 733004]
- Paxinos, G.; Franklin, KBJ. *The mouse brain in stereotaxic coordinates*. Vol. 2. San Diego: Academic Press; 2001.
- Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998;18:9996–10015. [PubMed: 9822755]
- Ricardo JA, Koh ET. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res* 1978;153:1–26. [PubMed: 679038]
- Saggu S, Lundy RF. Forebrain neurons that project to the gustatory parabrachial nucleus in rat lack glutamic acid decarboxylase. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R52–57. [PubMed: 17989138]
- Saper CB. Reciprocal parabrachial-cortical connections in the rat. *Brain Res* 1982a;242:33–40. [PubMed: 7104731]
- Saper CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. *J Comp Neurol* 1982b;210:163–173. [PubMed: 7130477]
- Saper CB, Swanson LW, Cowan WM. An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. *J Comp Neurol* 1979;183:689–706. [PubMed: 105019]
- Saper CB, Loewy AD. Efferent connections of the parabrachial nucleus in the rat. *Brain Res* 1980;197:291–317. [PubMed: 7407557]
- Shimura T, Tokita K, Yamamoto T. Parabrachial unit activities after the acquisition of conditioned taste aversion to a non-preferred HCl solution in rats. *Chem Senses* 2002;27:153–158. [PubMed: 11839613]
- Shipley MT. Insular cortex projection to the nucleus of the solitary tract and brainstem visceromotor regions in the mouse. *Brain Res Bull* 1982;8:139–148. [PubMed: 7066705]
- Shipley MT, Geinisman Y. Anatomical evidence for convergence of olfactory, gustatory, and visceral afferent pathways in mouse cerebral cortex. *Brain Res Bull* 1982;12:221–226. [PubMed: 6722597]
- Shipley MT, Sanders MS. Special senses are really special: evidence for a reciprocal, bilateral pathway between insular cortex and nucleus parabrachialis. *Brain Res Bull* 1982;8:493–501. [PubMed: 7116198]
- Smith DV, Ye MK, Li CS. Medullary taste responses are modulated by the bed nucleus of the stria terminalis. *Chem Senses* 2005;30:421–434. [PubMed: 15872146]
- Sugita M, Shiba Y. Genetic tracing shows segregation of taste neuronal circuitries for bitter and sweet. *Science* 2005;309:781–785. [PubMed: 16051799]
- Tokita K, Karádi Z, Shimura T, Yamamoto T. Centrifugal inputs modulate taste aversion learning associated parabrachial neuronal activities. *J Neurophysiol* 2004;92:265–279. [PubMed: 14973323]
- Tokita K, Shimura T, Nakamura S, Inoue T, Yamamoto T. Involvement of forebrain in parabrachial neuronal activation induced by aversively conditioned taste stimuli in the rat. *Brain Res* 2007;1141:188–196. [PubMed: 17276421]
- Touzani K, Tramu G, Nahon JL, Velley L. Hypothalamic melanin-concentrating hormone and alpha-neoendorphin-immunoreactive neurons project to the medial part of the rat parabrachial area. *Neuroscience* 1993;53:865–876. [PubMed: 8487959]
- Torvik A. Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures; an experimental study in the rat. *J Comp Neurol* 1956;106:51–141. [PubMed: 13398491]

- Travers JB. Efferent projections from the anterior nucleus of the solitary tract of the hamster. *Brain Res* 1988;457:1–11. [PubMed: 3167557]
- Travers JB, Norgren R. Afferent projections to the oral motor nuclei in the rat. *J Comp Neurol* 1983;220:280–298. [PubMed: 6315785]
- Travers JB, Herman K, Yoo J, Travers SP. Taste reactivity and Fos expression in GAD1-EGFP transgenic mice. *Chem Senses* 2007;32:129–137. [PubMed: 17082515]
- Travers SP. Quinine and citric acid elicit distinctive Fos-like immunoreactivity in the rat nucleus of the solitary tract. *Am J Physiol Regul Integr Comp Physiol* 2002;282:R1798–1810. [PubMed: 12010763]
- Travers SP, Norgren R. Organization of orosensory responses in the nucleus of the solitary tract of rat. *J Neurophysiol* 1995;73:2144–2162. [PubMed: 7666129]
- van der Kooy D, Koda LY, McGinty JF, Gerfen CR, Bloom FE. The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. *J Comp Neurol* 1984;224:1–24. [PubMed: 6715573]
- Veening JG, Swanson LW, Sawchenko PE. The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. *Brain Res* 1984;303:337–357. [PubMed: 6204716]
- Veening JG, Te Lie S, Posthuma P, Geeraedts LM, Nieuwenhuys R. A topographical analysis of the origin of some efferent projections from the lateral hypothalamic area in the rat. *Neuroscience* 1987;22:537–551. [PubMed: 2823175]
- Whitehead MC. Anatomy of the gustatory system in the hamster: synaptology of facial afferent terminals in the solitary nucleus. *J Comp Neurol* 1986;244:72–85. [PubMed: 3950091]
- Whitehead MC. Neuronal architecture of the nucleus of the solitary tract in the hamster. *J Comp Neurol* 1988;276:547–572. [PubMed: 2461969]
- Whitehead MC. Subdivisions and neuron types of the nucleus of the solitary tract that project to the parabrachial nucleus in hamster. *J Comp Neurol* 1990;301:554–574. [PubMed: 2177063]
- Whitehead MC, Bergula A, Hollyday K. Forebrain projections to the rostral nucleus of the solitary tract in the hamster. *J Comp Neurol* 2000;422:429–447. [PubMed: 10861518]
- Whitehead MC, Frank ME. Anatomy of the gustatory system in the hamster: central projections of the chorda tympani and the lingual nerve. *J Comp Neurol* 1983;220:378–395. [PubMed: 6643734]
- Whitehead MC, McPheeters M, Savoy LD, Frank ME. Morphological types of neurons located at taste-responsive sites in the solitary nucleus of the hamster. *Microsc Res Tech* 1993;26:245–259. [PubMed: 8241562]
- Whiteside B. Nerve overlap in the gustatory apparatus of the rat. *J Comp Neurol* 1927;44:363–377.
- Williams JB, Murphy DM, Reynolds KE, Welch SJ, King MS. Demonstration of a bilateral projections from the rostral nucleus of the solitary tract to the medial parabrachial nucleus in rat. *Brain Res* 1996;737:231–237. [PubMed: 8930370]
- Yamamoto T. Central mechanisms of roles of taste in reward and eating. *Acta Physiol Hung* 2008;95:165–186. [PubMed: 18642757]
- Zaidi FN, Todd K, Enquist L, Whitehead MC. Types of taste circuits synaptically linked to a few geniculate ganglion neurons. *J Comp Neurol* 2008;511:753–772. [PubMed: 18925565]
- Zaidi FN, Whitehead MC. Discrete innervation of murine taste buds by peripheral taste neurons. *J Neurosci* 2006;26:8243–8253. [PubMed: 16899719]

Figure 1.

(A) Fluorescent images of a representative injection site centered around the taste-responsive (waist) region of the PbN from caudal (left) to rostral (right) level (mouse 3). Scale bar = 500 μm. (B) Schematic diagram indicating PbN subnuclei at a caudal-intermediate level (*cf.* Fig 3M). (C) The location of the injection site of FG in each mouse.
^Ppressure injection; ^Iiontophoretic injection.

Figure 2.

Representative fluorescent images of labeled neurons in the major structures in the gustatory neural axis after an injection of FG into the PbN. All labeling is shown on the side ipsilateral to the injection site, except the PbN (E). A. Insular cortex. B. Bed nucleus of the stria terminalis. C. Central nucleus of the amygdala. D. Lateral hypothalamus. E. Parabrachial nucleus. F. Nucleus of the solitary tract. Scale bar = 500 μ m.

Figure 3. Diagrams illustrating the distribution of labeled neurons in the brain (rostral to caudal, A–R) after FG injections into the PbN (in mouse 1). Each dot represents one neuron in a single 40- μ m-thick section. Maps are adapted and modified from Paxinos and Franklin (2001). Scale bar = 1 mm

Figure 4.

The mean number of retrogradely labeled neurons in the insular cortex (IC), rostral nucleus of the solitary tract (rNST), bed nucleus of the stria terminalis (BST), lateral hypothalamus (LH) and central nucleus of the amygdala (CeA). * $p < 0.01$.

Figure 5.

(A and B) Fluorescent images of a representative elongate (A) and stellate (B) cells located in the rostral central subdivision of rNST. Arrowheads indicate cell body and arrows indicate dendrites. Scale bars = 20 μm. (C) Fluorescent image of rNST divided into the four subdivisions. Scale bar = 100 μm. (D) The mean number of retrogradely labeled neurons in the medial (M), rostral lateral (RL), ventral (V) and rostral central (RC) subdivisions of rNST. * $p < 0.01$.

Table 1
Number of retrogradely labeled neurons per section in each animal

ID	GC		BST		CeA		LH		rNST	
	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
Mouse 1P	35.5	7.2	192.4	16.3	211.8	21.4	141.7	45.1	102.2	8.1
Mouse 2P	21.3	6.3	163.2	7.1	201.8	19.2	115.5	29.6	128.7	35.1
Mouse 3P	43.1	11.5	121.6	12.4	219.5	7.7	75.9	63.1	95.2	16.3
Mouse 4P	57.2	17.2	169.7	3.1	231.1	8.2	61.8	36.1	87.8	38.2
Mouse 5P	42.6	28.7	166.2	6.1	239.7	19.4	229.3	75.8	178.1	32.3
Mouse 6I	20.2	2.8	95.5	2.5	120.5	3.1	34.6	4.5	63.2	15.5
Mouse 7I	28.8	2.6	92.1	8.4	186.4	5.8	70.9	35.2	91.6	31.2
Mouse 8I	43.3	17.3	164.1	9.5	185.6	2.5	116.1	26.6	86.5	16.1
Mouse 9I	26.6	3.4	106.8	5.7	130.5	5.5	93.2	15.6	143.4	23.1
Mouse 10I	37.3	9.4	143.4	9.9	193.2	7.1	101.4	15.9	118.5	14.2
Mouse 11I	58.2	26.2	126.6	7.7	197.6	15.1	131.8	54.7	111.3	20.3
Mouse 12I	66.3	20.9	171.8	11.4	188.2	20.7	191.3	66.6	105.5	31.1
Average	40.0	12.8	142.8	8.3	192.2	11.3	113.6	39.1	109.3	23.5
SEM	3.7	2.7	9.7	1.1	10.8	2.0	14.9	6.3	9.1	2.9

P – pressure injection; I – iontophoretic injection.