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ORGANIZATION OF ORAL AND GASTRIC REPRESENTATIONS IN THE PARABRACHIAL NUCLEUS OF THE RAT

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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ABSTRACT

The parabrachial nucleus (PBN) is a complex neural structure comprised of distinct subnuclei that mediate the transmission of most gustatory and visceral information from the medulla to the forebrain. That the transmission of gustatory and visceral inputs from the medulla to the forebrain is interrupted by a PBN synapse, implies further processing of this information in the brainstem. Gustatory function is represented in two anatomically segregated PBN regions that partially overlap with the general visceral representation, suggesting specialization of functions related to ingestive behavior. However, little is known about the inputs and outputs of these two regions, e.g. the contribution of gustatory PBN to medullary projections, or the nature and extent of overlap of gustatory and visceral inputs to the PBN. In the present set of experiments, physiologically defined afferent and efferent connections of the PBN were determined and potential sites for oral and gastric convergence were examined.

Anterograde and retrograde tracers were used to identify the efferent connections of gustatory sites in the PBN (chapter two). Injections centered on taste responsive sites in the ventral lateral and central medial subnuclei (‘waist-
region") resulted in projections to the parvocellular medullary reticular formation, in a region lateral to and partially overlapping with the distribution of retrogradely labeled lingual premotor neurons. In contrast, injections centered on taste and tactile responsive sites in the PBN external medial and lateral subnuclei ('external-region') produced few medullary projections. Significantly, both waist and external regions provided dense efferent projections to the thalamus and ventral forebrain. These findings suggest that the ascending gustatory projections from the NST become segregated in the PBN such that only specific PBN subnuclei sensitive to intraoral stimulation exert a direct influence on medullary substrates coordinating oromotor behavior.

To determine if the organization of gustatory function in multiple PBN regions represents a feature of other related visceral functions (e.g., gastric distension), the parabrachial distribution of terminal fields from caudal (gastric) and rostral (taste) regions of the nucleus of the solitary tract (NST) were compared (chapter three). Projections from caudal and rostral NST terminated throughout the anteroposterior extent of the PBN in many of the same subnuclei, including those in the waist and external regions. Although oral and gastric projections from NST reached many of the same subnuclei, the terminal fields exhibited only partial overlap. The results supported the concept of multi-regional representation of gastric function in the PBN revealing possible PBN substrates for oral and gastric convergence.
To characterize the physiological nature of these projections to the PBN, the response characteristics and subnuclear location of 55 single PBN neurons to taste, oral mechanical, and gastric distension stimuli were determined (chapter four). The results paralleled those of the anatomical experiments in that oral and gastric responsive neurons were identified in both the waist and external regions. Additionally, a subset of the PBN neurons responded to both modes of stimulation. Thus, the experiments in chapters three, and four confirmed the speculation that taste and gastric functions are represented in multiple PBN subnuclei that partially overlap in some regions and are segregated in others. Additionally, based on the results in chapter two, direct contributions to a descending medullary pathway may arise from a subset of oral responsive, gastric responsive, or convergent neurons. The overall results of these experiments indicate that oral and gastric functions represented in opposing poles of the NST converge within some PBN subnuclei, yet remain segregated in others. Anatomical overlap of NST inputs provides a mechanism for oral and gastric integration in the PBN that may influence brainstem mediated consummatory responses. Conversely, segregation of NST inputs to the PBN preserves a topographical representation of the alimentary canal as a feature of the ascending visceral pathway.
DEDICATION

to my parents, Ahmad and Effat for their unconditional love and support,
to my wife, Zahra for her love and devotion throughout this endeavor,
and
to our children, Behzad & Armon for making it all worth while.
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LIST OF ABBREVIATIONS

3V     3rd ventricle
AOC    anterior oral cavity
AN     ambiguus nucleus
AP     area postrema
bc     brachium conjunctivum
BD     biotynilated dextran
BLA    basolateral nucleus of amygdala, anterior
BNSt   bed nucleus of stria terminalis
CeL    central nucleus of amygdala, lateral division
CeLC   central nucleus of amygdala, lateral capsular division
CeM    central nucleus of amygdala, medial division
CNA    central nucleus of amygdala
cst    commissural stria terminalis
CG     central grey
cNST   caudal NST
Cu     cuneate nucleus
D3V    dorsal 3rd ventricle
DAB    3',3' diaminobenzidine
DCN    dorsal cochlear nucleus
DMN    dorsal motor vagal nucleus
FG     fluorogold solution
fr     fornix
Gi     gigantocellular reticular nucleus
Gr     gracile nucleus
Gu     gustatory thalamic nucleus
IO     inferior olive
IRt    intermediate reticular nucleus
KPBS   potassium phosphate buffered saline
Me5    mesencephalic trigeminal nucleus
mt     mammilothalamic tract
mV     trigeminal motor nucleus
MVe    medial vestibular nucleus
mVII   facial nucleus
mXII   hypoglossal nucleus
LC locus coeruleus
LH lateral hypothalamic area
LRT lateral reticular nucleus
MdD medullary reticular nucleus, dorsal part
MdV medullary reticular nucleus, ventral part
NRS normal rabbit serum
NST nucleus of the solitary tract
PB phosphate buffer
PBN pabrachial nucleus
PBS phosphate buffered saline
PCRt parvocellular reticular nucleus
PF parafascicular thalamic nucleus
PH posterior hypothalamic area
PHA-L phaseolus vulgaris-leucoagglutinin
POC posterior oral cavity
Pr5 principal sensory trigeminal nucleus
py pyramidal tract
rNST rostral NST
RF reticular formation
SI substantia innominata
Sp5 spinal trigeminal nucleus
SPF subparafascicular thalamic nucleus
SuVe superior vestibular nucleus
t solitary tract
VPM ventral posteromedial thalamic nucleus
vsc ventral spinocerebellar tract
WGA-HRP wheat germ agglutinin - horse radish peroxidase

cNST subnuclei:
c central subnucleus
dm dorsal medial subnucleus
m medial subnucleus
pc parvicellular subnucleus

rNST subnuclei:
rc rostral central subnucleus
rl rostral lateral subnucleus
m medial subnucleus
v ventral subnucleus

PBN subnuclei:
cl central lateral subnucleus
cm central medial subnucleus
dm  dorsal medial subnucleus
dl  dorsal lateral subnucleus
el  external lateral subnucleus
elo el outer portion
elii el inner portion
em  external medial
i  internal lateral subnucleus
KF  kölliker-fuse subnucleus
vl  ventral lateral subnucleus
vm  ventral medial subnucleus
s  superior lateral subnucleus

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CHAPTER 1

GENERAL INTRODUCTION

The central control of feeding behavior involves a distributed neural network throughout the brain [Grill Kaplan 90]. Neural substrates in the forebrain are implicated in the appetitive aspects of feeding while those in the brainstem control the consummatory phases of ingestive behavior. Thus, animals deprived of forebrain structures (decerebrates) do not spontaneously engage in food seeking behavior even when food deprived, indicating that forebrain processing is essential to regulating and guiding normal ingestion [Woods 64; Grill Norgren 78; Berntson Micco 76; Thexton Griffiths 79]. The lack of spontaneous feeding in decerebrates however, is not due to a complete lack of gustatory and visceral afferent processing. For example, behavioral experiments indicate that decerebrate rats can differentiate between palatable (e.g. sweet) and non-palatable (e.g. bitter) intraoral gustatory stimuli and correctly respond by making appropriate oral and facial expressions of ingestion and rejection [Grill Norgren 78; Grill Norgren 78a; Grill Kaplan 90]. During normal ingestion, the taste and texture of food determine its hedonic value and serve to promote feeding, while inhibitory signals from the gastrointestinal tract negatively influence food intake.
and contribute to satiation. During a meal, gastric signals (e.g., distension and rate of emptying) are among the most important parameters that determine meal size, whereas postingestive factors (e.g., glucose storage and availability) influence the intermeal interval {Sclafani Nissenbaum 85; Deutsch et al 78; Wirth McHugh 83; Kaplan et al 94}. Significantly, both intact and decerebrate rats proportionally decrease their intraoral intake of sucrose following gastric preloading, although only intact rats eat more when food deprived {Seeley et al 94}. Thus, the decerebrate’s abilities to discriminate between gustatory stimuli and to modify its intake of sapid intraoral stimuli based on gastrointestinal factors indicate that the fundamental components of sensori-motor integration underlying consummatory behavior must be contained in the brainstem, while the response to appetitive conditions such as food deprivation involve neural networks in the forebrain. Although potential brainstem sites for convergence of taste and visceral functions include the NST and the medullary reticular formation, many lines of evidence implicate the PBN in the integration of these inputs. The present series of experiments combined neuroanatomical and electrophysiological approaches to test the hypothesis that the PBN plays a role in organizing consummatory ingestive functions in the brainstem.

In the brainstem, oral and visceral inputs topographically synapse in opposing poles of the NST {Hamilton Norgren 84; Norgren Smith 88; Whitehead 88; Altschuler et al 89; Altschuler et al 91}. Anatomical studies indicate that
medullary projections from gustatory sites in the NST terminate extensively in the parvocellular (PCRt) and intermediate (IRt) areas of the reticular formation (RF) (Travers 88; Beckman Whitehead 91; Shammah-Lagnado et al 92; Halsell et al 96). These projections partially overlap with premotor neurons that in turn project to the motor nuclei that underlie the oral and pharyngeal phases of consummatory behavior (Travers Norgren 83; Ter Horst et al 91). That the RF mediates oral sensorimotor interactions is further emphasized by FOS immunoreactivity and neurophysiological recording experiments that indicate neuronal activation during chewing (Moriyama 87; Lund 91; Nakamura Katakura 95), licking (Travers DiNardo 92; Karimnamazi et al 94; Travers et al 97), and swallowing (Jean 90; Amri et al 91), as well as the orolingual movements of gaping during oral rejection of aversive gustatory stimuli (Travers DiNardo 92; DiNardo Travers 97). In addition to the local projections to the RF, ascending gustatory and visceral efferents from the NST converge in the PBN (Hermann et al 83; Herbert et al 90) prior to further transmission to the forebrain (Norgren 76; Voshart Van der Kooy 81; Lasiter et al 82; Fulwiler Saper 84; Bernard et al 93; Alden et al 94). This obligatory synapse in the PBN provides further processing of sensory inputs in the brainstem. Because the PBN is interposed between sensory inputs from the NST to the forebrain, it is in a position to influence both the forebrain mediated appetitive phase of ingestion as well as the consummatory behavior organized in the brainstem.
Although the role of the PBN in directing sensory inputs to the forebrain is not completely understood, lesion studies provide some compelling evidence for gustatory and visceral integration in the PBN. Learned associations between taste and visceral signals such as acquiring a salt appetite or a conditioned taste aversion (CTA) require intact forebrain structures {Grill Norgren 78b; Grill et al 86}. Electrolytic and cytotoxic lesions centered on taste activity in the PBN however, prevent the gustatory and visceral associations required in forming a salt appetite or a CTA {Spector 95}. This indicates that the PBN is required in forebrain-mediated appetitive phase of ingestive behavior. Convergence of inputs is an important neural mechanism in the integration of gastrointestinal signals {Chambert et al 93; Appia et al 86; Schwartz et al 95; Schwartz Moran 96}. Little is known however, about convergence of taste and gastric inputs in the PBN or the involvement of PBN in the organization of consummatory functions in the medulla.

An across study comparison of the terminal fields of NST projections in the PBN indicates that areas both medial and lateral to the brachium are targeted by ascending projections from both rostral and caudal regions of the NST {Norgren 78; Hermann et al 83; Fulwiler Saper 84; Herbert et al 90; Herbert Saper 90; Becker 92}. However, speculation about the degree of PBN overlap of NST projections is confounded. For example, not all studies related the distribution of NST projections to the subnuclear organization of the PBN. Further, dual
labeling experiments have not been done to investigate the extent of overlap or
segregation within those regions of the PBN where oral and visceral inputs seem
to be co-represented. Additionally, due to the complex viscerotopic
representation in the caudal NST, it is difficult to ascertain the functional nature
of these projections to the PBN from stereotaxically placed injections. Although
electrical stimulation of the vagus provides physiological evidence for gustatory
and general visceral convergence in the PBN {Hermann Rogers 85}, the specific
source of this visceral input is unclear. If convergence of taste and gastric inputs
to the PBN represents a brainstem mechanism for direct modulation of
consummatory reflexes in the medulla, then a descending projection from the
PBN to the medulla ought to exist. Although descending projections from the
PBN to the medulla have been reported, it is not known if gustatory responsive
neurons contribute to this projection {Saper Loewy 80; Herbert et al 90; Krukoff
et al 93}.

The experiments in chapters two and three combine neuroanatomical and
electrophysiological techniques to characterize the functional organization of the
afferent and efferent connections of the PBN with the medulla and forebrain.
The results indicate that the representation of oral and gastric functions overlap
in several PBN subnuclei, but are segregated in others. Only a subset of those
subnuclei directly exert influence over the medullary substrates implicated in oral
motor behavior. Chapter four further extends the findings in chapter three by
examining the physiological nature of single PBN neurons to taste, oral mechanical, and gastric distension stimuli, and characterizing the response of convergent neurons. In summary, convergence of oral and gastric inputs to the PBN provides a brainstem mechanism for control over intake and termination of a meal, whereas segregation of these functions preserves the topology of the gustatory and visceral systems in the forebrain.
CHAPTER 2

EFFERENT PROJECTIONS FROM GUSTATORY RESPONSIVE PARABRACHIAL NUCLEUS TO THE MEDULLA AND FOREBRAIN

Introduction

The parabrachial nucleus (PBN) consists of a heterogeneous complex of cells just ventral to the brainstem surface and surrounding the brachium conjunctivum (BC). Associated with the PBN are a wide range of gustatory {Norgren Pfaffmann 75; Ogawa et al 87; Halsell Travers 97}, see also review in {Travers 93}, visceral {Hermann Rogers 85; Kobashi Adachi 86; Yuan Barber 91; Suemori et al 94} autonomic {Bertrand Hugelin 71; Mraovitch et al 82; Ohman Johnson 89}, see also review in {Saper 95} and nociceptive {Cechetto et al 85; Hayashi Tabata 90; Bernard Besson 90} functions. Underlying these diverse functions is a complex anatomical system consisting of multiple subnuclei with widespread connections to both forebrain and hindbrain sites {Fulwiler Saper 84; Halsell 92; Herbert et al 90; Moga et al 90; Bernard et al 93; Alden et al 94}.

Because the transmission of gustatory information from the nucleus of the solitary tract (NST) to the forebrain is interrupted by a synapse in the PBN
{Norgren Leonard 73; Travers 88; Ricardo Koh 78; Hermann et al 83}, the PBN potentially plays a role in intrinsic brainstem functions involving taste.

Descending connections of the PBN are organized along two major pathways {Saper Loewy 80}. A ventrolateral medullary pathway carries the majority of descending output from the cardiorespiratory-related Kölliker-Fuse (KF) nucleus of the PBN {Herbert et al 90}. The other descending projection from PBN is via Probst’s tract, a bundle of fibers that arise from a more diffuse distribution of neurons in the dorsal pons that terminate mainly in the parvocellular reticular formation {Saper Loewy 80; Herbert et al 90; Shammah-Lagnado et al 92; Krukoff et al 93}. Although a comparison between the source of medullary projections originating from within the PBN and the location of gustatory responsive sites in the PBN suggests that some descending projections may well arise from taste responsive neurons, the exact source and extent of descending medullary projections from gustatory responsive PBN remains unclear. Previous studies either did not use electrophysiological guidance for placement of injection sites, or medullary projections were not reported {Herbert et al 90; Krukoff et al 93; Norgren Leonard 73; Norgren Pfaffmann 75}.

Thus, the current study was directed at determining the extent of projections from PBN neurons that process gustatory and oral somatosensory information to the medullary reticular formation. A second goal was to compare efferent
projections originating from different orally-responsive subdivisions of the PBN.

Gustatory function in the PBN is usually associated with neurons in and around the "waist area" {Van Buskirk Smith 81; Halsell Frank 91; Di Lorenzo Monroe 92} which we define here as neurons in either the ventrolateral or central medial subnuclei, as well as neurons scattered within the fibers of the brachium conjunctivum. There is, however both anatomical {Herbert et al 90; Becker 92} and physiological {Norgren Pfaffmann 75; Ogawa et al 87; Halsell Travers 97} evidence for both gustatory and orotactile representation in the 'external region' as well. The external region is composed of the external medial (EM) subnucleus and the inner subdivision of the external lateral (EL) subnuclei.

Thus, small amounts of neural tracers were injected into either the "waist area" that was sensitive to either anterior or posterior oral cavity stimulation, or an external region responsive exclusively to posterior oral cavity stimulation.

Materials and Methods

Nomenclature

The anatomical terminology in the present report was largely adopted from the Paxinos and Watson {Paxinos Watson 86} atlas of rat brain with some modifications in regards to the PBN and amygdala. Anatomical delineations of the amygdala are consistent with the study by Bernard et al (1993), while the subnuclear boundaries for the PBN were primarily drawn according to those of Fulwiler and Saper {Fulwiler Saper 84} with some modifications of the medial
subnuclei based on the study by Halsell and Frank (Halsell Frank 91) in the hamster. Inputs from rNST terminate in two mediolaterally segregated PBN regions (Herbert et al 90; Becker 92) henceforth define as: 1) the ‘waist region’, composed of the ventral lateral (VL), and central medial (CM) subnuclei and the neurons within the fascicles of the brachium conjunctivum that bridge these subnuclei and 2) the ‘external region’, composed of the external medial (EM) and the inner subdivision of the external lateral (EL) subnuclei that cap the brachium laterally (Halsell Frank 91; Fulwiler Saper 84; Bernard et al 93). A recent single unit electrophysiological mapping study indicated that gustatory and oral tactile evoked activity can be obtained from both the waist and the external regions of the PBN (Halsell Travers 97). Thus, to elucidate the organization of descending projections from oral somatosensory and gustatory responsive sites within PBN, we included both areas.

Experimental Design

A total of 38 adult, male, Sprague-Dawley rats weighing between 250-400 g were used for these experiments. Twenty-two provided useful data. In the majority of the experiments (19/22) we used 10-15% biotinylated dextran (BD: Lysine-fixable, MW=10 kDa, Molecular Probes Inc) as the anterograde tracer. Shorter survival times and less complicated histochemical reactions made the dextran our anterograde tracer of choice. In a few experiments (3/22), 2.5-10% phaseolus vulgaris leukagglutinin (PHA-L) was used to confirm the distribution of
anterograde fibers. Only experiments in which electrophysiological characterization of the injection sites was possible were included in the analysis. Although the primary technique relied on small injections of anterograde tracers made under physiological guidance, additional double-label experiments clarified the origin and destination of the descending pathway. In one set of experiments \((n=2)\) the distribution of descending PBN fibers relative to lingual premotor neurons was characterized by injecting BD into PBN waist region and a retrograde tracer \((2-4\% \text{ Fluorogold solution (FG)})\) into the hypoglossal nucleus. In a second set of double labeling experiments \((n=2)\) the morphology and organization of PBN descending projection neurons relative to the terminal fields of ascending gustatory inputs from rNST was characterized by iontophoresising BD into the (gustatory-responsive) rNST and pressure injecting FG into the medullary RF.

Surgical Preparation

The rats were food deprived overnight and injected with a prophylactic dose of penicillin on the day of surgery. Surgical anesthesia was obtained by sodium pentobarbital \((\text{Nembutal, 50 mg/kg; IP})\). Supplementary doses \((15 \text{ mg/kg})\) of this anesthetic were used as needed to maintain an areflexive state. Core temperature was monitored and maintained at \(37^\circ\text{C}\) throughout the experiment. For each experiment, the animal was mounted prone in a stereotaxic headholder equipped with non-traumatic earbars \((\text{Kopf})\). The dermis and periosteum on top
of the skull were reflected through a midline incision and the skull was leveled between bregma and lambda. For access to the PBN, a 2 mm trephine hole was centered 1.7 mm lateral to the sagittal suture and 0.5 mm anterior to lambda. The electrode was directed posteriorly 20° from the vertical axis to avoid the transverse sinus overlying the PBN. For access to NST, RF, and mXII the electrodes were directed along the vertical axis. For NST and RF penetrations, the trephine was centered at 4.5 mm posterior to lambda and 1.8 mm lateral to the midline. For mXII penetrations it was centered 6.8 mm posteriorly and 0.15 mm laterally. These coordinates were used as initial starting points for subsequent electrophysiological search. The initial trephine hole was extended to accommodate more rostral or lateral penetrations.

*Electrophysiological Identification*

The extracellular physiological responses of PBN neurons were recorded with glass coated tungsten electrodes (R=0.5-1.3 MΩ), grounded through a metal clip attached to the skin. When a single unit or robust multiunit activity was isolated, the responses to taste and tactile stimulation of the oral cavity were further tested using procedures similar to those described by others (Travers Norgren 95; Travers et al 86). Briefly, tactile testing consisted of lightly stroking the oral and pharyngeal mucosa with blunt glass probes. Gustatory stimuli were applied by flowing small amounts (10 - 200 µl) of a taste mixture (0.01 M saccharin, 0.3 M sodium chloride, 0.01 M hydrochloric acid, and 0.003 M quinine hydrochloride)
onto specific receptive fields of the anterior and posterior oral cavity with small camel hair brushes. Neural responses were amplified differentially (gain=10,000), monitored through an audio monitor (Grass AM8) and displayed on a cathode-ray storage oscilloscope. Electrophysiological identification of the waist area also served as a reference point in locating other PBN subnuclei. Thus, taste and orotactile responses in the external medial subnucleus were consistently identified 0.5 mm anterior and 0.5 mm lateral to the waist area, and rhythmic respiratory activity in KF was reliably recorded 1.1 mm anterior and 0.7 mm lateral to the gustatory activity in waist region.

Tracer Injection

After characterizing neural activity with the tungsten microelectrode, the electrode was withdrawn and replaced with a glass micropipette (o.d. at tip = 25-40 µm) containing a solution of BD dissolved in saline. Gustatory, orotactile and spontaneous respiratory activity could usually be recorded through the micropipettes. Although the signal was less robust than that recorded through the tungsten electrode, it was possible to verify physiological activity (Becker 92). An injection was then made by applying a 20 msec pulse of pressure at 20 psi using a Pico Spritzer (General Valve Corp) and confirmed by monitoring the meniscus of the micropipet through a surgical microscope with a calibrated ocular. Although BD is predominantly an anterograde tracer and remains stable long enough to transport to the medulla (Rajakumar et al 93) some retrogradely
labeled neurons were always observed in the forebrain and the brainstem. In order not to confuse anterograde projections with this retrograde label, we injected a 2-10% solution of PHA-L (a more specific anterograde tracer) dissolved in 1mM phosphate buffered saline (Gerfen Sawchenko 84). The PHA-L and some of the smaller dextran injections were iontophoretically made with 5 μA of anodal current with a 4 second duty cycle for 10-30 minutes. In the double labeling experiments, FG was either pressure injected or iontophoresed into RF and mXII using the same injection parameters as those for the BD and PHA-L. The tracer was injected into the RF based on stereotaxic coordinates that were 0.2 mm caudal, 0.05 mm medial, and 1 mm ventral to those of the rostral NST (see above). Following the injection, the micropipette was left in place for ten minutes to reduce unwanted spread into the track and was then slowly withdrawn. Identification of mXII was made by inducing observable lingual movements with microstimulation through a glass coated tungsten electrode (7 μA, 2500 pps, 0.1ms) and then replacing the tungsten electrode with a Fluorogold filled micropipette. Generally a higher current (15-20 μA, 2500 pps, 0.1 ms) was required to induce lingual movements through the micropipet. Upon completion of each experiment, the brain surface was covered with gelfoam and bone wax, an antibiotic ointment was applied and the incision was closed with surgical staples. All procedures were conducted under a protocol approved by the University Animal Care and Use Committee in a facility approved by the American Association for the Accreditation of Laboratory Animal Care.
Animals injected with BD survived for 7 days postoperatively; those injected with PHA-L and Fluorogold 12-14 days. After the appropriate survival time, the rats were deeply anesthetized with a lethal dose of Nembutal (150 mg/kg), and perfused transcardially with 0.1 M phosphate buffered saline (PBS) followed by a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer. The brains were removed and stored overnight in 20% sucrose at 4°C. Two (40 µm) or three (30 µm) transverse series of the hindbrain and forebrain were cut with a freezing microtome and placed in cold PBS.

To process the biotinylated dextran, free floating sections were rinsed in PBS and incubated for 45 minutes in 0.4% Triton in PBS. Using the ABC kit (10 µl/ml of Vectostatin elite ABC kit, Vector labs) the tissue was then incubated at room temperature for one hour to produce the avidin/avidin-peroxidase conjugates. After rinsing the tissue in phosphate buffer (PB), reaction product was visualized by the metal enhanced 3,3' diaminobenzadine HCl peroxidase reaction (DAB, Sigma Co). Tissue was incubated at room temperature for 15 min. in 0.05% DAB and 1% nickel ammonium sulfate in PB and 0.0005% H₂O₂ was then added for 2 to 5 minutes.

The reaction was monitored for the visualization of dark reaction products. Further intensification of the label was achieved by adding a 1% CoCl₂ (Fisher
Scientific Co) solution just prior to the hydrogen peroxide step. The reacted series were mounted on chrome-alum gelatin coated slides, and allowed to air dry. The unreacted series were mounted and stained with Cresyl Violet. In some of the experiments, one series of the reacted tissue was counterstained with neutral red and rapidly dehydrated in graded alcohols to allow for identification of injection sites and labeled fibers within specific anatomical subdivisions.

To process the PHA-L, the tissue was first incubated in 1% sodium borohydride (Fisher Scientific, Co) for 20 minutes to reduce background staining, and then rinsed 8 x 5 minutes. The sections were incubated overnight at 4°C in 2% normal rabbit serum (NRS: Vector Labs) and 0.4% Triton X-100 (Sigma) in potassium phosphate buffered saline (KPBS). The tissue was then transferred to wells containing the primary antibody (Vector Labs, goat Anti-PHA(E+L)), diluted 1:20,000 in a solution of 2% NRS in KPBS, and incubated at 4°C overnight. An initial dilution series showed that 1:20,000 was the optimal dilution of the primary antibody. Following the primary antibody incubation, the tissue was rinsed 5 x 5 min. in 0.2 M KPBS and incubated for 1 hour in the secondary antibody (rabbit Anti-Goat IgG; Vector Labs) diluted 1:200 with a 2% NRS in KPBS. After rinsing 4 x 5 min. in 0.2 M KPBS the sections were incubated at room temperature for 1 hour in a solution containing 10 _l/ml of the ABC Elite kit in 60 ml of 0.2 M KPBS. The tissue was then rinsed 5 x 5 min. in 0.2 M KPBS
and the label was visualized with the nickel intensified DAB reaction as before.
The sections were then rinsed 4 x 5 min. with 0.2 M KPBS and mounted on
chrome-alum gelatin slides.

Retrogradely labeled Fluorogold neurons were visualized with an
immunohistochemical reaction {Van Bockstaele et al 94}. After thoroughly
rinsing the cut sections in PBS (8 x 5 min.), the reaction was blocked with 10% sheep serum for 2 hours at room temperature. The sections were rinsed again with PBS (6 x 5 min.) and incubated in a 1:20,000 dilution of a primary antibody (Anti-FG Ab from rabbit; Chemicon International Inc.) for 2 days at 4° C. The tissue was then rinsed in PBS (10 x 3 min.) and incubated in a 1:600 dilution of a secondary antibody (Biotinylated Anti-rabbit IgG from goat; Vector Labs) for 1 hour at room temperature. After rinsing the tissue in 0.1 M PBS (6 x 5 min.) it was processed by the avidin-biotin peroxidase protocol (ABC kit, Vector Labs) as before. To color differentiate between the darkly labeled anterograde fibers and retrogradely labeled cells we used the Naphthol protocol with pyronine B as a chromogen {Peng et al 95}. Sections were rinsed in 0.1 M PB and incubated in a freshly made solution of 0.05% Naphthol and 0.1% ammonium carbonate in PB for 15 minutes. The oxidizing agent, 0.0003% H₂O₂ was then added for 2-5 minutes to catalyze the peroxidase reaction. The blue reaction product was then turned to pink by an additional incubation in 0.1% pyronine B in PB. The sections were differentiated in 70% ethanol for 1-2 minutes and rinsed with PB.
Data Analysis

Sections were viewed under brightfield and darkfield illuminations through a conventional microscope interfaced with a micro computer-based image processor through a video camera (Neurolucida, MicroBright field, Inc). To localize the labeled fibers, counterstained sections or stained alternate sections were used to define anatomical landmarks prior to drawing the labeled fibers. Thus, composites of the stained and reacted sections were produced to identify the labeled fibers in relation to anatomical structures. Individual fibers were anatomically traced at 100-200 magnification, whereas retrogradely labeled neurons in the PBN were marked by symbols. Axons could be classified into one of three categories, those that terminated within the field, those that passed through the field and showed evidence of axonal varicosities, and passing fibers that did not show evidence of any synaptic interaction within the field.

Throughout this study, only the former two categories of axonal specializations were analyzed and plotted.

Results

The location and extent of 14/20 BD and PHA-L injections are depicted at four different PBN levels in figure 2.1. Injection diameters in the transverse plane ranged from 150-350 μm in VL and CM (waist region), to 350- 450 μm in EM and EL (external region). Several larger injection sites that spread into adjacent PBN subnuclei or beyond the borders of the PBN (6/20) were not plotted to minimize
clutter, although projections from these injection sites were also analyzed. Injections centered on orally responsive sites in the PBN were clustered around two anatomically distinct regions. The first region consisted of injections into the "classical" waist region and were centered on anterior tongue gustatory responsive sites in CM (n=5) and a posterior tactile responsive site in VL (Fig. 2.1 A-C, 2.2 A). A second series of injection sites were clustered laterally in the external region and were centered on posterior oral cavity gustatory and tactile responsive sites in EM (n=4) and EL (n=2) (Fig. 2.1 B-C, 2.2 B). Unlike waist area injections in which both anterior and posterior taste activity could be detected, only posterior oral cavity taste and tactile responses were obtained in these lateral sites. At the most rostral level, a 3rd series of injections were centered on neural activity that correlated with respiration and corresponded anatomically to the KF nucleus (n=2) (Fig. 2.1 D, 2.2 C). Examples of injection sites together with evoked or spontaneous activity is shown in figure 2.2. Each of these functionally unique regions of PBN showed a specific efferent projection to the medulla.

**Descending Projections from the PBN**

**Waist Region Projections to the Medulla**

The distribution of medullary fibers from a small BD injection restricted to CM and centered on anterior tongue gustatory activity (Fig. 2.1 A: case 607), is shown in figure 2.3 A (1-4). In this dual tracer experiment, FG was also injected into the
hypoglossal nucleus (mXII) to relate the distribution of PBN fibers within the medulla to lingual premotor neurons. The distribution of PBN fibers and retrogradely labeled neurons was bilateral with the ipsilateral projections more prominent. At the level of the rostral pole of the NST (Fig. 2.3 A1), premotor neurons to mXII were clearly segregated mediolaterally from descending PBN fibers. Premotor neurons were concentrated in the intermediate zone of the reticular formation (IRt) compared to the distribution of descending PBN fibers concentrated more laterally within the parvocellular RF (PCRt). A similar distribution of anterograde label from a relatively large injection of BD centered on anterior tongue taste responses in CM (Fig. 2.4 A) is shown under darkfield illumination in figure 2.4 B. Although the size of this injection (500 μm rostrocaudally) was somewhat larger than those depicted in figure 2.1 or figure 2.3, the pattern of label in the medulla was similar to those from the smaller injections. Although many fibers coursed through the medial core of the RF and the ventrolateral medulla, these fibers were nearly devoid of axonal terminals or varicosities. In transverse sections, many fibers could be seen as they obliquely coursed across the PCRt from nucleus ambiguus (NA) dorsally towards the fourth ventricle. Fibers destined for more caudal levels of the medulla could be observed within the fiber bundles of PCRt.

At successively more caudal levels of the medulla (Fig. 2.3 A2-3) the segregation of afferent fibers and lingual premotor neurons decreased as premotor neurons
occupied somewhat more lateral RF sites. The location of descending fibers, however remained concentrated within lateral locations. At the most caudal level shown (obex), and caudal to it, fibers and premotor neurons were once again highly segregated with fibers distributed in the dorsal medullary reticular area (MdD) and premotor neurons concentrated more medially and ventrally in the ventral medullary subdivision (MdV) (Fig. 2.3 A4). With the exception of a few decussating fibers, Gi was mostly devoid of fibers and there was little evidence of synaptic terminals. An injection of BD into a site in VL responsive to posterior tongue mechanical stimulation (case 614 in Fig. 2.1 A-C) showed a similar pattern of labeling in the medulla (Fig. 2.5 A1-4) as cases centered in CM and responsive to anterior tongue taste stimulation. However, more fibers in IRt were labeled after injections into VL versus those into CM.

In addition to the medullary RF projections described above, projections from the PBN waist region were also consistently observed in rNST. These fibers were most dense ventrally and medially within the nucleus (Figs. 2.3 A2, 2.4 B, 2.5 A2), corresponding, in part, to the rostral central and ventral subdivisions (Whitehead 90; Halsell et al 96). At levels caudal to the fourth ventricle, NST projections diminished. In some experiments a few retrogradely labeled neurons also appeared in the rostral pole of NST (Fig. 2.4 C). In addition, these injections always produced labeling of axons in the spinal trigeminal nucleus (Figs. 2.3 A2, 2.4 B, 2.5 A2) as well as a few intermingled retrogradely labeled neurons.
External Region Projections to the Medulla

In contrast to the waist region, small injections of BD and PHA-L into the PBN external region (Fig. 2.1 B,C) labeled a minimum number of fibers within the medulla. Figure 2.3 (B1-4) depicts the medullary distribution of anterograde fibers from an injection primarily restricted to EM (case #603: Fig. 2.1 C), and centered on multiunit posterior oral cavity tactile responsive activity. Injection sites that extended into EL and KF resulted in somewhat more labeled fibers distributed ventrolaterally in the medulla. Those injection sites restricted to EM, however, resulted in the least overall amount of medullary label.

Although KF abuts the external region rostrally, it appears functionally distinct from other external PBN subdivisions. In anesthetized preparations, respiratory-related activity rather than intraoral taste or tactile responses were associated with this region. Injection sites of BD centered on this respiratory activity produced a distribution of dense projections ventrolaterally in the medulla (Fig. 2.5 B1-4) and extending into the lateral subdivisions of the facial nucleus. The density and distribution of KF projections was thus distinct from either the sparse medullary projection of the external region, or the more dorsal and laterally distributed RF projections from the waist region.

Distribution of RF Projection Neurons Relative to NST Input

Dual tracing experiments (n=2) were performed to further characterize the PBN
organization of RF projection neurons relative to rNST afferents. In these experiments, retrograde tracer injections (FG) into rostral PCRt (Fig. 2.6 D) were combined with small BD injections into anterior tongue gustatory responsive sites in rNST (Fig. 2.6 C). Thus in the same animal, the distribution of retrogradely labeled neurons in PBN was compared with rNST input (Fig. 2.6 A, B). Overall, the results of our previous experiments were confirmed in that PCRt projection neurons within PBN overlapped with rNST fibers in the waist region. Furthermore, the distribution of retrogradely filled PBN neurons from RF injection sites overlapped with ascending rNST projections in the PBN dorsal medial subnucleus (DM). As predicted from the anterograde studies, no retrogradely labeled neurons were observed in EM following medullary RF injections, although it was clearly a target of rNST ascending projections. In addition to DM and the PBN waist region, many retrogradely labeled neurons in mesencephalic and supratrigeminal nuclei were also labeled from the RF injections.

**Forebrain Projections from PBN Waist and External Regions**

Injections into both the waist region (n=5) and the external region (n=6) of PBN labeled fibers in the forebrain. These projections were most prominent in the gustatory subnucleus of thalamus (Gu) and central subnucleus of amygdala (CNA), although other areas such as bed nucleus of stria terminalis (BNSt), lateral hypothalamus, and zona inserta were also labeled. The largest
ascending projection from PBN to all forebrain areas originated from an injection of BD centered on posterior tongue responsive sites in VL (case 614, Fig. 2.1).

Parabrachial projections from both the waist and external regions to the thalamus were bilateral, however there was a pronounced asymmetry to the projections. Projections originating from VL or CM had a more pronounced ipsilateral representation in Gu, (Fig. 2.7 A) compared to those from EM and EL that were more contralateral (Fig. 2.7 B). In addition, projections that originated from EL were more sparse compared to the dense projections from VL and CM. Thalamic projections from the waist region were also observed in the parafasicular subnucleus caudal to Gu as well as in the mediodorsal subnucleus rostrally. In addition to Gu [the most medial pole of the ventroposteromedial (VPM)] subnucleus more lateral ventroposteriomedial regions also received PBN projections. These projections were most frequently seen with injections into the external region.

In contrast to the highly consistent pattern of label in the thalamus, hypothalamic projections from PBN waist region, when present (3/5 cases), were sparsely distributed in the ipsilateral lateral hypothalamic area (LH). Hypothalamic projections from the external region were also sparse, but more consistently present bilaterally in LH (5/6 cases). More rostrally, a group of fibers laterally pierced through the internal capsule towards the amygdala.
The PBN projections to the amygdala have recently been described in detail {Bernard et al 93}. Our results were similar to this study in that PBN waist region projections were concentrated in the central lateral (CeL) and central medial (CeM) subnuclei of CNA with some fibers extending ventrally into the cortical amygdaloid area. Figure 2.8 A shows this extensive amygdalar projection from a BD injection into VL (case #614 in Fig. 2.1). The density of fibers from this injection site greatly exceeded those resulting from injections centered on anterior tongue taste responsive sites in CM (n=4). However injections centered on either CM or VL resulted in a moderately dense pattern of labeled fibers just rostral to CNA and ventral to globus pallidus in substantia innominata (SI). The pattern of fibers arising from EL and EM seemed to target a more restricted portion of CNA compared to those arising from VL. Primarily, these fibers were seen terminating in the capsular area of the central lateral subnucleus (CeCL) and extending into the most ventrolateral region of CeM (Fig. 2.8 B). Projections to the SI from EL and EM were also evident. Dorsal to the CNA, an array of parallel fibers that seemed to be destined for higher forebrain regions was seen laterally in the stria terminalis. Further rostrally, fibers were directed medially and ventrally and terminated in the lateral bed nucleus of stria terminalis (BNSt).

As with the amygdala, VL was the greatest contributor of PBN projections to BNST. In contrast, CM had a very sparse and variable projection. The external region projections, though sparse, were consistent from preparation to
preparation. The most rostral forebrain region to receive direct projections from orosensitive regions of PBN was the insular cortex. Sparse ipsilateral projections were observed in the gustatory cortex following injections centered in the PBN waist region but not the external region.

Discussion

Our primary hypothesis that gustatory sites in the PBN directly project to the medullary RF was verified with both anterograde and retrograde tracer experiments. Although previous anatomical and physiological studies have examined the efferent connections of the PBN, descending projections originating from gustatory responsive sites within this nucleus were not specifically examined {Saper Loewy 80; Herbert et al 90; Krukoff et al 93}. Because the PBN is functionally heterogenous with gustatory {Norgren Pfaffmann 75; Ogawa et al 87; Halsell Travers 97} and visceral functions {Hermann et al 83; Hermann Rogers 85; Kobashi Adachi 86; Yuan Barber 91; Suemori et al 94} represented in close anatomical proximity to one another, it was essential to localize the injections to physiologically identified sites.

Descending Projections from the PBN

Projections from the waist region (VL and CM) to the medullary RF were preferentially distributed within the PCRT and MdD. This projection is lateral to the major source of direct hypoglossal projection neurons located in either the
IRt of the rostral medulla, or more medially in the RF in the caudal medulla. This pattern echoes local projections from gustatory-responsive sites in the NST{Becker 92; DiNardo Travers 97; Travers 88} and reinforces the view that lateral sites within the medullary RF process orosensory information earlier in a gustatory-motor circuit related to the consummatory phase of ingestion (reviewed in {Travers et al 97}). Thus, the increased responsiveness to higher concentrations of a preferred gustatory stimulus, as well as the rejection of a normally unpalatable QHCI solution in a chronic decerebrate rat {Grill Norgren 78} could be mediated by converging gustatory inputs from both the rostral nucleus of the solitary tract and the waist region of the PBN onto a common substrate in the PCRt. We speculate that this substrate then influences interneurons in the IRt that have direct access to the oral motor nuclei that produce the responses of ingestion and rejection.

Despite the overlapping terminal fields in the PCRt from both the rostral NST and PBN waist region, projections from the PBN are likely to contribute more integrated orosensory processing compared to the NST. Neurophysiological and anatomical studies, for example, indicate that the PBN but not the NST is a site for convergence of gustatory and visceral inputs {Hermann et al 83; Hermann Rogers 85; Karimnamazi et al 97}. Chronic decerebrate rats decrease their intake of a sucrose solution in response to a gastric preload of milk {Seeley et al 94}. Thus, it may be through descending medullary projections that integrated
visceral and gustatory information in the PBN waist region affects ongoing consummatory behaviors organized in the medulla.

The distribution in the medulla from the waist region of the PBN contrasted markedly with projections from the external region that produced only a minimum of labeling in the RF. Although both the waist and external regions receive input from the rostral NST (Herbert et al 90; Becker 92), and we even occasionally observed single fibers from NST injections that branched to terminate in both regions, there is a degree of sorting or segregation of afferent input that takes place within different PBN subnuclei. Specifically, the external region that responds to posterior tongue and tactile information (Halsell Travers 97), also receives spinal and trigeminal inputs implicated in nociception (Bester et al 95; Cechetto et al 85; Slugg Light 94; Bernard et al 95; Feil Herbert 95; Menendez et al 96), and preferentially expresses FLI in response to a conditioned taste aversion (Yamamoto et al 94). Overall, the external region appears specialized to process aversive (oral) stimuli. The lack of descending projections from this nucleus compared to the waist region, however, suggests that forebrain pathways are critical to the expression of external region functions.

Injections into the KF, which abuts the external region, verified that closely spaced injections into physiologically distinct PBN locations could produce highly differentiated patterns of label. Despite the close anatomical relationship
between KF and the external region, efferent projections from these areas were very different. Unlike external region injections, injections of BD into respiratory-related sites in KF resulted in dense labeling in the ventrolateral medulla. A similar pattern of label also resulted from those larger injections into the external region that extended beyond the borders of EM and EL. Although detailed investigations of sensory activity in KF are lacking, it is unlikely that this area processes sensory information from the oral cavity. In the present study, injections of anterograde tracers into orally responsive NST or PBN did not label fibers in KF. Nevertheless, the KF receives input from the caudal NST and is a major source of PBN descending projections to the medulla and spinal cord {Saper Loewy 80; Stevens et al 82; Fulwiler Saper 84; Herbert et al 90; Gang et al 90}. These projections innervate a wide range of neural substrates related to cardiorespiratory control {Chamberlin Saper 94; Saper 95}.

*Ascending Projections from the PBN*

Differences in forebrain projections from the external and waist regions were more subtle compared to those observed in the medulla. For example, projections to the gustatory thalamus from both the external and waist regions were bilateral, however the external region projections were concentrated more contralaterally, while those from the waist region had a more ipsilateral representation. These differences in the laterality of projections to the thalamus confirm previous studies that also used electrophysiological guidance to target
gustatory sites in the PBN waist region {Norgren Leonard 73; Halsell 92}, as well as two other studies of projections from EM based on stereotaxic injections of anterograde and retrograde tracers {Saper Loewy 80; Cechetto Saper 87}, see also reviews {Saper 95; Travers 93}. Although some have argued that the major thalamic input from the PBN arises from the contralateral EM {Fulwiler Saper 84}, also review {Saper 95}, the present results confirm the original description {Norgren Leonard 73} of a strong ipsilateral projection from the gustatory-responsive waist region, see discussion in {Travers 93}.

Projections from the external and waist regions to the amygdala also differed. External region projections were primarily to the capsular region of the CNA, while those from the waist region, in particular those from VL, projected more diffusely throughout CeL and CeM. Injections into CM, based on anterior oral cavity responses, produced very little CNA label but did label the substantia innominata (SI). This projection from CM appears consistent with an electrophysiological study of anterior tongue responsive gustatory neurons in rat PBN that were antidromically activated from SI but not CNA {Norgren 74}, as well as anterior oral cavity gustatory responses in the amygdala found primarily in the dorsal-most part of CNA and in the SI of rabbit {Block Schwartzbaum 83}. In addition, an anatomical study {Moga et al 90}, (figure 11B,C) clearly shows a more massive projection to the CNA following an external lateral injection of WGA-HRP compared to medial subnucleus injections.
Other studies, however, have described a dense projection from the medial PBN to the central medial subnucleus of amygdala that continued rostrally to SI (Bernard et al. 1993). The origin of this projection may reflect the precise mediolateral extent of the injection sites within CM. Most of our injections in the waist region were centered on anterior tongue gustatory responsive sites within CM that resulted in injection sites in the lateral half of the subdivision. In contrast, the injections into PBN medial subnucleus by Bernard et al. extended further mediolaterally, and it remains possible that different functionally defined CM regions have different ascending projections. More medial sites in CM may have a posterior oral cavity representation (Becker 1992; Halsell Travers 1997) and more lateral sites in VM may have a non gustatory function. Although we did not place any injections into posterior oral cavity responsive sites in CM, such an injection centered in VL did produce dense labeling in CNA, a pattern very similar to that reported by Bernard et al. (1993). Thus, it may be that taste related CNA projections from PBN arise primarily from posterior oral cavity responsive sites, regardless of which subnuclei they are located. Small physiologically guided injection sites will be necessary to determine whether posterior oral responsive sites in CM project to CNA as they do from EM.

Functional Organization of Gustatory-Responsive PBN

Although anatomical studies now confirm differences in both the afferent input and efferent targets of the external and waist region, the functional significance
of these differences has yet to be fully evaluated. Despite an extensive literature showing that PBN lesions can impair many taste or ingestive related behaviors, lesion studies have generally not been targeted to functionally differentiate among the various PBN subnuclei {Scalera et al 95; Spector et al 95; Spector et al 92; Flynn et al 91a; Flynn et al 91b}. Overall there is little correlation between lesion location and the type of gustatory or ingestion-related functional deficit encountered, reviewed in {Spector 95}. One exception to this is the demonstration that serotonergic antagonists infused into the lateral PBN increased sodium intake in response to an experimentally induced salt appetite {Menani et al 96}. Infusions into the medial PBN subdivision had no effect.

The organization of the PBN also appears to accentuate differences in central projections of neurons responding to posterior versus anterior tongue stimulation. An overview of these pathways is depicted in figure 2.9. The external region with its exclusive posterior tongue responsiveness, provides a larger projection to the CNA than waist region sites responsive to the anterior tongue. Also present in the CNA are projections from posterior tongue sites in VL. Thus, the CNA ought to be more responsive to posterior rather than anterior tongue stimulation. Likewise, the SI may prove to have predominantly anterior tongue representation, although it too receives projections from posterior tongue responsive sites in either the waist or external region. Such differences in anterior and posterior tongue central representation may subserve fundamentally
different functions {Frank 91}. It has been proposed, for example, that the
posterior tongue innervated by the glossopharyngeal nerve, plays a more
prominent role in aversive reflex function compared to facial nerve innervated
taste buds of the anterior oral cavity that contribute more to quality
discrimination. The results of the present study provide additional evidence of
different central representation of these intraoral sites.
Figure 2.1

Schematic representation of BD and PHA-L injection sites in PBN waist region, external region, and Kölliker-Fuse subnucleus. Figure 2.1C corresponds approximately to figure 54 (the interaural -0.16 mm plane) in the Paxinos and Watson atlas {Paxinos Watson 86}. Sections are separated by about 240 µm. Level A is about 480 µm rostral to where Mo5 begins. A) most of the label at this level represented spread from injections centered at level B. B) most injections at this level were centered on anterior tongue taste activity in CM, but extended into VL. At this level, most label in the external region represented spread from injections centered at level C. C) EM at this level is prominent; most injections in the external region targeted posterior oral cavity responses in EM but spread somewhat into EL. D) small injections into KF at this level were mostly restricted to this subnucleus.
Figure 2.1
Figure 2.2

Brightfield photomicrographs of BD injections into the PBN and the corresponding electrophysiological activity recorded with injection pipettes just prior to injection.  **A1-2)** injection no. 516 centered on anterior tongue taste responses in PBN waist region; arrow in A2 corresponds to onset of taste stimulus application;  **B1-2)** injection no. 616 centered on posterior tongue tactile responses in the PBN external region; arrows in B2 correspond to onset of mechanical stimulations of ipsilateral foliate papilla. **C1-2)** injection no. 535 centered on spontaneous respiratory related activity in KF subnucleus. Scale bar is 0.5 mm.  Time markers are 1 sec.
Figure 2.3

Schematic representation of medullary projections from PBN waist and external regions. A1-4) in this dual tracer experiment our smallest BD injection restricted to CM subnucleus of the waist region (607 in Fig. 2.1) was combined with FG injection into mXII (shaded area in A3-4) in order to compare the distribution of retrogradely labeled lingual premotor neurons (closed symbols) with anterograde PBN projections. B1-4) sparse distribution of fibers in the medulla after injection of BD into EM (603 in Fig. 2.1).
Figure 2.4

A) Brightfield photomicrograph of a large BD pressure injection centered on anterior tongue taste activity in PBN waist region. Scale bar is 0.5 mm. B) darkfield photomicrograph of medullary projections (about 1.8 mm rostral to obex) from injection in A. Scale bar is 0.25 mm. C) brightfield photomicrograph of medulla (about 2.7 mm rostral to obex) showing PBN projections intermingled with a dense cluster of retrogradely labeled neurons in rostral pole of NST (arrow head) and scattered within PCRt (arrows). Scale bar is 0.25 mm. D) Brightfield photomicrograph of passing fibers with axonal varicosities (thin arrow), terminal arborization (arrow heads), and passing fibers without synaptic interactions (wide arrow). Scale bar is 50 μm.
Figure 2.5

Medullary projections from PBN ventrolateral subnucleus

A1-4) anterograde labeling observed in IRt and PCRt following BD injections centered on posterior tongue (foliate) mechanical stimulation responsive sites in VL (614 in Fig. 2.1); B1-4) dense pattern of label in ventrolateral medulla (VLM) observed with injections centered on respiratory activity in KF (535 in Fig. 2.1).
Figure 2.5
Schematic representation of a dual tracer experiment in which a small injection of BD (shaded area in A1) centered on anterior tongue taste in the NST was combined with a large injection of FG centered on PCRt/IRt (shaded and stippled area in A2). B1) this PBN level corresponds to figure 2.1 C. At this level, RF-projection neurons (closed circles) in the PBN waist region appear equally distributed in both CM and VL but are completely absent in EM. Nevertheless, NST afferent fibers terminate in both the waist region and EM. B2) at this level (about 200 μm caudal to B1); a small cluster of neurons and fibers are also labeled in DM in addition to the waist area; Me5 was also labeled with anterograde fibers and retrogradely filled neurons.
Figure 2.6
Figure 2.7

PBN Projections to the Thalamus

A) The waist region projects more ipsilaterally (right) to the gustatory subnucleus in the thalamus (Gu) B) the external region projects more contralaterally (left) to Gu.
Figure 2.8

PBN Projections to the Amygdala

A) Brightfield photomicrograph of PBN projections to CeL and CeM from VL (injection 614 in Fig. 2.1). B) PBN projections from the external region (injection 616 in Fig. 2.1) to the amygdala terminate primarily in ventrolateral regions of CNA including the CeLC subnucleus. Scale bar is 0.25 mm.
Figure 2.9

Schematic diagram summarizing some of the major differences in projections from anterior and posterior oral cavity representation in the PBN. Anterior oral cavity (AOC) representation in PBN is primarily in the waist area in VL and CM, somewhat lateral to posterior oral representation (POC). There is a prominent descending projection to the PCRt and an ascending projection to SI and ipsilateral GU, as well as a lesser projection to contralateral GU. Posterior oral cavity representation in the PBN is in both the waist region and the external subnuclei of EM and EL. The waist region has a descending projection but the external region does not. Both thalamic and amygdalar projections differ somewhat as well. The external region has a dominant contralateral GU projection with a lesser ipsilateral one, the waist region is the converse. The waist region projects throughout CeM and CeL, the external region is primarily to the capsular region of CeL. Both regions project to the SI.
CHAPTER 3

ORAL AND GASTRIC PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT TO THE PARABRACHIAL NUCLEUS

Introduction

In rodents, most oral and visceral projections from the NST reach the forebrain after first synapsing in the PBN. It is becoming increasingly evident that morphologically distinct subnuclei in both the NST and the PBN underlie specific oral and visceral modalities (Whitehead 90; Altschuler et al 89; Herbert et al 90). The rostral NST processes oral sensation (Travers et al 86; Travers Norgren 95), and can be divided into several subnuclei (Whitehead 90; Halsell et al 96) of which some (e.g. rostral central: RC) transmit gustatory and oral sensory information to the PBN, and others (e.g. ventral: V) are more involved in local medullary circuits. The rostral NST is anatomically distinct from the more caudal intermediate; and ventrolateral subnuclei in the NST that process respiratory inputs (Bianchi Grelot 94; Altschuler et al 89), the commissural and the caudal part of the medial subnucleus that process cardiovascular inputs (reviewed in Ciriello et al 94), as well as the rostral part of the medial subnucleus that receives gastrointestinal afferents (Hermann et al 83; Shapiro Miselis 85; Leslie et al 82; Norgren Smith 88; Altschuler et al 89). Each of these NST subnuclei
have more or less specific PBN connections {Herbert et al 90}. The results of chapter two indicated that gustatory function in the PBN was distributed in multiple subnuclei with descending projection from some gustatory responsive PBN sites but not others. The implication of these results is that the waist region subnuclei are potentially more involved in local brainstem circuits in contrast to the external region. It is unclear how ascending visceral pathways map onto the gustatory representation in the PBN. To determine if convergence of NST inputs in the PBN may provide a neural mechanism for subsequent modulation of brainstem circuits, the present chapter compared the parabrachial distribution of projections from gastric and taste responsive regions of caudal and rostral NST.

Initial descriptions of projections to the PBN indicated a mediolateral segregation of taste and visceral functions in the PBN {Ricardo Koh 78; Norgren 78}. However, later studies indicated a more complex relationship of these inputs including potentially overlapping terminal domains in some PBN substrates {Hermann et al 83; Fulwiler Saper 84; Herbert et al 90; Herbert Saper 90; Karimnamazi et al 97}. The organization of NST inputs to the PBN is complicated in that ascending fibers from both rostral and caudal NST terminate in two mediolaterally distinct regions in the caudal part of the PBN {Herbert et al 90; Herbert Saper 90; Becker 92}. Furthermore, both of these PBN regions are targets of projections from anterior and posterior oral cavity sites in the rostral NST {Becker 92}. However, only posterior oral cavity responsive neurons are
found in the external region, whereas the waist region contains neurons that respond to both anterior and posterior oral cavity \cite{NorgrenPfaffmann75, HalsellTravers97}. Within this complex topographical arrangement, anterior tongue taste dominant neurons are clustered in a core that is surrounded by a shell of posterior oral cavity dominant responses \cite{HalsellTravers97}. This organization parallels an anatomical pattern produced by injections centered on POC responses in the NST that result in fibers more medial in the PBN waist region compared to those originating from anterior tongue taste regions of NST \cite{Becker92, Travers93}. These more medial regions are also the site of visceral projections from the caudal NST \cite{Hermannetal83, HerbertSaper90, Herbertetal90}. Thus, potential PBN regions of overlap between oral and gastric projections from the NST include medial sites in the waist region (i.e. CM/VL) representing the classical pontine taste area \cite{NorgrenLeonard73}, as well as more lateral sites in the external subnuclei. The external subnuclei were originally associated with cardiovascular regulation and other visceral functions including gastric distension \cite{ChamberlinSaper94, YuanBarber91}, but recently, based on FOS immunoreactivity and neurophysiological studies, have also been implicated in oral sensation \cite{Yamamotoetal94, HalsellTravers97}.

Despite neurophysiological evidence for gustatory and visceral convergence in the PBN waist region \cite{HermannRogers85}, the extent of this overlap and the functional nature of these inputs is unclear. It is also unclear if intranuclear
projections of the NST or projections to the subjacent reticular formation, potentially contribute to orovisceral convergence in the brainstem. Although the focus of the present chapter was on the organization of oral and visceral functions in the PBN, anterograde injections into gastric responsive sites in the caudal NST and oral responsive sites in the rostral NST resulted in intranuclear NST projections and local projections to the RF which were also analyzed.

Materials and Methods

Adult, male, Sprague-Dawley rats weighing between 250-400 g were used for these experiments. Rats were food-deprived overnight and anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg) injections into the peritoneal cavity. Supplementary doses (15 mg/kg) of this drug were used as needed to maintain an areflexive level of anesthesia. Animals were also injected with a prophylactic dose of penicillin (0.08 ml S.Q) approximately one hour prior to surgery and the dorsum of the head and ventral region of the belly were shaved and treated with antiseptic solution (Povidone-Iodine, L.T. York Co.) in preparation for surgery. Core temperature was monitored and maintained at 37 ± 1°C throughout the experiment.

Construction of Gastric Balloon

Prior to surgery, a gastric balloon was constructed out of the small finger of a petite size latex glove tied to approximately 5 inches of a flexible plastic tubing.
with Teflon tape (Tygon, o.d. = 1/8", VWR Scientific). An extension line (PE 190 & 280 Becton Dickenson Co.) was used to attach (miniature connectors; General Valve Co.) the balloon apparatus to a 10 cc syringe outside the abdominal cavity. This allowed for controlled volumetric expansion of the stomach wall.

Surgical Procedures

Abdominal surgery was performed to implant the gastric balloon prior to placement of the animal in the stereotaxic stage. The animal was placed in a supine position and the abdominal cavity was opened through a midline incision. The stomach was then located and gently raised to expose its dorsal surface. Care was taken not to overstretch the gastric innervation. A small clamp was used to pinch an approximately 5 mm area of the dorsal surface of the stomach at the site of incision to limit bleeding and allow ease of gastric manipulation during surgery. Just anterior to the clamp, an incision was made with a sterile surgical blade (Paragon #11; Sheffield, England) which penetrated approximately 3-5 mm into the rostral third of the greater curvature. A cotton applicator was use to maintain the patency of the incision. While being careful not to damage the abdominal lining, the residual gastric contents were gently removed with a small spoon excavator. The balloon was then implanted by tightly wrapping it around the small end of a cotton applicator and gently inserting it into the stomach. After removing the applicator, a piece of suture was used to tie the incision around the shaft of the balloon as it exited the stomach.
This method was more efficient and less traumatic to the tissue than using purse string sutures {McCann Rogers 92}. After closure of the stomach, the balloon was tested over a range of volumes (2-10 ml) to make sure it functioned properly and that the gastric contents did not leak outside the stomach. The stomach was then repositioned in its natural posture and the abdominal wall was closed with continuous sutures around the gastric catheter. Prior to closing of the abdomen, the peritoneal fluids lost during surgery were replace with physiologic saline (5 ml of 0.9% NaCl). The overlying skin was closed with surgical staples and antibiotic ointment was applied externally to the wounds.

After completion of gastric surgery, the animal was mounted prone in a stereotaxic head-holder equipped with non-traumatic earbars (Kopft). The skull was exposed through a midline incision and was leveled with respect to the anteroposterior and mediolateral axes. Access to the brainstem was achieved through an opening in the skull that extended posteriorly from the transverse suture to the occipital plate where the cervical muscle attachments were reflected to open the cisternae magnum.

_Stimulation, Recording & Lesioning Procedures_

The extracellular electrophysiological response of NST neurons were identified with glass coated tungsten microelectrodes (R= 0.5-1.3 MΩ) grounded through a metal clip attached to the skin. The signal was amplified (10K) and recorded on
magnetic tape for off-line analysis. For access to the gustatory responsive sites in the rostral NST, the skull was maintained parallel to the horizontal axis whereas for identification of gastric responses in the caudal NST the incisor bar of the head holder was lowered to allow a 10 degree inclination of the head relative to the horizon. This allowed direct visualization of the fourth ventricle and access to calamus scriptorius (CS) which was used as a brain surface landmark for identification of gastric stretch responsive sites in the caudal NST. Gastric distension was achieved by volumetric expansion (2-10 ml) of the stomach via the gastric balloon. The distended posture of the stomach was maintained for 10 seconds and then released. At least 30 seconds separated repeated gastric distensions. Typical mediolateral (ML) and anteroposterior (AP) millimeter measurements for starting the search for gastric responses in the caudal NST were ML = 0.3 and AP = 0.3 from CS. When identifying gustatory responses in the rostral NST, a bony landmark (lambda) was used as reference, because CS is relatively distant from the rostral pole of NST. The oral sensory stimulation procedures were similar to those used by Travers et al (1986) and are briefly summarized here. The tactile test consisted of lightly stroking the oral and pharyngeal mucosa with a small blunt glass probe. The gustatory test consisted of flowing a small amount (1 ml) of a taste mixture (0.3 M sucrose, 0.3 M sodium chloride, 0.01 M hydrochloric acid, and 0.003 M quinine hydrochloride) onto specific anterior and posterior oral cavity receptive fields with small, soft paint brushes. Typical millimeter coordinates for identifying AOC responses in
the rostral NST were ML = 1.7 / AP = 3.9, and for POC responses in rostral NST were ML = 1.6 / AP = 4.5 from lambda. In an initial set of experiments (n=4) electrolytic lesioning techniques were used to map the gastric stretch responsive sites in the caudal NST. This guided the subsequent series of tracer injection experiments. In the mapping experiments, after characterization of a caudal NST site, small electrolytic lesions (3 μA x 3 ms) through the recording electrode were used to mark the site of electrophysiological activity (Fig. 3.1).

Injection of Neural Tracers

Upon qualitative characterization of a neurophysiologic site (i.e. oral or gastric responsive), the tungsten electrode was withdrawn and replaced with a glass micropipet that contained a neural tracer. In a series of eight experiments, 10% Biotinylated Dextran (BD: Lysine-fixable, MW = 10 kDa, Molecular Probes Inc) dissolved in 0.5 M KCl was injected into the gastric responsive regions of the caudal NST to identify the organization of gastric projections in the PBN. In addition, in four of the experiments 15% Rhodamine Dextran (RD: tetramethylrhodamine, 3000 MW, anionic, lysine fixable, Molecular Probes Inc) dissolved in 0.9% NaCl was also injected into the gustatory responsive sites in the rostral NST of the same animals. All injections were iontophoretically made with 3-5 μA of anodal current and a 4 second duty cycle for 10 - 20 minutes. After each injection the pipet was left in place for approximately 5 minutes, to reduce unwanted spread into the tract, and then withdrawn.
**Perfusion & Histology**

For the mapping experiments, at the end of the recording session, the animals were given a lethal dose of Nembutal (150 mg/kg), and perfused with phosphate buffered saline (PBS) and 10% buffered formalin, and the brains were extracted and stored in 20% sucrose formalin overnight. Two parallel series of 52 μm transverse sections were prepared with a freezing microtome and the sections were mounted on chrome alum slides and allowed to air dry. The series were alternately stained with Cresyl Violet and Weil stains to distinguish different neural structures and coverslipped with Permount.

In the neural tracing experiments, after appropriate survival time (7-14 days) the rats were perfused with PBS and 4% paraformaldehyde and the brains were immediately recovered and stored in 20% sucrose overnight. Coronal 40 μm section of the brainstem were cut with a freezing microtome into 2 series and placed in cold PBS. Free floating sections from one of the series were rinsed in PBN and incubated for 45 minutes in 0.4% Triton in PBS. The tissue was then incubated in the ABC solution (10 μl/ml of Vectostatin Elite ABC kit + 0.1% bovine serum albumin, Vector Labs) at room temperature for one hour to produce the avidin/biotin-peroxidase conjugates. After rinsing the tissue in phosphate buffer (PB), reaction products were visualized by the metal enhanced 3,3' diaminobenzadine HCl peroxidase reaction (DAB, Sigma Co). The tissue was incubated at room temperature for 15 min. In 0.05% DAB and 1% NiNHSO₄.
in PB and 0.0005% H₂O₂ was then added for 2 to 5 minutes while the reaction was monitored for the visualization dark reaction products. Further intensification was achieved by adding a 1% CoCl₂ (Fisher Scientific Co) solution just prior to the hydrogen peroxide step. The unreacted series was mounted and stained with Cresyl Violet for visualization of Nissl substance. In the dual tracing experiments, due to the fluorescent nature of the RD tracer only the BD histochemical reaction was required to visualize both label. In these experiments, the tissue was cut into three series one of which was not reacted but coverslipped with a non-fluorescent medium (Cytoseal) to visualize the RD label only. The other two series were reacted to also visualize the BD label and one of the latter series was also counterstained with neutral red to distinguish other neural structures on the section. Thus, series A was the counterstained sections, series B was dually labeled with both tracers, and series C was fluorescent only.

**Data Analysis**

The slides were viewed under bright field and dark field or under fluorescent illumination through a conventional microscope interfaced with a microcomputer based image processor through a video camera (Neurolucida, Micro Brightfields Inc). Composite sections were drawn to localize the labeled fibers within topographical landmarks of the NST, RF, and PBN. An atlas (Paxinos Watson 86) was consulted to assign label to specific anatomical structures. To reduced
error due to thickness when relating the distribution of BD and RD fibers to neural structures across the three series, and to minimize fading of the fluorescence, the RD label in series B (dual labeled sections) was plotted first. Series C (fluorescent only sections) was used to control for fading of the fluorescence during the BD reaction phase, and series A was used to plot the anatomical structures onto series B. Finally, the BD label in series B was plotted in relation to the RD label and the anatomical structures.

Results

Distension of the stomach activated neurons in a restricted region of the medial subnucleus of cNST. The photomicrograph in figure 3.1 shows two electrolytic lesions placed at sites of neural activity in cNST that were activated by gastric stretch. The mean stereotaxic coordinates for identifying gastric responses in cNST were: AP = 0.5 mm anterior to calamus scriptorius, ML = 0.35 mm lateral to the midline, and DV = 0.5 mm ventral to the brainstem surface. These are typical coordinates for electrophysiological identification of gastric distension activated neurons in the NST {McCann Rogers 92; Chambert et al 93}. Different physiological responses helped localize neurons that were activated by distension. Thus, gastric activated neurons were dorsoventrally interposed between somatosensory responsive sites in gracile nucleus that were activated by tactile stimulation of the lower extremities, and spontaneously active cells, presumably in the DMN, that were transiently inhibited with distension ventrally.
Further, respiratory activity was consistently identified lateral to gastric responsive neurons. Figure 3.2 A shows a composite of eight different BD injections into gastric responsive sites in the caudal NST (cNST). In four of these experiments, RD was also injected into anterior (AOC: n=2) and posterior (POC: n=2) oral cavity taste and tactile responsive sites in (Fig. 3.2 B,C). The photomicrographs in figures 3.3 and 3.4 represent one such dual injection experiment (case 15 in Fig. 3.2 A,B) that combined an injection of BD into a gastric responsive site in cNST (Fig. 3.3) with an injection of RD into a POC (foliate) responsive site in rNST of the same animal (Fig. 3.4). These injection sites were separated by approximately 1.7 mm from each other and each injection spread no more than 200 μm in the anteroposterior dimension. The electrophysiological responses recorded with the injection pipets prior to iontophoresis are illustrated in figure 3.5. Although the gastric response recorded in this experiment was partially masked by a relatively high spontaneous rate (Fig. 3.5 A), typical gastric responsive neurons in cNST had a very low spontaneous rate (~ 1 spike/sec) and their evoked responses were typically time locked with the duration of gastric stimuli. Similarly, tactile responses in rNST were tightly coupled to stimulus duration (Fig. 3.5 B), but taste responsive units had a higher evoked response that outlasted the gustatory stimulus and returned to baseline after the water rinse.
Projections to the PBN

The distribution of fibers in the PBN from a representative dual injection experiment (Fig. 3.2: case 13) that combined a BD injections into a gastric responsive site in cNST with a RD injections into a POC responsive site in rNST is shown in figure 3.6. At the most rostral levels of the PBN (Fig. 3.6 A,B) fibers from cNST were segregated lateral and dorsal to those from rNST. Further caudally, this segregation of fibers became less apparent as oral and gastric projections became interdigitated across multiple subnuclei in a laminar fashion. Thus, overlap was observed in CM, DM, VL, CL, EM, and ELi, as well as the interstitial neurons in the waist area (Figs. 3.6 C-E). However, separation of fibers was seen in DL, and Elo (Figs. 3.6 A-C) which were almost exclusively labeled by gastric projections (Fig. 3.8) as well as VM (Figs. 3.6 C, D) which primarily received afferents from rNST.

Tracer injections into AOC responsive sites in rNST produced a similar pattern of label across different PBN subnuclei and consequently similar patterns of overlap with gastric projections from cNST. Thus, AOC and gastric afferents (Fig. 3.7 F,G) overlapped in CM/VL, EM/ELi, DM, and CL in the caudal PBN (Fig. 3.7 C-E) but were segregated further rostrally (Fig. 3.7 A,B). Additionally, certain differences resulting from AOC/POC injections were also observed within individual PBN subnuclei. Specifically, a topography was observed in CM at the second most caudal levels of the PBN (Figs. 3.6,3.7 D). At this PBN level,
gastric projections heavily labeled the medial third of CM, whereas the intermediate and lateral thirds were consistently labeled by POC and AOC fibers respectively. A partial overlap of rNST and cNST fibers was observed at the transition zone between the medial and intermediate parts of CM. Thus, the alimentary canal seems to be inversely represented from medial to lateral in CM. At the most caudal level of the PBN however, this topography was replaced by a more complete overlap of oral and gastric projections to CM (Figs. 3.6, 3.7 E).

Projections to the Reticular Formation

Efferent projections from the NST reached the PBN by ascending through the medullary and pontine reticular formation. In transverse sections, NST projections were restricted to fiber bundles in the pontine RF and did not show evidence of synaptic interaction. However, NST projections showed segmental innervation of the medullary RF. Thus, cNST projections to the RF were heaviest caudally in the dorsal reticular nucleus (MdD), the periambiguus region, and the ventrolateral medulla, and diminished further rostrally in parvocellular (PCRt) and intermediate (IRt) reticular areas (Fig. 3.9). The projection from rNST was complementary to that of cNST in that the more rostral RF areas were heavily labeled in PCRt and IRt, and diminished further caudally in MdD and in the ventrolateral medulla [not shown].
Intraneuronal Projections of the NST

Injections of BD into gastric responsive sites in cNST produced a distinct pattern of label in rNST (Fig. 3.9 A, B) such that the rostral medial (M), rostral central (RC), and to a lesser extent the ventral subnucleus (V) were densely labeled with axons that contained numerous varicosities. This pattern of label extended up to the rostral pole of rNST and was bilateral with an ipsilateral dominance. Conversely, RD injections into rNST did not result in a significant projection to gastric responsive sites in cNST, although a moderate projection to the intermediate NST at the level of the fourth ventricle was consistently observed [not shown].

Discussion

In the present experiments, small physiologically guided iontophoretic injections into oral and gastric responsive NST sites resulted in a pattern of both segregation and overlap of oral and gastric representations in the PBN. Thus, specific PBN subnuclei in both the waist (CM/VL) and external regions (EM/ELi) are potential sites for integration of these inputs, whereas sites in VM, DL, CL, and ELo subnuclei showed more segregation of oral and gastric afferents. Further, evidence for intraneuronal projections in the NST and local projections to the reticular formation was identified. The functional implications of these connections are discussed below.
Localization of NST Neurons Activated by Oral and Gastric Stimulation

Anatomical and physiological experiments indicate that oral and gastric functions are segregated to the rostral and caudal portions of the NST {Hamilton Norgren 84; Norgren Smith 88; Altschuler et al 89; Altschuler et al 91; Whitehead 88; McCann Rogers 92; Travers Norgren 95}. The left and right gastric branches of the subdiaphragmatic vagus nerve carry mechanosensitive afferents from the anterior and posterior regions of the stomach to the medial and dorsomedial subnuclei of the caudal part of NST {Shapiro Miselis 85; Norgren Smith 88; Altschuler et al 89}. Physiological experiments indicate that gastric distension activates neurons in a restricted region of cNST just dorsal to the dorsal motor nucleus of the vagus {McCann Rogers 92; Chambert et al 93}, an area that contains PBN projection neurons {Whitehead 90; Herbert Saper 90}. Similarly, gustatory fibers in the facial, trigeminal, glossopharyngeal, and laryngeal branch of the vagus, terminate in the rostral NST in an orderly fashion {Hamilton Norgren 84} such that the rostral central and lateral NST subnuclei receive the bulk of inputs from chorda tympani and glossopharyngeal nerves and subsequently project to the PBN {Whitehead 88; Whitehead 90; Halsell et al 96}. Gustatory neurophysiology is largely confirmatory with these anatomical descriptions and indicates that neurons in the central and lateral regions of rNST respond predominantly to gustatory and tactile stimulation respectively and that these responses are topographically organized according to the oral receptive fields {Ogawa Hayama 84; Travers Norgren 95} see reviews {Travers 93;
Norgren 95}). In the present study, the distribution of physiologically guided BD and RD injections in the caudal and rostral NST (Fig. 3.2) correspond well with the anatomical and physiological descriptions (above). Based on this highly topographical representation of the alimentary canal in the NST, direct convergence of primary afferents is highly unlikely, prompting the present investigation of NST efferent projections to the PBN. However, recording experiments indicate the visceral modulation of gustatory activity in the NST {Glenn Erickson 76; Giza Scott 83; Jacobs et al 88}, perhaps suggesting that intranuclear projections within the NST should also be considered. Alternatively, other possible sources of modulation of gustatory activity in the NST include those from the forebrain {Halsell 97; Van Der Kooy et al 84} and from the PBN [see chapter 2].

Medullary and Pontine Connections of Oral and Gastric NST

Projections to the parabrachial nucleus

The PBN consists of a series of morphologically distinct subnuclei {Fulwiler Saper 84; Halsell Frank 91} that receive topographically complex terminal fields, reflecting the multi-functional nature of its afferent source in the NST. Although the initial descriptions of ascending oral and visceral projections from NST to PBN suggested a mediolateral segregation of these inputs {Ricardo Koh 78; Norgren 78}, other studies emphasized the potential overlap of these projections in the PBN {Hermann et al 83}. Many studies have reported on the afferent and
efferent connections of the PBN with the medulla and the forebrain {Norgren 76; Norgren 78; Voshart Van der Kooy 81; Lasiter et al 82; Saper Loewy 80; Loewy 81; King 80; Holstege 88} however, only more recently have these connections been related to the subnuclear topography of the PBN {Fulwiler Saper 84; Herbert et al 90; Moga et al 90; Herbert Saper 90; Bernard et al 93; Halsell 92; Alden et al 94; Feil Herbert 95; Karimnamazi et al 96}. A recent study based on the subnuclear origin and termination of these connections between the NST and PBN indicates both overlap and segregation in a complex organization involving specific morphologically distinct subnuclei {Herbert et al 90}. Thus, projections from the general visceral and the gustatory parts of NST overlapped in CL and the waist area, compared to gustatory and visceral segregation evident in VL/ EM and DL/ELi respectively. The results of the present study confirmed these findings and indicated that segregation of oral and gastric projections occurred at the most rostral levels in EL, DL, and CL (Fig. 3.6, 3.7 A, B) such that projections from gastric responsive NST were further dorsal and lateral in relation to projections from oral responsive NST. Conversely, projections from oral and gastric responsive NST overlapped in the caudal PBN waist area and in CM/VL (Fig. 3.6, 3.7 D, E), as well as further rostrally in EM/ELi, CL, and DM (Fig. 3.6, 3.7 C). In a previous study that predates the subnuclear parcelation of the PBN, similar findings were reported with regards to PBN projections from hepatic and taste responsive sites in the caudal and rostral NST respectively {Hermann et al 83}. That is, axons from both the hepatic and
gustatory regions of the NST terminated densely in the caudal dorsomedial part of the PBN, whereas at anterior levels, the gustatory and visceral NST projections were mediolaterally separate.

In addition to ascending projections from the NST, the PBN receives nociceptive inputs from the spinal trigeminal nucleus in the medulla, and the dorsal column nuclei of the spinal cord (Allen et al 96; Menendez et al 96; Bester et al 95; Bernard et al 95; Feil Herbert 95; Cechetto et al 85). Parabrachial terminal fields of nociceptive projections in the external region as well as CL and DL subnuclei overlap with some gustatory and gastrointestinal projections from the NST, suggesting possible PBN convergence of oral, visceral, and spinal nociceptive processes. This view is consistent with the hypothesized role of the PBN in body-wide regulatory processes rather than specific organs or autonomic reflexes, organized in the medulla, or behavioral processes, dependent on the forebrain (Saper 95).

In the present study, a consistent mediolateral topography was observed in CM at level D (Fig. 3.6, 3.7) such that gastric projections were located in the medial third, posterior oral cavity projections in the middle third, and anterior oral cavity projections in the lateral third of this subnucleus. These patterns of convergence and segregation suggest functional specialization in the PBN; an observation supported by comparing the afferent terminal fields of forebrain and medulla.
There seems to be a correlation between medullary and forebrain afferents to the PBN. In some instances, the terminal fields of forebrain and medullary afferents almost completely overlap in some PBN subnuclei. For example, injections into gastric responsive sites in the medial subnucleus of cNST resulted in projections to EL, EM, CL, and waist subnuclei in a pattern nearly identical to that seen after injections of anterograde tracers into the central nucleus of amygdala, and bed nucleus of stria terminalis {Moga et al 90}. Thus, because some efferent projections of these PBN subnuclei terminate in the central nucleus of the amygdala and the bed nucleus of stria terminalis, there appears to be reciprocal connectivity between these forebrain sites and the PBN {Bernard et al 93; Alden et al 94}. These findings further emphasize that functionally related afferent connections of the forebrain and the medulla converge in specific PBN subnuclei that are also the source of reciprocal projections. A pattern of convergence and segregation of afferent projections to the PBN does not seem to be restricted to the mammalian brain, because in fish both anatomical segregation and physiological convergence of facial and vagal inputs to the secondary gustatory nucleus have been reported {Morita Finger 85; Lamb Caprio 92}. Anatomical convergence provides a mechanism for orogastric interactions in the PBN that may influence brainstem mediated consummatory responses (discussed in chapter 2), whereas segregation preserves the topographical representation of the alimentary canal in the ascending visceral pathway.
Intranuclear Projections of Gastric NST

Injection of BD into gastric responsive sites in the caudal NST resulted in a specific pattern of intranuclear projections to the intermediate and rostral portions of the nucleus. Although intranuclear connections of the NST have not been explicitly studied, evidence for such connections are evident in the published figures of other anatomical studies that have examined the efferent connections of cNST. For instance, injections of radiolabeled amino acids into the caudal (commissural) NST resulted in a pattern of label restricted to the medial half of the rostral NST similar to that observed in the present study (Ross et al 85).

Gastric projections from cNST terminated predominantly on neurons in the rostral medial (M), central (RC) and the medial part of ventral (V) subnuclei In the NST. Neurons in RC receive the bulk of afferent inputs from the chorda tympani and the lingual branch of glossopharyngeal nerve (Hamilton Norgren 84), respond to gustatory stimulation ([McPheeters et al 90] discussed in [Halsell et al 96]), and provide the greatest contribution to the ascending gustatory limb from the NST to the PBN (Whitehead 90; Halsell et al 96). Thus, a subset of gustatory responses in the PBN (e.g. the gastric modulated oral responses in chapter 4) may reflect the effects of visceral modulation of gustatory neurons in rNST. Other physiological studies that demonstrate gustatory, visceral, or humoral interactions in the NST, further support a possible functional role for intranuclear NST projections. Thus, modulation of gustatory responses in rNST
by sustained gastric distension {Glenn Erickson 76}, IV glucose and insulin infusion {Giza Scott 83; Giza Scott 87}, and sodium deprivation {Jacobs et al 88; Nakamura Norgren 95} are potentially mediated by intranuclear NST connections. Other connections such as descending projections from the forebrain {Halsell 97} or PBN (see chapter 2) however, must also be considered. Regardless of the nature of visceral modulatory influences on gustatory neurons in the NST, direct convergence of primary afferents is unlikely because electrical stimulation of afferent fibers in the vagus nerve did not prove effective in activating gustatory responses in the NST {Hermann et al 83}. Additionally, the alterations in NST gustatory responses that occur with visceral manipulations require several minutes (e.g. sustained gastric distension) to several days (e.g. sodium depletion) to produce effects that do not return to baseline for a relatively long time. This further implicates the involvement of complex pathways possibly through polysynaptic connections to the forebrain or through short projections to the medullary reticular formation.

The terminal distribution of cNST intranuclear projections also extended into the ventral subnucleus of rNST, indicating possible gastric modulation of neurons in this subnucleus as well. Anterograde injections into taste responsive sites in the PBN (chapter 2) indicated a descending component that terminates in the ventral part of rNST. Similar findings have been reported with descending projections from the central nucleus of the amygdala that terminate in the medial half of RC
and extends into V {Halsell 97}. Despite a paucity in studies of intranuclear connections of NST subnuclei, a recent study {Hu et al 97} suggests a possible connection for sensory inputs to V that involves intranuclear projections from the overlying RC and RL subnuclei. The dendritic extension of V neurons into these overlying subnuclei provide yet another mechanism for synaptic interactions {Whitehead 88}. Thus, increasing evidence suggests that the ventral subnucleus of rNST may represent a secondary receiving site for afferent sensory information from interneuronal sources within rNST. Based on the segmental efferent connections of V to the subjacent reticular formation {Travers 88; Whitehead 90; Beckman Whitehead 91; Becker 92; Halsell et al 96} neurons in V are hypothesized to contribute to local brainstem functions that include differential oromotor responses to ingestive and aversive gustatory stimuli {DiNardo Travers 97; Travers et al 97}.

*Projections to the Reticular Formation*

Projections from the rostral and caudal NST to the RF extended from the lateral border of NST to the ventrolateral medulla. At rostral levels the fibers were primarily comprised of rNST projections to the PCRt and IRt that diminished further caudally and were replaced by cNST projections to MdD that in turn tapered off rostrally. This relative segregation between rostral and caudal NST projections to the medullary reticular formation suggests that the RF may not be a likely site for direct convergence between oral and visceral projections from the
NST. Many studies have examined the local interconnections of NST and RF
{Travers 88; Ter Horst et al 91; Shammah-Lagnado et al 92; Whitehead 88;
Beckman Whitehead 91; Becker 92; Halsell et al 96; DiNardo Travers 97}. Some
studies have suggested a segmental organization that involves local projections
from the NST to the immediately subjacent RF {Halsell et al 96}, that may
resemble a columnar pattern of innervation as reported in other species
(discussed in {DiNardo Travers 97}). As such, the rostral extent of NST-RF
connections may represent an oral sensorimotor coupling mechanism whereas
the caudal connections may underlie more visceral interactions that involve the
coordination of swallowing, digestion, and cardiorespiratory functions.
Figure 3.1

Subnuclear Organization of caudal NST

This brightfield photomicrograph shows a medullary section approximately 500 μm anterior to obex. Arrows depict electrolytic lesions at sites of recording of multiunit (dorsal) and single unit (ventral) gastric activated responses in the medial subnucleus of the caudal NST. Section was stained with Cresyl Violet. Asterisks indicate center of lesions. Scale bar = 250 μm. For abbreviations refer to List of Abbreviations section.
Figure 3.2

Schematic representation of injection sites in NST. A) Eight BD injections were centered on gastric responsive sites in the medial subnucleus of cNST. In four of the experiments, RD was also injected into POC (B) and AOC responsive sites in rNST of the same animal.
Figure 3.2
Figure 3.3

A BD Injection Site Centered on Gastric Responses in NST

Brightfield photomicrograph of a BD injection site (case 15 in Fig. 3.2 A) centered on gastric responses in cNST of a dual injection experiment. The electrophysiological activity at this site is displayed in Fig. 3.5 A. The PBN projections resulting from this injection are displayed in Fig. 3.6 and the medullary projections are depicted in Fig. 3.9. Scale bar = 250 µm. For abbreviations refer to List of Abbreviations section.
Figure 3.4

The dense fluorescent label in this photomicrograph depicts a RD injection into a POC responsive site in rNST (case 15 in Fig. 3.2 B) as part of a dual labeling experiment. The randomly scattered fluorescent cells are most likely humoral cells that have phagocytized the fluorescent molecules. The physiologic response at this site is depicted in figure 3.5 B. Projections from this injection are depicted in figure 3.6.
Figure 3.5

Electrophysiological Records of Activity Prior to Injection

**A)** Electrophysiological record depicts spike activity recorded with the BD injection pipet in cNST just prior to tracer injection (Fig. 3.3). Long bar indicates duration of gastric stretch (GS) with 2 ml of air. **B)** Integrated multiunit activity recorded with RD micropipet positioned at a POC (foliate) responsive site in rNST (Fig. 3.4). Arrowheads correspond to mechanical stimulation of the ipsilateral foliate. Scale bars = 1 sec.
Figure 3.6

Distribution of POC and Gastric Afferent in the PBN

Overlapping terminal fields in the caudal PBN (levels 3-5) following injection of BD in gastric distension responsive cNST (grey) and RD in foliate responsive rNST (black). Fiber distributions are more segregated at rostral levels (A & B). Note the relative segregation of NST terminal fields in CM at PBN level D.
Figure 3.6 (continued)
Distribution of AOC and Gastric Afferents to the PBN

PBN series from a double labeling experiment that combined a RD (black) injection centered on an anterior tongue taste responsive site in rNST with a BD (grey) injection in a gastric responsive site in cNST. Note the extent of RD label in CM and its relative distribution to the BD label.
Figure 3.8

Distribution of Gastric afferents in the PBN

Photomicrograph depicts the distribution of BD from a gastric distension responsive site in cNST. Note the laminar arrangement of fibers dorsoventrally in the lateral subnuclei. Section corresponds to figure 3.7 B. Scale bar =100 μm.
Figure 3.9

Intranuclear Projections of Caudal NST

Tracing of medullary series of a dual labeling experiment depicting projections from a BD injection (E) into a gastric responsive site (case 15 Fig.3.2, Fig. 3.3). The shaded area next to the injection site in (E) contained a very high density of fibers emerging from the injection site. The dark area in (B) corresponds to the RD injection site in rNST. Only the BD label has been plotted on these sections. Numbers next to sections indicate distance anterior to obex. Scale bar = 500 μm.
Figure 3.9
CHAPTER 4

ELECTROPHYSIOLOGICAL RESPONSE OF SINGLE NEURONS IN THE PARABRACHIAL NUCLEUS TO ORAL AND GASTRIC STIMULATION

Introduction

Oral and visceral functions must interact to produce a balanced nutritional state but neural signals generated in oral and visceral structures are carried to the brainstem by different cranial nerves (discussed in chapter 3), indicating central convergence. Physiological studies indicate that multimodal convergence of primary afferents from abdominal viscera (e.g. gastric and hepatic) or within oral structures (e.g. lingual and palatal) occur in the caudal and rostral NST respectively {Chambert et al 93; Appia et al 86; Ogawa et al 84; Travers Norgren 95}. Further, visceral signals such as sustained gastric distension can modulate the response of gustatory neurons in the rostral NST {Glenn Erickson 76; Giza Scott 87; Jacobs et al 88; Nakamura Norgren 95}. Behavioral experiments indicate that similar to normal controls, decerebrate rats correctly decrease their intake of a sucrose stimulus with a gastric preload with milk {Seeley et al 94}. Although the underlying mechanism for this type of modulation is not clear {Hermann et al 83; Bereiter et al 81}, the topographical segregation of oral and visceral functions in the NST suggest that direct neural convergence of primary
afferents in the NST is unlikely {Hermann et al 83}. The segregation of primary
gustatory and vagal inputs in the rodent brainstem is even more apparent in
some species of fish {Finger 76}. However, in both rodents and fish, segregation
of oral and vagal inputs is considerably reduced in the secondary gustatory relay
{Lamb Caprio 92; Herbert et al 90; Hermann et al 83; Karimnamazi et al 97} [also
discussed in Travers, 1993].

In rodents, the PBN is the secondary gustatory relay in the transmission of NST
inputs to the forebrain. Disruption of inputs to the forebrain by PBN lesions
selectively dissociates some aspects of gustatory and visceral integration, further
implicating the PBN in motivated aspects of ingestive behavior that require
gustatory and visceral convergence {Scalera et al 95; Spector et al 95; Spector
et al 92; Flynn et al 91a; Flynn et al 91b;} [also see review Spector, 1995].
Conversely, descending forebrain inputs represent another pathway that may
modulate PBN neurons that receive gustatory and visceral input {Moga et al 90;
Herbert et al 90}. For example, descending projections from the insular cortex
terminate extensively in the waist region {Moga et al 90}, an area that also
receives dense projections from rostral and caudal NST. This suggests possible
forebrain modulation of gustatory and visceral information at this PBN locus.

Although electrophysiological evidence has demonstrated that a subset of PBN
neurons can be coactivated by electrical vagal and oral stimulation {Hermann
Rogers 85), the functional nature of the vagal signal and the extent of gustatory or oral somatosensory stimuli in coactivating PBN neurons is unclear. Electrical stimulation of the cervical vagus activates multiple visceral afferents to the brainstem, precluding interpretation of the nature of the convergence. Thus, it cannot be concluded if gastric manipulation in general [e.g., thermal, chemical, osmotic] and distension per se are effective in activating these convergent orovisceral responses in the PBN. Electrical stimulation of the gastric nerves as well as volumetric distension can be effective stimuli in activating neural responses in the PBN of anesthetized preparations (Yuan Barber 91; Suemori et al 94). Although the location of gastric activated neurons in previous reports was not related to the subnuclear organization of the PBN, regions that correspond to the dorsal, central, and external lateral subnuclei that were extensively labeled by gastric projections in chapter 3, seem to be involved. However, more medial and caudal regions of the PBN that also receive gastric projections from cNST were not targeted for recording experiments in the previous studies.

In light of the anatomical results of chapter three that showed considerable overlap of PBN afferents from rostral (oral) and caudal (gastric) NST, it was hypothesized that a subset of PBN neurons may be coactivated by oral and gastric stimuli. The purpose of the present study was to identify and record single neurons in the PBN that responded to both oral and gastric stimulation and relate their location to the subnuclear organization of this nucleus. Thus,
volumetric expansion of the stomach, within physiological parameters, was used in conjunction with a battery of oral receptive field specific gustatory and mechanical stimuli, to evoke responses in the PBN.

**Materials and Methods**

Adult, male Sprague Dawley rats (n=20) weighing between 250 and 450 g were used in these experiments. Animals were maintained on a 12 hr. light/dark cycle, and feed at libitum up to the day of surgery. This was done to insure that hunger or food deprivation did not confound the results of manipulation of experimental variables. Surgical anesthesia was obtained with sodium pentobarbital (Nembutal, 50 mg/kg, IP). Supplemental doses of this anesthetic (0.1 ml) were used to maintain an areflexive level of anesthesia when tested by pinching of the hindlimb or touching the cornea. Body temperature was monitored and maintained at 37 ± 1°C throughout the experiment.

**Surgical Preparation**

After an adequate level of anesthesia was obtained, the animal was shaved and placed in a supine position on a temperature regulated plate. Abdominal surgery was performed and a gastric balloon was inserted according to the procedures outlined in chapter 3. The balloon was tested for proper inflation and deflation and the abdominal incision was closed with continuous sutures.
Oropharyngeal Surgery

This phase of surgery consisted of tracheotomy, insertion of an oral drain tube, and placement of mouth sutures. Tracheotomy was performed by placing a midline incision on the ventral surface of the throat area to reflect the cervical musculature and expose the thyroid cartilage and the trachea. Two sutures spaced about 5 mm from one another were passed under the trachea mediolaterally. The ventral part of the trachea was transversely cut and opened to insert the curved end of a flexible polyethylene tubing (PE 205, 10 cm). The distal suture passing under the trachea, was used to secure the canula in place, and the more proximal suture was used to tie off and occlude the proximal end of the trachea. A modified dental instrument with a blunt contra-angle tip was passed through the oral cavity into the larynx and through the cricothyroid membrane to exit rostral to the tracheal tube. The drain tube (PE 205, 20 cm) was attached to end of the instrument and was retracted through the larynx into the oral cavity. The proximal end of the drain tube was attached to a small (1 cm) piece of PE tubing flared at one end to acted as an intraoral funnel for the collection of oral fluids, and the distal end of the tube was attache to a vacuum pump. The drain-tube was retracted distally to position the intraoral component in the center of the pharyngeal opening, and the cervical incision was closed around the tubes. After closing the incision, to increase access to the oral cavity, sutures were placed at the corners of the mouth and around the mandibular incisors and the coronal portions of the maxillary incisors were removed. In
some of the experiments, a lingual suture was also placed approximately 2 mm anterior to the foliate papilla. Retraction of the sutures exposed the entire oral cavity and stretched the tongue enough to fully expose the taste buds within the foliate papilla.

_Cranial Surgery_
In each experiment, the animal was returned to the prone position and placed in a stereotactic frame (Kopf). The skull was exposed with a midline incision, leveled with respect to the anteroposterior axis, and trephined 1.7 mm lateral and 0.4 mm anterior to lambda to expose the cortical surface of the brain. The opening was further enlarged medially up to the midline and caudally to the transverse sinus to allow full access to the PBN. In all experiments, due to the likelihood of interruption of afferent fibers in the right vagus nerve resulting from surgical manipulation of the dorsal (posterior) side of the stomach, the PBN recording sites were restricted to the animal's left side.

_Recording_
The stimulation and recording protocol in the present study were similar to those of Travers et al {Travers Norgren 95; Halsell Travers 97}. Glass coated tungsten microelectrodes (R = 0.5-2.5 MΩ) were used to record extracellular activity of single neurons in the PBN. A time/amplitude window discriminator was used to isolate single neurons based on a consistent amplitude and waveform. Neural
activity was amplified (1K-10K), monitored on a storage oscilloscope, and stored on magnetic tape. Voice commentary was also recorded on a different channel and used to designate stimulus markers. The electrode was inclined 20° posteriorly to prevent rupturing of the transverse sinus and advanced automatically using a piezoelectric microdrive (Inchworm Systems, Burleigh Instruments). The electrode was advanced at a moderate rate through spontaneously active sites in the cortex and auditory (voice) responsive sites in the inferior colliculus to reach the fourth ventricle which was usually identified by a sudden decrease in neural activity. The electrode was then slowly advanced through the ventricle to reach the brainstem surface marked by the resurgence of spontaneously active neurons. During the search, single neurons in the brainstem, were tested with whole mouth gustatory stimuli, intraoral tactile stimuli, and gastric distension. After a response was detected, it was systematically tested according to the procedures outlined below.

**Stimulation**

The stimulation algorithm used in these experiments is represented in flowchart format in appendix A. A taste mixture consisting of 0.3 M NaCl, 0.3 M sucrose, 0.01 M HCL, and 0.003 M QHCl, representing the four basic tastes, was used as a gustatory search stimulus. A syringe attached to a blunt needle (18 G), was used to stimulate the entire oral cavity with approximately 2 ml of the taste mixture bracketed by water stimulation (2 ml) and rinsing (4 ml). Tactile
stimulation of the oral cavity was achieved by stroking individual receptive fields (below) with a glass probe. A dissection microscope was used to monitor fluid delivery and mechanical stimulation of the oral cavity. The oral stimulation was followed by a mid-range (5 ml) gastric stimulus. If a neural response to either the oral taste and tactile stimuli or the gastric stimulus was detected, the best stimulus in the relevant oral or visceral receptive field was determined. The best gastric stimulus was determined by inflating the gastric balloon over a 2 to 7 ml volume to identify the most efficacious stimulation volume. The best gustatory stimulus was determined by applying the individual components of the taste mixture (above) with a syringe to the whole mouth. Once the best oral and gastric stimuli were determined, coactivation of the neuron was achieved by applying the best gustatory stimulus before, during, and after the best gastric stimulus. If the response of the neuron to the oral response was to the tactile and not the taste stimulus, the best tactile stimulus was used in the coactivation step. When the neural response was stable, the coactivation was repeated two more times and then the best oral receptive field was identified by using small nylon brushes or stroking the lingual surface with a blunt glass probe to test the individual oral receptive fields in the anterior tongue (AT), nasoincisor duct (NID), foliate (FOL) and circumvallate (CV) papillae, and the soft palate (SP). The protocol was repeated until the response became unstable or isolation was lost.
Neurophysiological Data Analysis

The data was analyzed in a similar manner to other analysis of gustatory neurophysiological data published previously by others {Travers Norgren 95; Halsell Travers 97}. The electrophysiological data was analyzed off-line by digitizing the spike train and accumulating it into 500 msec bins with peristimulus time histogram software [Modular Instruments]. The neurophysiological response to taste or gastric stimuli were analyzed for 10 seconds of activity. However, analysis of only the first 5 seconds of activity was usually sufficient to determine a response measure. The criterion for a response was a change in the mean evoked firing rate that was >2SD from the mean spontaneous rate. Because the stimulation procedures were usually repeated many times before the isolation of the unit became unstable, the mean spontaneous and evoked responses represent the average unit activity over several time periods. This also allowed a statistical measure in addition to the response criterion measure. Thus, paired t-tests and multivariate techniques (Systat) were used to compare spontaneous with evoked responses or gastric conditioning effects on the oral response before, during and after distension of the stomach. Responses to oral mechanical stimuli were measured during one second of peak activity because the stimulation periods were variable and the interstimulus intervals usually were not long enough for 5 second analysis. The mean response during one second of peak activity was compared to the mean spontaneous rate per second measured at several 5-10 second intervals. The response criteria to mechanical
stimulation was a mean evoked response during 1 second of peak activity that was >2SD of the mean spontaneous activity averaged over 1 second. All neurons that did not show a spontaneous rate met another criterion of a change in firing rate >1/sec. All levels of significance were set at p<0.05 and all deviations were calculated as standard deviations from the mean.

*Histological Reconstruction*

Electrolytic lesions (anodal current: 3 μA x 3 msec) were used to mark the recording sites or a site that was a specific distance below the recording site (Fig. 1). When using electrolytic lesions to mark recording sites, the number of lesions in a particular track or in a region of interest must be balanced with the size and location of the lesions so that excessive coagulation of neural structures that potentially obliterate other sites of interest are minimized. To that end, lesioning at the site of recording in the present study, was reserved for sites that were of most interest to the study. Thus, almost all (84%) neurons that showed significant responses to both the oral and the gastric stimuli were lesioned at the site of recording. Additionally, most of the gastric responsive neurons (56%) as well as the oral responsive neurons that were modulated during gastric distension (57%) were lesioned at the site of recording or a specified distance below it. The location of other neurons including those that only responded to oral stimulation and were not modulated by gastric distension were reconstructed from the electrode tracks and lesions in adjacent tracks. At the end of the
recording session, the animal was given a lethal dose of Nembutal (150 mg/kg) and perfused through the aorta with 500 ml of 0.9% saline and 200-400 ml of 10% buffered formalin. The head was removed and the skull was reflected to expose the brain. In order to section the brain at the same angulation as the electrode track, the head was replaced in the ear bars and the brain was blocked at 20° by replacing the electrode with a sharp blade in the microdrive. Once blocked, the brain was removed and scored on the contralateral side with a pinhole and cryoprotected overnight in 20% sucrose at 4° C. Alternate sections were stained according to the Weil (Fig. 1A) and Cresyl Violet (Fig. 1B) protocols to distinguish the myelinated fibers and cellular components in each section. The Weil stains were useful in lesion identification and the Cresyl Violet stains allowed localization of the lesions within morphologically distinct PBN subnuclei {Fulwiler Saper 84; Halsell Frank 91}.

Results

Location of Cells

A total of 98 single neurons in 17 different animals were recorded in the PBN and adjacent structures. All cells were tested with gastric and oral stimuli. Fifty five cells that were specifically activated by gastric distension or oral taste and mechanical stimulation were also tested for orogastric coactivation and were further analyzed. The other 43 neurons responded to thermal, jaw stretch, respiratory activity, or were uncharacterizable and were thus excluded from
further analysis. Figure 4.2 shows the anatomical distribution of the neurons that
were activated by oral and/or gastric stimuli. An equal proportion of the cells
(29%: 16/55) were present ventral to and within the brachium. The remaining
cells (42%) were located dorsal to it. Because the brain was sectioned parallel
to the 20 degree angle of the electrode penetrations, PBN subnuclei dorsal to
the brachium were approximately 200 µm rostral to those ventral to the
brachium. Thus, compared to transverse sections cut orthogonal to the
horizontal plane of section, the location of gastric neurons in figure 4.2 appear
further rostral.

Overall, the location of gastric activated neurons were dorsal compared to orally
activated units. This was especially apparent at levels 2 and 3. While some
PBN subnuclei such as the internal (il) or superior lateral (sl) subnuclei were
completely devoid of any oral or gastric responsive neurons, others such as
dorsal lateral (dl) and the most caudal extent of central medial (cm) contained
neurons that responded to either gastric distension or oral stimulation
exclusively. Other subnuclei that were dorsoventrally interposed between the DL
and CM contained cells in close proximity to one another that were activated by
either or both stimulation modalities. The close proximity between cells that
responded only to gastric or oral stimuli and those that responded to both
stimulation modalities was clearly evident in the external subnuclei.
Gastric Only Cells

A total of 16 single neurons from 11 preparations showed excitatory responses to gastric distension (D) but not to oral stimulation. These units were scattered throughout PBN levels 2-4, primarily dorsal to the brachium (Figure 4.2). Thus, D cells were identified in VL, CL, DL, and EL at levels 2 and 3 and in EL, EM, and VM at level 4. No D units were identified at PBN level 1. Although many cells appeared within the anatomical boundaries of different PBN subnuclei, others did not. Thus, to further describe the relative distribution of these cells, an arbitrary rectangular coordinate system was superimposed on the center of the brachium with the vertical axis parallel to the midline and the horizontal axis orthogonal to it (Figure 4.2). Relative to these coordinates, D units were equally represented medial and lateral to the origin at PBN level 2, compared to levels 3 and four where this activity was identified exclusively lateral to the origin. Furthermore, it was only at the most rostral level of the PBN (i.e. level 4) that we found D units ventral to the brachium. Thus the distribution of the PBN neurons relative to the center of the brachium was such that at the most caudal level of the PBN (level 1), D units were primarily ventromedial to the origin of the quadrant system (Figure 4.2). At level 2 they were more equally distributed in all quadrants. At level 3 the distribution moved to the lateral half of the PBN, and the activity recorded at the most rostral level (level 4) was primarily restricted to the ventrolateral quadrant. The stereotaxic coordinates of D neurons are categorized according to PBN level in Table 4.1. The overall mean stereotaxic
coordinates at a 20° angle for D units in the PBN were: 0.74 ± 0.07 mm anterior to and 1.97 ± 0.15 mm lateral to lambda, and 6.73 ± 0.55 mm ventral to brain surface.

Neuronal responses to gastric distension were qualitatively classified into phasic, tonic, and sustained excitatory responses (Figure 4.3). Relative to spontaneous activity, six D neurons showed a phasic increase, eight a tonic increase, and two a sustained increase in firing rate that outlasted the gastric stimulus by approximately 5 seconds. Gastric distension did not produce any inhibitory responses in D units of the present study. The average gastric response of the D units over the initial 5 seconds of activity was 6.22 ± 1.24 spikes/sec and the spontaneous rate over a similar period was 2.71 ± 1.12 spikes/sec. In 75% of the cells (12/16) the evoked response of D cells to gastric distension was greater than 2 standard deviations from the mean spontaneous rate of the cells. Although the remaining 25% (4/16) of the D cells did not meet this criteria, they were included in this group because they consistently showed an increase in discharge rate during distension of the stomach. A relatively fast spontaneous rate of a tonically activated unit, or a slow onset of the response, prevented the response rate from meeting the 2 SD criteria in 2 of these cells. The mean response rate and spontaneous rates of all of the D cells during the first 5 seconds of activity was averaged across all PBN levels and is shown in Table 4.2. The average spontaneous and evoked responses of D units at level 2 were
approximately twice those at other levels. At level 2, the spontaneous and evoked responses of oral units appeared much higher than units located at more rostral levels.

**Oral Only Cells**

The majority of cells analyzed (33/55) responded exclusively to oral cavity taste and/or tactile stimulation. More than 63% (21/33) of the oral units responded primarily to taste stimuli (G) while the rest (12/33) were activated by mechanical stimulation (M) of the oral cavity. Most gustatory cells were spontaneously active with an average spontaneous rate of 4.58 ± 1.34 spikes/5 sec. When tested with the taste mixture applied to the whole mouth (WM:Mix), the average evoked response across all gustatory neurons was 24.37 ± 3.90 spikes/sec. In addition to a WM:Mix response, more than 76% (16/21) of gustatory only cells had a best taste stimulus response. Of those, eleven responded best to NaCl (N), three to sucrose (S), and two to HCl (H). Four of the N-best cells also had a second best response to H, two of which further responded to S and Q. All of the S-best neurons also had a second best response to N. The H-best cells responded to all other taste stimuli.

All cells designated as taste responsive units responded to stimulation of the whole mouth with the taste mixture. In 16/21 cells it was also possible to determine the best intraoral receptive fields. Figure 4.4 shows the topographical
distribution of these cells according to their best receptive fields. In this figure. Neurons that responded best to posterior oral cavity or gastric stimulation were located at more rostral levels of the PBN, primarily dorsal or lateral to the brachium. In contrast, anterior oral cavity neurons were primarily within and ventral to the brachium at more caudal levels. Table 4.3 displays the mean responses to WM:Mix during the initial 5 seconds of activity categorized according to best receptive fields. Thus, the arrangement of best receptive fields based on mean response to WM:MIX, was: AT>FOL>NID>WM.

Many gustatory cells (10/21) also responded to oral tactile stimulation following whole mouth rinsing with water (n=8) or mechanical stimulation with a glass probe (n=2). All tactile responses of gustatory units were phasic and transient and were primarily present in cells that responded best to anterior oral cavity stimuli. The average peak response to a whole mouth water stimulus was 5 ± 2.68 spikes/sec. This response rate was close to the mechanical response of a foliate best unit (4.5 ± 1.73 spikes/sec) but much lower than the mean peak response rate to mechanical stimulation of the anterior tongue best units (25.4 ± 2.5 spikes/sec.).

The response of G neurons to mechanical stimulation were similar to that of M neurons. Table 4.4 shows the distribution of spontaneous rate and evoked responses of M neurons categorized according to best receptive field. Although
a subset of G neurons responded to anterior oral cavity mechanical stimulation (above), M neurons (Fig. 4.5) responded exclusively to mechanical stimulation of the posterior oral cavity. The mean spontaneous rate (1.18 ± 0.37 spikes/sec.) and mean evoked response (9.19 ± 2.01 spikes/sec.) of M neurons were similar to the D neurons, but were much lower than those of G neurons. Analysis of M neuron responses revealed that the peak response to mechanical stimulation of the CV was the highest followed by SP, WM, and FOL respectively. Although convergence between receptive fields of M neurons was not routinely tested, convergence between CV and FOL lingual receptive fields was common.

**Oral Cells Modulated by Gastric Distension**

A statistical comparison (t-test) of the response characteristics of G and M neurons before and during gastric distension conditions, revealed that the response to oral stimulation of a subset (21% = 7/33) of these cells was significantly modulated with gastric distension (p < 0.05). These neurons were scattered throughout the PBN (Figure 4.5). Based on modality, their topographical location followed the overall topographical organization of oral representation in the PBN. Thus, at the more caudal and ventral PBN regions, gastric distension modulated a subset of gustatory neurons; at the more rostral and lateral regions the cells that responded to oral mechanical stimulation were modulated by gastric distension. Four of the modulated neurons responded to taste stimulation and three neurons responded to mechanical stimulation of the
posterior oral cavity and were thus included in the initial analysis of G and M response rates (Table 4.3). However, when the responses of these neurons were averaged together and compared to the average response of the remaining G and M neurons (Tables 4.5, 4.6), the modulated gustatory response (G-MOD) was slightly higher than that of other G neurons, but the modulated responses to mechanical stimulation (M-MOD) was clearly lower than that of other M neurons. In calculating the mean evoked responses of non-modulated neurons, all responses to gustatory and mechanical stimuli before, during and after gastric distension were grouped together. Neural responses to gustatory or mechanical stimuli were consistently increased (p<0.05) during gastric distension in 5/7 modulated neurons (e.g. Figure 4.6 B,C), and were decreased (p<0.05) in the other two (e.g. Figure 4.6 A). In 3/7 modulated cells, the neuronal activity in response to oral stimulation was similar before and after gastric distension (e.g. Figure 4.6), whereas in the other 4 cells the oral response before gastric distension was consistently either higher or lower than that after gastric distension, while spontaneous activity remained the same. Figure 4.7 depicts the response of one such neuron to repeated gastric stimulations during the gastric conditioning tests of the experiment. Although the spontaneous activity of this cell remained stable throughout the experiment, repeated gastric stimulations resulted in an oral response to anterior tongue stimulation with NaCl (stim. 79) that was approximately twice the initial response (stim. 16), indicating some long lasting excitatory effects of gastric stimulation.
**Orogastric Coactivated Cells**

A total of six neurons scattered throughout the PBN at levels 2 and 3 responded to both oral and gastric stimuli independently (Figure 4.4). All neurons in this group meet the 2SD criteria (Table 4.7) for both oral and gastric stimuli (Figure 4.8). Four of these neurons responded to oral mechanical stimulation; three to posterior oral cavity (Figure 4.8 D-F) and one to anterior tongue (Figure 4.8 C). The response of one such neuron to oral and gastric stimulation is shown in figure 4.9. Note that this neuron had a tonic response to gastric distension. This was true in 4/5 coactivated neurons that showed an excitatory response to gastric stimulation. Of the other two neurons in this category, one showed a phasic excitatory response similar to that in figure 4.3A and the other a suppression of spontaneous activity during gastric distension. All of the POC responsive neurons were activated by foliate stimulation, two of which also responded to soft palate stimuli. The other two cells in the coactivated group responded to whole mouth application of the taste mixture (Fig. 4.8 A,B). All coactivated neurons had excitatory responses to gastric distension except for one, which showed suppression of spontaneous activity during gastric stimulation (Fig. 4.8 F). The response to oral mechanical stimuli in this cell were not significantly different during gastric distension compared to the response before or after distension. The average oral and gastric responses of coactivated neurons were similar but generally smaller than those of oral only and gastric only neurons (Table 4.7).
Discussion

The major finding in the present study was that gastric distension significantly coactivated or modulated approximately 20% of neurons that responded to gustatory or mechanical stimuli of the oral cavity. This supports the hypothesis that convergence of oral and gastric inputs in the PBN may represent a brainstem mechanism for modulation of ongoing consummatory behavior. A second result that D neurons were located in both the waist and external regions extends the results of a previous report of this activity in the PBN (Suemori et al 94), and indicates that the afferent gastric signal selectively activates some neurons while coactivating others in the same subnucleus.

Gastric volume is a physiological factor with significant behavioral consequences (Sclafani Nissenbaum 85; Wirth McHugh 83; Deutsch et al 78). Repeatable changes in gastric volume can be achieved experimentally by distension of the stomach wall (by introducing air or liquid into the stomach with or without a gastric balloon), and has been used to investigate gastric responses in peripheral nerves and central subnuclei (vagus: (Schwartz Moran 96; Schwartz et al 95) NST: (Appia et al 86; Chambert et al 93; McCann Rogers 92) and PBN: (Suemori et al 94)). Thus, the use of gastric distension is an appropriate experimental tool for the physiological assessment of oral and visceral integration in the PBN.
Dual Representation in the PBN Waist and External Regions

The anatomical organization of the PBN based on the subnuclear arrangement of the afferents and efferents discussed in chapter 3 was further reflected in the organization of physiological responses following oral and gastric stimulation. Previous electrophysiological recording studies have characterized the response profile and topographical organization of oral responsive neurons {Norgren Pfaffmann 75; Van Buskirk Smith 81; Schwartzbaum 83; Ogawa et al 87; Travers Smith 84} more extensively than gastric responsive neurons {Suemori et al 94; Yuan Barber 91}, or perhaps any other physiologic response in the PBN. Although the original descriptions of gustatory responses in the PBN {Norgren Leonard 73; Norgren Pfaffmann 75} predate more recent parceling of this nucleus {Fulwiler Saper 84}, two recent studies have related the topographic location of orosensitive neurons to the morphologic descriptions of the PBN in both hamster and rat {Halsell Frank 91; Halsell Travers 97}. These studies allow a more detailed comparison of physiologic responses in different PBN subnuclei. The initial descriptions of gustatory activity in the PBN were restricted to the responses of neurons in a caudal and medial region {Norgren Leonard 73; Norgren Pfaffmann 75; Ogawa et al 87; Schwartzbaum 83; Travers Smith 84}. More recently this part of the PBN has been identified as a complex of neurons in the central medial and ventral lateral subnuclei that span across a narrow part of the brachium [waist area] dorsoventrally {Halsell Travers 97}. This complex was defined as the waist region in chapter 2. Additionally, based on FOS
immunoreactivity, afferent input, and neurophysiology, a second gustatory region [the external region] of the PBN has recently been identified {Herbert Saper 90; Becker 92; Yamamoto et al 94; Halsell Travers 97} that is primarily activated by posterior oral cavity stimulation. The results of the present study confirm observations by Halsell and Travers (1997) that G and M neurons in the external subnuclei responded almost exclusively to stimulation of POC receptive fields and thus support the conceptualization of a dual representation of gustatory activity in the PBN. Differences in the anatomical connections of waist and external regions with forebrain (e.g. thalamus: {Fulwiler Saper 84; Saper 95}; amygdala: {Bernard et al 93}) and medullary sites (see chapter 2) implicated in feeding behavior parallel the neurophysiological differences between waist and external regions.

If, as suggested by gustatory elicited Fos following conditioned taste aversion {Yamamoto et al 94}, neurons in the external subnuclei represent the negative hedonic properties of gustatory stimuli rather than the gustatory quality code, gastric responses in the external region may serve to augment the negative hedonics associated with gastric filling and satiation. Alternatively, based on the additional afferent connections of the PBN external region with nociceptive substrates in the trigeminal and spinal systems {Cechetto et al 85; Berkley Scofield 90; Feil Herbert 95; Ding et al 95}, the gastric responses in the external region may represent a nociceptive component of the gastric stimulus. However,
this is unlikely because the volumes used to distend the gastric corpus in the present study (2-7 ml) are within the physiological limits of intake during normal ingestion in the rat {Fraser et al 95}. Yet, another caveat in the interpretation of gastric responses in the PBN is that distension of the stomach may effect cardiovascular physiology which is also represented in the external region of the PBN {Chamberlin Saper 94; Saper 95}. Since blood pressure was not monitored in the present experiments the possibility that some gastric responses may reflect changes in blood pressure remains. Nevertheless, the relatively short duration of the gastric stimulus (<10 sec) was probably not long enough to initiate a cardiovascular effect {Grundy Davison 81}. Additionally, in some of the experiments heart rate was monitored and a correlation between distension and heart rate was not qualitatively apparent.

Response Profiles and Topographic Location

In the present study, gustatory neurons comprised the largest proportion of orally responsive neurons (63%) as well as the largest proportion of all cells analyzed (38%). However, these counts may be biased because of the relatively faster spontaneous and evoked responses of neurons that responded best to gustatory stimulation of the anterior tongue (AT) that tended to promote their identification. Most gustatory units responded to AOC stimulation and were primarily located in the waist region at the caudal levels of the PBN. Two anterior tongue-best neurons in the present study showed significant modulation with gastric
distension while the other modulated taste neurons responded best to FOL/NID or WM gustatory stimulation. The average spontaneous rate and evoked response of G neurons that were modulated by gastric distension was higher than that of other G neurons. Although the reason for this is unclear, a higher spontaneous rate in neurons may allow for response decrements which can be used to further code neural information. This type of decrement was indeed observed in some, but not all modulated responses of oral responsive neurons (Fig. 4.6 B).

When all suprathreshold responses were considered, approximately 24% of gustatory neurons in the present study showed convergence between intraoral receptive fields. All but one of these showed convergence between AOC and POC receptive fields. These findings are at odds with previous reports that have quantified the incidence of convergent receptive fields of gustatory neurons in the NST and the PBN {Travers Norgren 95; Halsell Travers 97}. Although the latter studies reported a similar proportion of convergent neurons (~50%) in the two brainstem relays, convergence within AO or PO receptive fields was more prevalent than convergence between them. These differences may be related to a less complete sampling of gustatory responsive units in the present study. Because gustatory activity was often used as a neurophysiological landmark to probe other PBN regions for orogastric coactivation, a less than exhaustive evaluation of gustatory neurons in the PBN may have lead to only a partial
sampling of G neurons. Although a difference in intraoral convergence exists between the present results and those of Halsell and Travers (1997), the percentage (~50%) and response of G neurons that also responded to mechanical stimuli were similar in the two studies. In addition, the spontaneous and evoked responses of M neurons, and the mechanical response of G neurons were similar to those reported by Halsell and Travers. Although M neurons were intermingled with G neurons in the waist region, rostrally they were the more prevalent oral response in the external region.

The external region receives direct projections from the spinal trigeminal nucleus (Cechetto et al 85; Feil Herbert 95), suggesting more tactile input to this part of the PBN compared to the waist region that lacks direct trigeminal inputs. The role of somatosensory input in the gustatory system is speculative, but could be important in signaling bolus consistency in the oral cavity or the spacial and temporal processes of chewing, swallowing, and subsequent progress of ingesta through the esophagus and gastrointestinal tract. The spontaneous and evoked responses of the M neurons were similar to those of D neurons, suggesting possible similarities in peripheral transduction mechanisms. In contrast to the fast spontaneous and evoked responses of AT neurons, the oral and gastric mecanoresponsive neurons had relatively slow spontaneous and evoked responses. However, the decrement in response rate of oral and gastric mechanical responses does not reflect those found in peripheral nerves. The
response frequency of single fibers in the vagus to gastric distension \cite{SchwartzMoran96}, or the glossopharyngeal nerve to oral mechanical stimuli \cite{Frank91}, was higher than those recorded in the NST \cite{Grundyetal81;TraversNorgren95} or PBN \cite{presentstudy,alsosee{HalseLLTravers97}}. This difference between response rates suggests a central inhibitory mechanism. The response profiles of M neurons in the present study were comparable to other reports of gustatory and mechanical responses in the PBN \cite{HalseLLTravers97;Ogawaetal87}. Although other quantitative data on the response rate of gastric neurons in the PBN is lacking \cite{Suemorietal94}, a visual comparison indicates similar firing rates between excitatory responses in the Suemori et al report and those observed in the present study. In addition, the response of D units in the present study was similar to those reported in the NST \cite{Grundyetal81}. Similarly, the tonic and phasic response characteristics of D neurons in the present study were also reported by Suemori et al and seem to reflect those in the NST \cite{McCannRogers92}. Although D neurons of the present study always showed excitatory responses to gastric distension, inhibitory responses to gastric stretch have been documented in both the PBN and the NST \cite{Suemorietal94;McCannRogers92}. The only instance of gastric inhibition of oral activity in the present study was that of a coactivated neuron that also responded to mechanical stimulation of the soft palate. Although generalization of this finding is premature, it may be that inhibitory responses in other studies involved coactivated cells as well.
For both coactivated and gastric only cells, the average response to gastric stimulation was similar (Tables 4.2, 4.7). This suggests that similar NST inputs reach both categories of neurons. This agrees well with the results of chapter 3 which showed that terminal fields in DL, VL, and CM all resulted from the same caudal NST source. Thus, the topographical distribution of gastric responsive and coactivated neurons corresponds to the anatomical projections from the caudal NST. The location of gastric responsive neurons in EL, CM, and DL subnuclei in the present study confirm a previous report (Suemori et al 94), although gastric responsive neurons were not specifically localized to PBN subnuclei. Additionally, in the present study D neurons were identified further medially in VL and DM, more ventrally within the brachium, and in the medial part of CM. Gastric nerve stimulation and Fos immunohistochemistry further indicate that these more ventral and medial PBN areas are indeed substrates for gastric function (Yuan Barber 91; Kobashi et al 93). The subnuclear distribution of gastric representation in the neurophysiological experiments further emphasizes the anatomical observations (chapter 3) that showed a segregation of oral and gastric terminations in some PBN subnuclei but overlap in others. Thus, the parabrachial terminal fields of gastric related NST projections presented in the previous chapter are indeed related to gastric function in the PBN and further delineate the functional topography to this nucleus. Within this organotopic representation, D and G/M neurons are segregated in DL and the lateral part of CM respectively, but intermingled in other PBN subnuclei such as
DM, VL, CL, EM, EL, as well as the waist area which also contain both coactivated and modulated neurons. However, not all PBN sites that received oral or gastric inputs showed a physiological response to these stimuli. For instance, single units responsive to gastric stretch were not recorded at sites caudal and medial in CM, where gastric projections were consistently observed (chapter 3: figures 3.6, 3.7 D). Similarly, oral stimulation did not activate neurons in the more rostral and medial levels of the PBN that receive axonal projections from rostral NST. The discrepancies between anatomical projections and physiological findings may be related to oral and gastric functional modalities (e.g. thermal) not examined in the acute experiments of the present study.

**Gastric Modulation of Taste Responses**

The location of gastric modulated taste neurons in the PBN (Fig. 4.5) correlated well with the anatomical results of chapter 3 that showed an overlap of gastric and taste projections from the NST in the waist region, i.e. medially in CM, within the brachium, and in VL. One such neuron at the ventral edge of the brachium (PBN level 2) responded best to whole mouth application of sucrose and NaCl and significantly increased its firing rate (p=0.024) during gastric distension compared to before or after the gastric stimulus (Fig. 4.6 B). Many neurons that respond well to sucrose and NaCl have previously been reported in the PBN of awake and behaving animals {Nishijo Norgren 90}. However, when a non-caloric sweet stimuli such as sodium saccharin was used in anesthetized preparations,
only a single sweet-best neuron was identified {Halsell Travers 97}, suggesting a metabolic rather than a hedonic mechanism for this type of convergence in the PBN. It is conceivable that this type of neuron may participate in signaling mechanisms of satiety, because both gastric distension and sucrose metabolism have pronounced satiating effects during feeding. The other three taste responsive neurons in the PBN which showed modulatory effects with gastric distension responded best to NaCl. Gastric distension can produce hormonal changes mediated by the hypothalamus and the pituitary, resulting in antidiuresis and sodium excretion in water loaded rats {Young Huang 92}. A subset of gastric modulated NaCl best neurons recorded in the present study may comprise a PBN substrate for neural control of natriuresis in response to gastric distension. Alternatively, AT neurons that respond to NaCl may participate in an amiloride sensitive sodium pathway necessary in sodium appetite (discussed in {Halsell Travers 97}).

Cross Modality Within Oral or Gastric Receptive Fields

Both the glossopharyngeal nerve and superior laryngeal branch of the vagus carry taste and somatosensory afferents from PO and laryngeal receptive fields, and contain some multimodal fibers that respond to both gustatory and mechanical stimulation {Frank 91; Sweazey Bradley 89}. Multimodal fibers are also reported in the subdiaphragmatic vagus which provide gastrointestinal inputs to the caudal NST {Blackshaw Grundy 93}. Multimodal fibers in the
vagus respond phasically to mechanical stimulation of the gut mucosal lining as well as stimulation with chemical metabolites such as organic and inorganic acids, water, alcohol, casein hydrolysate, hypertonic saline and ammonium chloride {Clarke Davison 78}. The tonic and phasic nature of gastric responses reflect slow and rapidly adapting stretch receptors in the muscular and submucosal layers of the stomach. The gradual distension of the stomach wall activates the slowly adapting receptors whereas flow of small particulate matter against the mucosal lining is thought to activate the rapidly adapting receptors {Schwartz Moran 96; Blackshaw Grundy 93}. Rapidly adapting mechanoreceptors in the mucosal lining of the gut are not only activated by discrete mechanical stimuli, but they also respond to the chemical attributes (e.g. pK) of metabolites {Clarke Davison 78}. This is remarkably similar to the taste and tactile multimodality of the glossopharyngeal nerve as well as NST and PBN neurons {Frank 91; Ogawa et al 84; Ogawa et al 87; Travers Norgren 95; Halsell Travers 97}. Thus, during feeding, chemical and mechanical qualities of ingesta interact both peripherally and centrally to affect pre- and post-ingestive signals generated in different compartments of the alimentary canal. One may speculate that phasic responses to gastric distension in the present study reflect multimodal responsive fibers in the vagus, although we have no evidence to support this.
Convergence of Related Visceral Functions in the PBN

The PBN is an important substrate in the central coordination of many autonomic reflexes {Saper 95}. Through reciprocal connections with the medulla, the PBN is in a key position to control medullary visceral efferents. Projections from respiratory and cardiovascular parts of the NST for example, overlap in KF, which in turn is reciprocally connected to the ventrolateral medulla implicated in cardiorespiratory functions {Herbert et al 90}. Microstimulation of the lateral areas of the PBN including EL and KF, results in a complex alteration of cardiovascular and respiratory functions that include change in blood pressure and modulation of respiratory phase {Lara et al 94; Holmes et al 87; Dick et al 94; Chamberlin Saper 94; Mraovitch et al 82}. These changes are mediated through medullary circuits {Segers et al 85}, and occur independently of peripheral interactions between cardiorespiratory systems {Lara et al 94}. Somewhat analogous to the dual representation of gustatory activity in the PBN waist and external regions (discussed above), respiratory function in the PBN is also represented in two segregated regions, rostrally in the medial parabrachial region {Bertrand Hugelin 71}, and further caudally and laterally in Kolliker Fuse {Dick et al 94}. These parabrachial respiratory areas are designated as 'pneumotaxic centers' because prolonged inspiratory phase (apneustic breathing) occurs with selective lesioning of KF or the medial parabrachial area (reviewed in {Dick et al 94}) and phase-switching between inspiration and expiration occurs with microstimulation of these structures {{Holmes et al 87};
The PBN control of respiratory phase is important in the coordination of respiration with other related functions such as vocalization (Farley et al. 92; Kirzinger Jurgens 91; Wild Arends 87). Oral and visceral integration in the PBN may reflect a similar phase switching mechanisms [i.e., between ingestion and satiation] as in cardiorespiratory integration. Descending control of KF over medullary circuits that coordinate respiration with vocalization may represent a general parabrachial mechanism that contributes to the coordination of oral and visceral functions in the medulla.

The PBN waist region is reciprocally connected to parts of the medullary RF implicated in ingestive behavior (see chapter 2), suggesting that a subset of orogastric responsive neurons in this PBN region may directly modulate the RF premotor neurons that organize the oromotor activity of ingestion and rejection of gustatory stimuli (Travers Norgren 83; Travers DiNardo 92; Travers et al. 97). Coactivated neurons in the waist region observed in the present study confirm and extend the results of a previous study by Rogers and co-workers that demonstrated oral and vagal convergence in the interstitial zone of the PBN that appears to correspond to the waist area (Hermann Rogers 85). In contrast to a previous NST study by the same group (Hermann et al. 83), the investigators were able to identify a set of neurons in the caudal PBN which responded to both gustatory and visceral inputs. These convergent neurons were clustered within the fascicles of the brachium in the waist area of the PBN and constituted the
majority (31/47) of the gustatory cells tested. However, interpretation of the results of that study is limited by the fact that a single gustatory stimulus (i.e. 0.3 M NaCl) was used to activate the taste receptors in only one oral receptive field (i.e. anterior tongue), and visceral stimulation was induced by electrical stimulation of the cervical vagus rather than with a more natural physiological stimulus. In addition, electrical stimulation of the cervical vagus activates cardiorespiratory, hepatic, gastrointestinal, and pancreatic afferents to the brainstem, precluding interpretation as to the nature of the taste and visceral convergence. This may account for the discrepancy between the larger proportion of convergent neurons reported by Rogers et al (66%) and those reported in the present study (22%).

Role of PBN in Ingestive Behavior

Based on reciprocal connections of the PBN with forebrain substrates implicated in feeding behavior (e.g. hypothalamus {Fulwiler Saper 84; Norgren 95}; amygdala {Bernard et al 93}) and medullary circuits implicated in oromotor reflexes (e.g., {Travers Norgren 83; Herbert et al 90}), one may speculate a role for the PBN in both long term appetitive functions that require an intact forebrain, as well as the ongoing consummatory functions that are organized in the brainstem. Appetitive mechanisms, such as hunger that motivate the animal to search for food require forebrain interaction because a decerebrate animal (CD) does not express such behavior and will starve if not artificially fed {Grill Norgren
The CD rats also fail to acquire a conditioned taste aversion (CTA) when palatable gustatory stimuli are repeatedly paired with aversive visceral stimuli (Grill Norgren 78b). Further, decerebration eliminates the expression of sodium appetite normally expressed in response to sodium deficiency (Grill et al 86). More restricted lesions centered on gustatory responsive sites in the CNS implicate specific forebrain substrates as well as the main afferent source of gustatory and visceral inputs to the forebrain (i.e. PBN) in the formulation of CTA and sodium specific appetite (reviewed in Spector 95). Thus, electrophysiologically guided ibotinic acid lesions of gustatory neurons in the PBN results in a severe impairment in acquiring a CTA even with multiple pairings, an effect that lasts many months following the lesion (Scalera et al 95). These deficits cannot be explained by a simple absence of gustatory detectability or a change in gustatory quality because PBN lesioned rats are not ageusic and can respond appropriately to increasing concentrations of gustatory stimuli (Spector et al 95). Further, although PBN lesions are effective in impairing the acquisition of a new CTA, those formed prior to lesioning can still be expressed, suggesting that the PBN is a requisite to the formation but not the expression of CTA (Grigson et al 93). The impairments in CTA acquisition with PBN lesioning are also generalized to the formation of a salt appetite when animals are salt deprived (Spector et al 95). Thus, the same rats with PBN lesions that fail to express CTA also fail to formulate salt appetites, suggesting similar gustatory/visceral associative mechanisms in the PBN.
Although several lines of evidence indicate that gustatory responses in the NST can be modified by visceral manipulations including CTA, sodium deprivation, and sustained gastric distension, as well as changes in serum glucose and insulin levels {Glenn Erickson 76; Giza Scott 83; Jacobs et al 88; Giza Scott 87; Nakamura Norgren 95}, the PBN has not received this level of physiological analysis. However, based on the anatomical results that indicate considerable overlap of oral and visceral functions in the PBN, visceral manipulations may have an even greater effect on gustatory neurons in the PBN. The results of the present study support the role of the PBN not only in guiding long term processes as implied by lesion experiments (above), but also in more immediate integration of orovisceral signals that determine feeding duration and consequently meal size. The decerebrate’s ability to monitor short term changes in gastrointestinal parameters such as gastric preload, but not long term metabolic changes with starvation {Seeley et al 94; Grill Norgren 78b} implies sufficiency of brainstem substrates to respond to short term gastrointestinal signals but not long term appetitive mechanisms that involve the forebrain. That the PBN bridges afferent inputs from the medulla to the forebrain positions it in a key location to effect both the long term effects of food that require forebrain processing as well as short term consummatory effects during a meal that are mediated through oromotor reflexes organized in the medullary RF.
Figure 4.1
Electrolytic Lesioning of the Recording Site

A) Brightfield photomicrograph of a Weil Stained 52 μm section through the PBN. Arrow indicates site of recording of cell 1902 in figure 4.5 B. Arrowheads point to the electrode tracks. Asterisk mark center of lesions. The ventral lesion marks an uncharacterized site approximately 500 μm below the more dorsal lesion. B) Photomicrograph of adjacent section in A, stained with Cresyl Violet. Arrow marks the same recording site in A, localized to the CL subnucleus in this section. Scale bars are 250 μm for both A and B.
The PBN was divided into 4 different levels which were separated by approximately 200 μm. Circles and diamonds represent oral and gastric units respectively. Squares represent oral responses that were modulated during gastric distension and units that were independently activated by either oral or gastric stimuli. 

A) This is the most caudal level of the PBN. The cells at this level were mostly oral only cells and were located medially with respect to the coordinate system.

B) At level 2 the cells were equally distributed medial and lateral to the vertical axis. The dorsolateral subnucleus first appeared at this PBN level.

C) At this level of the PBN the internal lateral subnucleus is prominent. The distribution of neurons recorded at this level shifted lateral to the vertical axis.

D) At the most rostral level of the PBN all of the recorded neurons but one were represented lateral to the vertical axis and ventral to the horizontal. The external medial subnucleus is clearly recognizable at this PBN level.
Figure 4.2
Figure 4.3

Three types of responses to gastric distension in the PBN

Peristimulus Time Histogram of PBN unitary responses to gastric distension. Bin width = 500 msec. A) A phasic response that was time locked with the onset of stimulus. B) A neuron that showed a tonic response that was delayed relative to stimulus onset. C) A neuron that showed sustained activation that outlasted the stimulus by several seconds.
Figure 4.3
The neurons that responded to oral cavity stimulation have been divided into anterior (AO) or posterior (PO) oral cavity or whole mouth (WM) responsive units and have been symbolized accordingly.
Figure 4.4
Figure 4.5

Topographical Organization of PBN Neurons Based on Response Modality

The oral responsive neurons in this figure have been further divided into gustatory and mechanical responsive units. The oral responses of a subset of these neurons were modulated by gastric distension and are represented by filled squares or filled circles in this figure. Filled diamonds represent neurons that responded independently to oral and gastric stimuli.
Figure 4.5
A subset of oral responsive neurons modified their response to oral stimuli during gastric distension. For each neuron the spontaneous (white bar) and evoked (dark bar) responses to oral stimulation before, during, and after gastric distension is represented by histograms.  

A) Gastric distension seemed to dampen the excitatory response of this PBN neuron which was activated by the application of NaCl or HCL to the anterior tongue.  

B) This neuron which responded to whole mouth application of taste mixture showed an increased response during gastric distension than before or after it.  

C) This neuron which responded to mechanical stimulation of the soft palate showed an increased response during gastric stretch.
Before During After

Figure 4.6
Figure 4.7
Gastric Modulation of Taste Activity

A single unit response to taste that was modulated by gastric distension. Although this taste activated neuron (cell 2405 Fig. 4.5) did not show a response to gastric distension, repeated coactivation resulted in the modulation of the taste response during gastric distension that lasted even after the gastric stimulus was removed. NaCl, Suc, Q, and HCl represent individual taste stimuli outlined in the methods section. WM is whole mouth stimulation. AT, NID, and FOL represent anterior tongue, nasoincisor duct and foliate receptive fields respectively. B,D,A refer to the before, during, and after gastric stimulus conditions. White bars indicate spontaneous activity. Black bars indicate NaCl stimulations. Shaded bars indicate stimulations with taste mixture. Striped and stippled bars indicate other gustatory stimuli. Numbers on the horizontal axis represent stimulus number in the sequence of stimulations. See methods section and Appendix A for further clarification.
Figure 4.8
Orogastric Convergent Cells

All neurons in this group independently responded to oral and gastric stimuli. The white, cross hatched, and dark bars indicate spontaneous, gastric, and oral responses respectively. **A,B)** Neurons represented in these panels were activated by gastric distension and whole mouth stimulation with taste mixture. **C)** This neuron showed suprathreshold responses to gastric distension and mechanical stimulation of the anterior tongue. **D,E)** Both of these neurons responded to gastric distension and mechanical stimulation of the ipsilateral foliate papilla. The neuron in panel E further responded to soft palate mechanical stimulation. **F)** This neuron exhibited inhibition of spontaneous activity during gastric distension. The oral response to soft palate mechanical stimuli were not significantly different during gastric distension than before or after it.
Figure 4.8
Peristimulus time histogram representation of neural responses of a coactivated neuron (Fig. 4.5, cell 2505) to oral and gastric stimulation. The spikes were accumulated with a 500 msec. time bin. A) Arrowheads represent onset of oral stimuli. AT:M = mechanical stimulation of the anterior tongue; W and T represent stimulation of the anterior tongue with water and taste mixture respectively, and R refers to rinsing of the tongue with water. B) Solid line represents the duration of gastric stretch (GS) with 5 ml of air.
Figure 4.9
Gastric Distension Neurons in the PBN

<table>
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<th>LEVEL</th>
<th>n</th>
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<th>ML</th>
<th>DV</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
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<td>0.68 ± 0.19</td>
<td>1.8 ± 0.2</td>
<td>6.58 ± 0.38</td>
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<tr>
<td>L3</td>
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<td>0.73 ± 0.44</td>
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<tr>
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<td>1.97 ± 0.19</td>
<td>6.73 ± 0.55</td>
</tr>
</tbody>
</table>

Table 4.1- Stereotaxic Coordinates used to identify single neurons that increased their response to gastric stretch. These neurons were not found on level 1. Levels refer to those designated in Figure 4.2.
## Gastric Stretch Neurons Across Different PBN Levels

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>n</th>
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<td>43.24 ± 9.77</td>
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<tr>
<td></td>
<td></td>
<td>(8.65 ± 1.95)</td>
<td>(3.69 ± 1.68)</td>
</tr>
<tr>
<td>L3</td>
<td>6</td>
<td>24.62 ± 3.8</td>
<td>9.91 ± 3.93</td>
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<tr>
<td></td>
<td></td>
<td>(4.92 ± 0.77)</td>
<td>(1.98 ± 0.79)</td>
</tr>
<tr>
<td>L4</td>
<td>4</td>
<td>22.72 ± 4.31</td>
<td>11.62 ± 3.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.54 ± 0.86)</td>
<td>(2.32 ± 0.78)</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>31.13 ± 6.18</td>
<td>13.54 ± 5.59</td>
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<tr>
<td></td>
<td></td>
<td>(6.22 ± 1.24)</td>
<td>(2.71 ± 1.12)</td>
</tr>
</tbody>
</table>

**Table 4.2**- Spontaneous and evoked responses to gastric distension during the initial 5 sec. of activity at different levels of the PBN (Figure 4.2). Quantities in parenthesis represent average responses per second.
Table 4.3- Spontaneous and evoked responses to the taste mixture applied to the whole mouth. Neurons were classified according to their best response in a particular oral receptive field. Discharge rates were accumulated during the first five seconds of response. Quantities in parenthesis represent average spike rate in 1 sec. of response.
Mechanical Responses in the PBN

<table>
<thead>
<tr>
<th>Field</th>
<th>n</th>
<th>Mean Response Rate</th>
<th>Mean Spontaneous Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>3</td>
<td>12.49 ± 5.18</td>
<td>0.52 ± 0.76</td>
</tr>
<tr>
<td>S. Palate</td>
<td>7</td>
<td>8.81 ± 3.62</td>
<td>1.75 ± 1.00</td>
</tr>
<tr>
<td>WM</td>
<td>1</td>
<td>7.5 ± 3.08</td>
<td>0.33 ± 0.82</td>
</tr>
<tr>
<td>FOL</td>
<td>1</td>
<td>3.66 ± 2.53</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>9.19 ± 2.01</td>
<td>1.18 ± 0.37</td>
</tr>
</tbody>
</table>

Table 4.4- Spontaneous and evoked responses of mechanical only neurons in the PBN. Mean response rates represent spikes during one second of peak response.
Gastric Modulation of Gustatory Neurons in the PBN

<table>
<thead>
<tr>
<th>Field</th>
<th>G</th>
<th>G-MOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>152.33 ±19.49 (30.47 ± 3.90) n=9</td>
<td>185.96 ± 47.91 (37.19 ± 9.58) n=2</td>
</tr>
<tr>
<td>FOL</td>
<td>80.0 ± 41.0 (16 ± 8.2) n=1</td>
<td>127.4 ± 26.16 (25.48 ± 5.23) n=1</td>
</tr>
<tr>
<td>WM</td>
<td>40.30 ± 12.52 (8.6 ± 2.50) n=4</td>
<td>52.25 ± 14.22 (10.45 ± 2.84) n=1</td>
</tr>
<tr>
<td>AVG.</td>
<td>115.16 ± 19.04(23.03 ±3.81) n=14</td>
<td>137.89 ± 34.05 (27.58 ± 6.81) n=4</td>
</tr>
</tbody>
</table>

Table 4.5- Average responses during the initial 5 seconds of activity of a subset of gustatory neurons (G) which showed a modulation of their responses with gastric stretch (G-MOD). The cells are categorized according to their best response to the taste mixture applied to particular receptive fields. Quantities in parenthesis are average spikes during one sec of activity.
### Gastric Modulation of PBN Cells That Responded to Intraoral Mechanical Stimulation

<table>
<thead>
<tr>
<th>BEST RF</th>
<th>Evoked</th>
<th>Spontaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>M-MOD</td>
</tr>
<tr>
<td>CV</td>
<td>13.48 ± 5.67</td>
<td>10.50 ± 4.28</td>
</tr>
<tr>
<td>n=2</td>
<td>n=1</td>
<td></td>
</tr>
<tr>
<td>S.PALATE</td>
<td>10.66 ± 4.68</td>
<td>4.18 ± 1.36</td>
</tr>
<tr>
<td>n=5</td>
<td>n=2</td>
<td></td>
</tr>
<tr>
<td>AVG.</td>
<td>11.46 ± 4.96</td>
<td>6.29 ± 2.33</td>
</tr>
</tbody>
</table>

**Table 4.6** - Average responses during the initial 5 seconds of activity of a subset of PBN cells that responded to mechanical stimuli (M) and showed a modulation of their activity with gastric distension (M-MOD). The cells are categorized according to best receptive field.
Table 4.7 - Responses of cells that were independently activated by oral and gastric stimuli averaged over the initial 5 seconds of activity. All responses meet the 2SD criterion. Quantities in parenthesis indicate mean response per second. Responses to oral mechanical stimulation were calculated during 1 sec. of peak activity.
CHAPTER 5
SUMMARY & CONCLUSIONS

The present study of the subnuclear organization of afferent and efferent connections of the parabrachial nucleus demonstrates that functionally related NST projections (i.e. oral and gastric) maintain a segregated representation in some PBN areas while terminating in overlapping zones in others. Further, oral and gastric functions are both represented in two mediolaterally distinct PBN regions. These PBN regions have similar forebrain efferents but only one has a descending medullary component.

The segregation of oral and gastric projections observed at the most rostral levels in DL, EL, and CL were such that projections from gastric responsive NST were further dorsal and lateral in relation to projections from oral responsive NST. At these PBN levels, the exclusive representation of gastric responsive neurons in DL was consistent with the anatomical observations that indicated terminal fields from gastric but not oral responsive regions of NST in DL. Further caudally, NST projections overlapped in the external and waist regions and in CL and DM. This anatomical overlap correlated well with the representation of
coactivated neurons at these PBN levels. For instance, in the middle third of CM, identification of coactivated neurons were medial to oral only units. The location of these coactivated neurons corresponded with the anatomical topography of CM, i.e. gastric projections predominated in the medial third, oral projections predominated in the lateral third, and there was partial overlap in the middle third. Thus, the overlap of NST fibers at the junction of medial and middle thirds of CM provides a substrate for integration of oral and gastric inputs that may directly modulate medullary interneurons in the RF that program the oral and pharyngeal phases of ingestion.

The distribution of forebrain projections from the PBN followed a predictable pattern based on NST inputs. Thus, those subnuclei that received convergent inputs projected to similar forebrain sites compared to those that received segregated inputs. For example, EM and VL received convergent inputs from the rostral and caudal NST and projected to similar regions of the VPM nucleus of thalamus, bed nucleus of stria terminalis, and central nucleus of the amygdala. However, ventral forebrain projections from the lateral part of CM which only received oral inputs were different in that they terminated in substantia innominata and not CNA. Similarly, forebrain projections from DL which primarily received gastric and not oral inputs from the NST, terminated in the lateral hypothalamus, and parafascicular thalamic nucleus {Fulwiler Saper 84}, regions that do not receive substantial inputs from the waist or external subnuclei.
Although the afferent source and the efferent forebrain connections of EM and VL were very similar, the projections to the medulla were quite different in that VL contributed to a dense projection to the PCRt, whereas EM did not have a significant descending projection. Taken together, the above results indicate that segregation and convergence of oral and gastric representations in the PBN provide mechanisms for functional specialization so that different subnuclei may attend to complementary functional tasks. For instance, convergent inputs in CM and VL may directly modulated ongoing oral motor reflexes organized in the medulla, while the external subnuclei regulate the emotional, motivational, and mnemonic aspects of ingestion that require forebrain interaction.

**Future Directions**

The present results suggest at least three likely brainstem sites for convergence of oral and gastric functions: 1) intranuclear NST projections, 2) NST projections to the medullary reticular formation, and 3) projections to the PBN, the major focus of the present study. Although several studies indicate that sodium deprivation, alterations in serum glucose and insulin levels, and sustained gastric distension produce specific shifts in gustatory response profiles of NST neurons, {Glenn Erickson 76; Giza Scott 83; Jacobs et al 88; Giza Scott 87; Nakamura Norgren 95} the underlying neural mechanism for such humoral or visceral modulation of gustatory responses in the NST is unclear. Although the convergence of primary taste and visceral afferents in the NST is unlikely
{Hermann et al 83}, the anatomical proximity of the medial subnucleus in the caudal NST to the area postrema (a circumventricular organ that lacks a blood brain barrier) and its rich neural connections with vagal structures in the periphery, make it a candidate for the neural transmission of visceral and humoral signals to other central structures. Thus, the present results provide two other possible neural pathways, within the NST and through the RF, for such modulation. The neurophysiological characterization of these other targets of cNST projections will further aid in the elucidation of gustatory and visceral organization in the brainstem.
APPENDICES
APPENDIX A

ORAL AND GASTRIC STIMULATION PROTOCOL

Test Response to WM: Mix & tactile Stim.

Response to 5 ml Gastric Stretch?

N

STOP

Y

"GASTRIC BEST-STIMULUS"
Test Volume Series: 2, 5, 7 ml

N

Respose to WM: MIX?

Y

"TASTE BEST-STIMULUS"
WM: 4 STANDARD TASTES:
W-Stim-Rinse (2 ml, 10 sec)
1 min inter-taste-interval

Test Coactivation: Test "Best" Taste Stimulus before, during and after "Best" gastric stimulus:
15 sec gastric and 10 sec taste; 1 min inter-stim-interval

Test Individual Receptive Fields with Taste Mix
1) AT 2) NID 3) FOL 4) S. Palate
Brush No.: #5, #00, #00, #1

161
APPENDIX B
TASTE MIXTURE

Taste Mix. (1L)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Formula Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>.3 M NaCl</td>
<td>17.53g</td>
<td>FW=58.94</td>
</tr>
<tr>
<td>.003 M QHCl</td>
<td>1.19g</td>
<td>FW=396.92</td>
</tr>
<tr>
<td>.3M Sucrose</td>
<td>102.7g</td>
<td>FW=342.3</td>
</tr>
<tr>
<td>.01M HCl</td>
<td>10ml 1M HCl</td>
<td></td>
</tr>
</tbody>
</table>

Bring up to desired volume in a graduated cylinder or volumetric flask.

Base Mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>1L</th>
<th>2L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>35.06g</td>
<td>70.12g</td>
</tr>
<tr>
<td>HCl</td>
<td>20ml 1M</td>
<td>40ml 1M</td>
</tr>
<tr>
<td>QHCl</td>
<td>2.38g</td>
<td>4.76g</td>
</tr>
</tbody>
</table>

Bring up to desired volume in a graduated cylinder or volumetric flask.

To make 100ml Taste Mix from Base Mix
50ml Base Mix
Add 10.27g Sucrose
Bring volume up to 100ml in a graduated cylinder or volumetric flask

To make 250ml Taste Mix from Base Mix
125ml Base Mix
Add 25.67g Sucrose
Bring volume up to 250ml in a graduated cylinder or volumetric flask.


projection to nucleus raphe magnus and adjacent tegmental field. Brain
Res. 447:154-158.

neurons in the nucleus of the solitary tract. Soc Neurosci Abstr 23
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from the gustatory responsive parabrachial nucleus to medullary reticular

23:1038.

characterization of neuronal activity in the medullary reticular formation of

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influenced by hepatoportal afferent signal to parabrachial nucleus. J.


