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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Medullary Neural Circuits related to the Nucleus of the Solitary Tract in the Mouse Model

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science

in

Biology

by

Vanessa Johanna Cicchini

Committee in charge:

Professor Mark Whitehead, Chair
Professor Jill Leutgeb, Co-Chair
Professor Stefan Leutgeb

2012

The thesis of Vanessa Johanna Cicchini is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

_____

Chair

University of California, San Diego

2012

I dedicate this thesis to my parents,

Joseph and Suzanne,

for their

continuous love and support.

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I am fortunate to have been given the opportunity to work with Dr. Whitehead, and I greatly appreciate his continuous support and patience. Performing research in his lab has fueled my interest in neuroanatomy, and learning research techniques under his guidance has been truly beneficial.

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I would also like to thank my committee members, Dr. Stefan Leutgeb and Dr. Jill Leutgeb, for their feedback and support throughout the program.

ABSTRACT OF THE THESIS

Medullary Neural Circuits related to the Nucleus of the Solitary Tract

in the Mouse Model

by

Vanessa Johanna Cicchini

Master of Science in Biology

University of California, San Diego, 2012

Professor Mark Whitehead, Chair
Professor Jill Leutgeb, Co-Chair

The medullary connections of the subdivisions of the rostral and caudal nucleus of the solitary tract (NST) of the medulla were elucidated in the mouse model. Tract tracing experiments involving injections of Cholera toxin subunit B delivered by iontophoresis aimed at subdivisions of the NST, the area postrema, or the reticular formation investigated the hypothesis that the subnuclei within the NST have differential connections. Subsequent visualization of transported anterograde labeling of axonal endings and retrograde labeling of cell bodies throughout the medulla was accomplished with immunohistochemical techniques. Labeled cells and endings were plotted in relation to an atlas of cell groupings revealed with a routine histological counterstain. The results show intra-NST connections and differential connections depending on the sites injected.

Introduction

Utilizing neuroanatomy for mapping circuits within the brain has diverse applications in neurobiology and the health sciences. Studying taste neuroanatomy in the mouse model contributes to the understanding of specific connections within the mouse nervous system as well as the greater understanding of human conditions involving appetite, loss of taste, obesity, and diseases of the nervous system. Neuroanatomical studies also contribute to further understanding of the human "Connectome," an extensive project seeking to understand the vast neuronal connections composing the human brain (Sporns et al., 2005). The importance of the Connectome Project stems from the current lack of knowledge on the specific circuits of the brain and the patterns of neuronal projections.

The historical basis for research in neuroanatomy was built upon the studies by Santiago Ramón y Cajal and his identification of the structural components of individual neurons and their specific networks (Purves et al., 2008). Neurons possess dendrites for receiving electrical stimuli, a cell body for accomplishing protein synthesis, and an axon for directing signals in the form of action potentials away from the cell body toward the axon terminal for communication with other neurons. Specific clusters of neurons in regions of gray matter throughout the central nervous system are known as "nuclei." Groups of axons within the central nervous system are referred to as "tracts." My project focuses on the region of the nucleus of the solitary tract (NST) within the medulla oblongata of the brainstem. The brainstem serves important functions in monitoring consciousness as well as serving as the central nervous system starting point for sensory and motor-related cranial nerves. The cranial sensory nerves are directed toward the sensory cranial nerve nuclei of the brainstem; cranial nerve motor nuclei serve as the origin of the cranial motor neurons (Purves et al., 2008).

The nucleus of the solitary tract (NST) is a sensory nucleus. Information is brought to the NST via specific cranial nerves, including the trigeminal (V), facial (VII),

glossopharyngeal (IX), and vagus (X) nerves (Bromley, 2000). The management of the information arriving at the central nervous system related to taste begins at the NST. The receptors for taste are contained in taste buds on the tongue surface, fungiform papillae, and within trenches surrounding small protuberances on the tongue known as the 'circumvallate papillae.' They are also present on the soft palate. The signals detected by the taste buds are transmitted to the NST via branches of the facial, glossopharyngeal, and vagus nerves (Chiras, 2012). The NST is also important in the behavior related to food consumption because neurons responding to taste stimulation affect the response of the oral cavity related to food intake (Whitehead, 1994).

A brief overview of anatomical directional terms is necessary prior to delving into the connections and cytological characteristics of the nucleus of the solitary tract (NST). The rostral direction refers to regions toward the frontal lobe of the brain, while the caudal direction refers to regions further back past the cerebellum and toward the spinal cord. The dorsal direction refers to the back of the brainstem, while the ventral direction refers to the front of the brainstem (Castro and Sergeant, 2010). Another important anatomical region involved in directionality is the region of the obex, and this caudal medullary structure designates the boundary between the opening of the fourth ventricle rostrally and the emergence of the central canal and dorsal column nuclei caudally. The fourth ventricle refers to a hollow space filled with cerebral spinal fluid that becomes continuous with the central canal at further caudal levels (Butler and Hodos, 2005).

Previous anatomical studies have shown that the "special" visceral afferent fibers responsible for smell and taste, in contrast to the "general" fibers responsible for bringing information from pain sensations or touch, project to the rostral NST as well as the lateral NST. The rostral portion of the NST is especially important for taste input, and the caudal portion has a role in bringing information to the brain from general viscerosensory sources,

such as receptors for blood pressure or feedback from the gut. The NST has important functions in taste as well as involuntary visceral sensation, or pertaining to the internal organs of the abdomen or chest. Connections between the rostral and caudal NST would be accomplished by interneurons and enable communication between various regions related to the visceral organs and taste (Purves et al., 2008).

The cellular composition of the NST is not homogeneous. The accompanying cell structures and neuronal connections of the different regions of the NST have not been fully investigated in the mouse model. Previous studies with routine cell stains have identified four subdivisions of neuronal distributions composing the rostral NST. These include the rostral central subdivision (RC), the ventral subdivision (V), the rostral lateral subdivision (RL), and the medial subdivision (M). The caudal NST also contains a medial subdivision (M) as well as five additional subdivisions including the caudal central subdivision (CC), the caudal lateral subdivision (CL), the dorsal lateral subdivision (DL), the ventral lateral subdivision (VL), and the commissural subdivision (COM) (Whitehead, 2012). The locations of these subdivisions throughout the medulla are defined by their relative distances in millimeters rostral or caudal to the obex (See Figures S1 and S2 on following page).

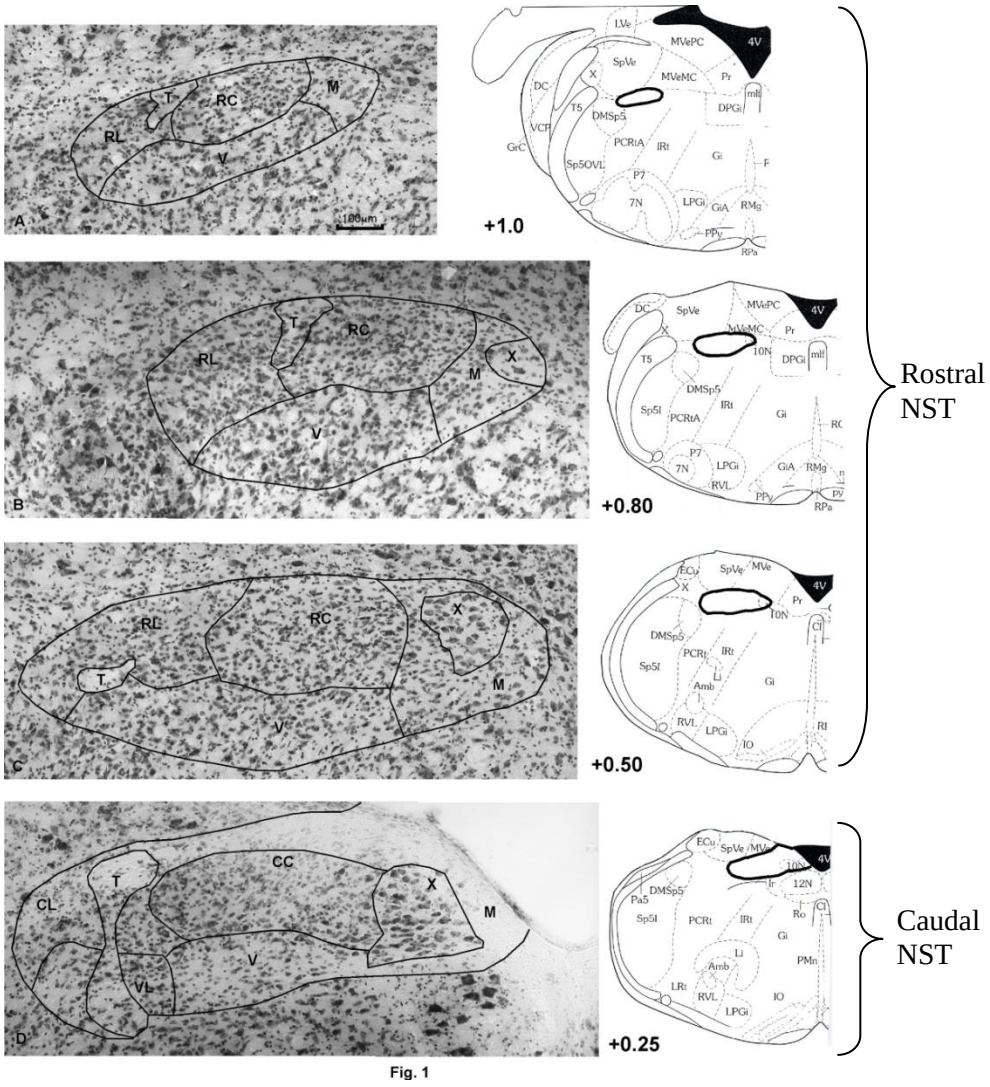


Fig. 1

<u>Figure S1</u>: Rostral subdivisions and initial caudal subdivisions of the NST. From *The Mouse Nervous System* (pg. 575) by Mark Whitehead, 2012, San Diego: Elselvier.

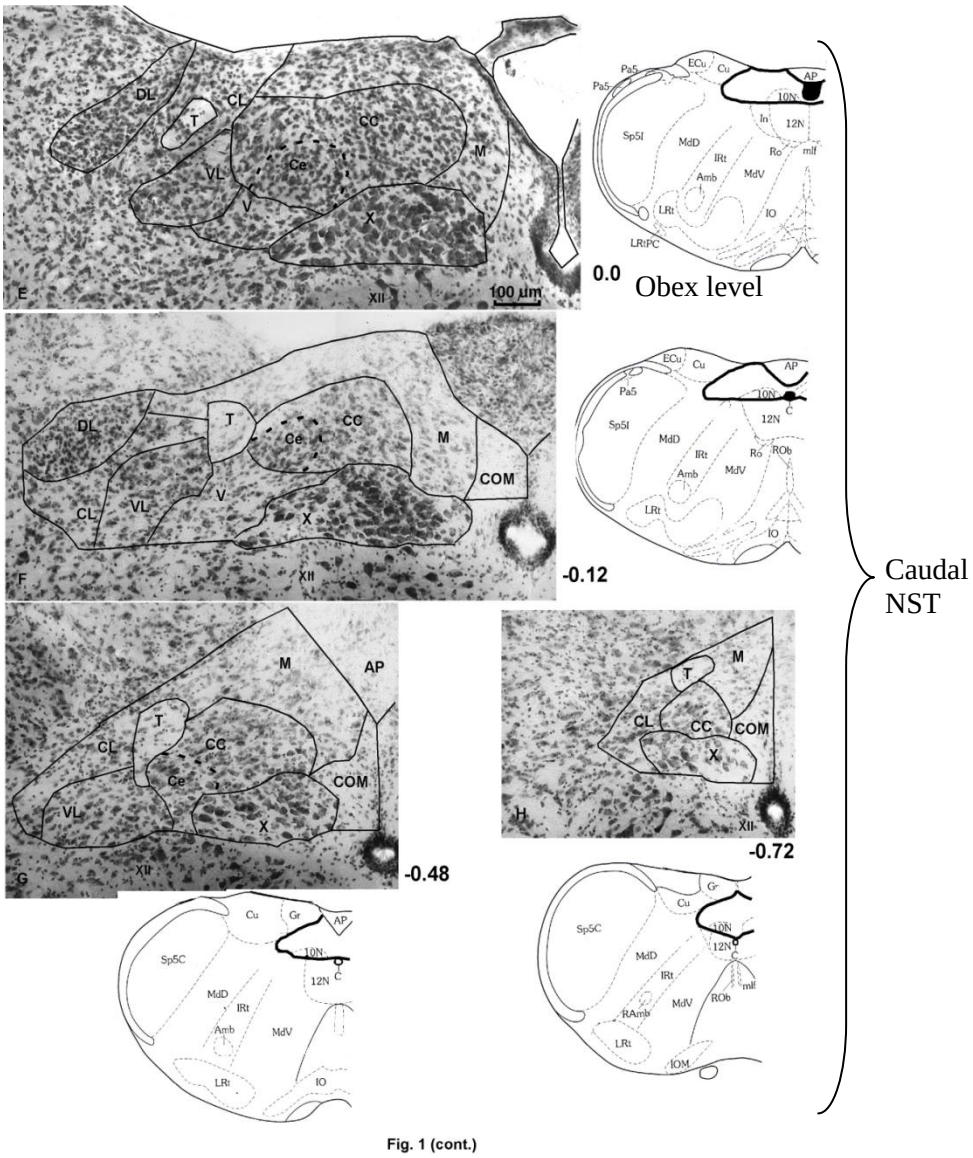


Fig. 1 (cont.)

<u>Figure S2</u>: Caudal subdivisions of the NST (continued). From *The Mouse Nervous System* (pg. 576) by Mark Whitehead, 2012, San Diego: Elselvier.

Previous studies have utilized injections of the B subunit of Cholera toxin (CTb) in the rostral NST in order to examine the sources of forebrain inputs and their effect on taste response due to evidence of projections from the forebrain to the NST (Whitehead et al., 2000). CTb functions as an anterograde and retrograde neuronal tracer, which enables the

labeling of the path from the cell body down the axon (anterograde) as well as from the axon terminal back up to the cell body (retrograde) (Zaborsky et al., 2006). My studies will rely on CTb injections targeting regions of the NST at levels rostral and caudal to the obex in order to examine projections within the NST subdivisions and between the NST and other brainstem nuclei. The brain injections will be performed in the medulla of mice after a craniotomy procedure and aspiration of the cerebellum at specific coordinates. Subsequent immunohistochemistry on coronal brain tissue sections will visualize the injection site as well as the transported retrograde labeled cell bodies and anterograde labeled endings. The tissue sections will be counter-stained for cell groups. This will enable patterns of labeling to be related to the subnuclei within the NST and nuclei in the medulla. This will allow me to relate my findings to neuroanatomical landmarks using published and unpublished atlases of the mouse medulla and the nucleus of the solitary tract. My project aims to reveal further information regarding the existence of neural circuits between the NST and other key cell groups within the medulla in order to test the hypothesis that there are differential projections of the subnuclei within the NST. The study will be organized according to injections of the rostral NST, the caudal NST, the area postrema, and regions of the reticular formation.

Limited studies exist regarding the projections of the caudal most region of the NST known as the commissural subnucleus in the mouse model. Otake and colleagues examined this subdivision in cats using injections of the neuronal tracer, biocytin, and reported the presence of many labeled nerve endings in the dorsal motor nucleus of the vagus nerve (1992). This was consistent with previously accepted data regarding the commissural subnucleus of the NST and its receipt of neuronal input originating from the lungs and other internal organs receiving input from the vagus nerve. Their results proposed the dual involvement of the commissural subdivision of the NST in regulating both autonomic tasks as well as respiratory functions (1992). One aspect of my study will draw comparisons between the tract tracing

results of the commissural subnucleus in mice to the connections of the subdivisions observed in the cat nervous system.

Additional investigation of the neural circuits existing between the neurons of the NST and the neurons of the area postrema are necessary because limited studies describe the connections between these regions. Initial studies by Morest in 1960 defined a region near the fourth ventricle to be the location of the area postrema. Morest also noted the characteristic flattened ependymal cells unique to this region, as well as its importance in triggering the vomiting response (1960). This region is also characterized by a relatively more permeable blood brain barrier (Shapiro et al., 1985). Studies by Shapiro and colleagues on the area postrema of the rat determined the existence of connections between the area postrema and the NST as well as the nucleus ambiguus and the dorsal motor nucleus of the vagus nerve at levels rostral and caudal to the obex. They also noted the possibility of faint projections from the caudal NST to the area postrema (1985).

My project will also examine the connections between the rostral and caudal NST and oral motor nuclei within the medulla. The facial motor nucleus serves to stimulate muscles for the support of movements of the jaw as well as moving the face and whispering. The function of the motor nucleus of the trigeminal nerve allows jaw movements such as opening of the mouth, mastication, and prompting swallowing. The hypoglossal nucleus is responsible for both projecting the tongue and returning it to its normal resting position during chewing of food and it facilitates swallowing by lifting the rear portion of the tongue. The dorsal motor nucleus of the vagus nerve has an important role in maintaining certain connections of the parasympathetic nervous system for assisting peristalsis during swallowing in the smooth muscle of the esophagus (Whitehead, 1994). It also functions in gut motility and heart and lung functions. Studies by Rogers and colleagues also reported the dorsal motor nucleus of the vagus nerve works with the NST in order to develop various ‘vago-vagal reflexes’ (1980).

This includes the ability of the stomach to prepare for storing an incoming meal through relaxation of its smooth muscle in response to expansion of the esophagus swallowing a bolus (Hornby and Wade, 2011).

The injections resulting in anterograde labeled endings present in the dorsal column nuclei, the gracile nucleus and the cuneate nucleus, were also investigated since they were involved in some of the adjacent NST injections. The gracile nucleus is important for relaying information in the pathway for distinguishing touch and body position from the lower limbs (Orrison, 2008). The cuneate nucleus is the more lateral subdivision and is important for bringing information from the limbs of the upper body as well as the trunk and neck to the central nervous system (Purves et al., 2008).

Another less studied area of the medulla includes the nucleus ambiguus, which is a distinct group of motor neurons located within the reticular formation. Nucleus ambiguus is classified as a visceral motor nucleus, referring to its projections providing innervation to smooth muscle or glands, as well as a brachial motor nucleus, which refers to its embryological relationship to the brachial arches and its involvement in developing muscles and bones of the craniofacial region (Purves et al., 2008). Additional studies have reported the involvement of the nucleus ambiguus in swallowing through its participation with glossopharyngeal and vagal motoneurons reaching the striated muscle of the pharynx and esophagus (Watson, 2012). Possible connections between the nucleus ambiguus and the NST were reported by Travers and colleagues in 1983 in the rat through their identification of anterograde labeled nerve terminals in the NST following injection of horseradish peroxidase into nucleus ambiguus. Further tracing studies by Bieger and Hopkins in 1987 reported the presence of nerve endings in the nucleus ambiguus originating from the central subnucleus of the NST.

Additional regions that will be investigated for the presence of anterograde and

retrograde labeling include the nucleus prepositus and the vestibular nuclei. The prepositus nucleus functions to link stimuli reached by the visual system with stimuli of balance and spatial orientation from the vestibular system. For example, this nucleus may be involved in maintaining appropriate visual responses with changes in the position of the head (Beitz and Anderson, 2000). The vestibular nuclei also play an important role in head position and spatial orientation (Siegel and Sapru, 2010). Previous studies by Fornai and colleagues investigated stimulation of the vagus nerve as a treatment for epilepsy and they noted the pathway in which the NST sends information from the vagus nerve to the locus coeruleus through the paragigantocellular nucleus and the prepositus nucleus (Fornai et al., 2011).

Two of the mice involved in the study will receive injections within the reticular formation in order to explore connections between the NST and the reticular formation. The reticular formation consists of a meshwork of neurons along the brain stem spanning regions of the spinal cord as well as in the opposite direction toward the brain. This region is involved in a variety of respiratory and cardiovascular functions as well as pain moderation (Silverthorn, 2010). Previous studies have reported that over 1000 neurons innervate a single neuron of the reticular formation, and each neuron subsequently connects to about 10,000 neurons (Jacobson and Marcus, 2011). Various nuclei within the reticular formation will be investigated for transported labeling of anterograde labeled endings and retrograde labeled cell bodies for determining possible connections with the NST. For example, the intermediate reticular nucleus of the medulla is involved in regulating functions such as heart rate and respiration, and the parvocellular reticular nucleus is especially responsive to visual stimuli (Farmer-Dougan et al., 2010); it also functions in oromotor behaviors.

Studying the results of the reticular formation injections, such as the retrograde labeling of NST neurons, will provide further insight on possible projections from the NST to the reticular formation. This will also verify previous cases in which anterograde labeled

endings were detected in the reticular formation as a result of an injection of the NST.

The presence of transported labeling to the parabrachial nucleus of the pons will also be evaluated throughout all cases. Previous studies, such as in the hamster nervous system, have described projections from the caudal NST to the parabrachial nucleus, as well as from the rostral central subdivision of the rostral NST. Fewer connections have been reported that originated from the rostral lateral and ventral subdivisions, but this conclusion will be investigated further within my study (Whitehead, 1990).

Materials and Methods

Experiments using nine C57BL/6J mice were performed according to lab procedures approved by University of California at San Diego Laboratory Animal Care and Use Committee and National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Injections into the medulla used purified Cholera toxin B subunit (CTb) isolated from *Vibrio cholera* (Product #103b, List Biological Laboratories, Inc.).

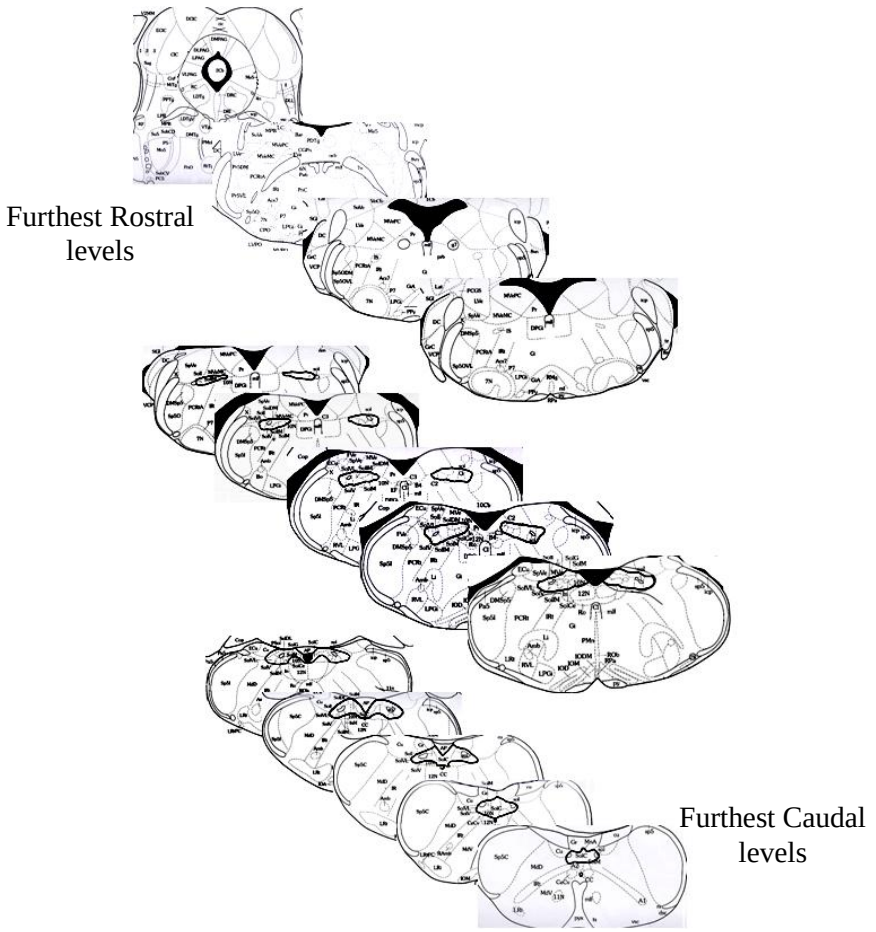
Animals were calmed in a bell jar containing isoflurane vapors, and then anesthetized with intrapleural injections of Ketamine/Xylazine (0.1mL anesthetic per 10g mouse). Animals were placed on a warming platform with their heads stabilized in a nose clamp. The mice received CTb through iontophoretic injections administered through a glass micropipette (tip diameter approximately 15-20μm) attached to a micromanipulator (Fine Science Tools) following craniotomy and aspiration of the cerebellum.

Animals surviving 24 hours after injection of CTb were perfused transcardially with 4% paraformaldehyde in phosphate buffer (pH 7.4). The brain tissue was allowed to soak in 30% sucrose for 1-3 days, and then it was sliced into 40μm thick coronal sections using a microtome.

Detection of the CTb began with treatment of free-floating tissue sections with a primary antibody solution of phosphate buffered saline, 0.3% Triton X-100 (Fisher Scientific, lot# 036262), 5% normal rabbit serum (Thermo scientific, lot # LG13290213), goat-anti-CTb primary antibody diluted 1:10,000 (Product #703, List Biological Laboratories, Inc.) left rotating overnight. The following morning, after 3 washings of the tissue in phosphate buffered saline, a secondary antibody, rabbit biotinylated anti-goat IgG (Product #BA-500, Vector Laboratories) was applied. After additional washes, an avidin and biotin system was used to further detect the signal (Vectastain Elite ABC kit, Vector Laboratories). In order to localize the CTb, a routine peroxide diaminobenzadine immunohistochemistry (Catalog

#98068, MP Biomedicals, ImmunO 3,3-diaminobenzidine tablets, catalog #98068) was performed utilizing the avidin and biotin system and amplified the signal.

Anterograde labeled axonal endings and retrograde labeled cells were visualized with standard light microscopy following counterstaining of the divisions of various nuclei throughout the medulla with a Giemsa stain. The locations of labeled endings and cell bodies within the regions of nuclei were plotted according to the atlas of the coronal series of the mouse brain by Paxinos and Franklin (2004).



<u>Figure S3</u>: Sections of rostral and caudal medulla to plot transported label.

Adapted from *The Mouse Brain in Stereotaxic Coordinates* by George Paxinos and Keith Franklin, 2004, San Diego: Elselvier.

Results

Each case was grouped according to the injection unique to each case. Cases 1, 2, and 3 studied injections of the rostral NST, and Cases 4, 5, and 6 investigated injections of the caudal NST. Additional injections of the area postrema and reticular formation were included in this study as well. The coronal series specific to each case displays the distributed anterograde labeled endings and retrograde labeled cell bodies resulting from each injection. The plots of the coronal series are made on representative medulla section levels adapted from those included in the atlas of the mouse brain by Paxinos and Franklin (2004). The symbols illustrated in the table below will be used to indicate labeled endings, cell bodies, and regions of injection throughout each coronal series.

Table 1: Legend for plots of transported labeling

Symbol	Definition	Unit
	Anterograde labeled endings	**10 endings**
	**Retrograde labeled cell bodies**	**4 cell bodies**
	**Center of injection site (see plot)**	**(encircles region)**
	**Edge of injection site (on sections very close to injection center)**	**(encircles region)**
	Center of injection site (see summary tables)	**(highlights center)**
	Dividing line between rostral and caudal NST	**(see summary tables)**

Part A: Injections of the Rostral NST

Case 1 – Center of injection in the medial subdivision of the rostral NST

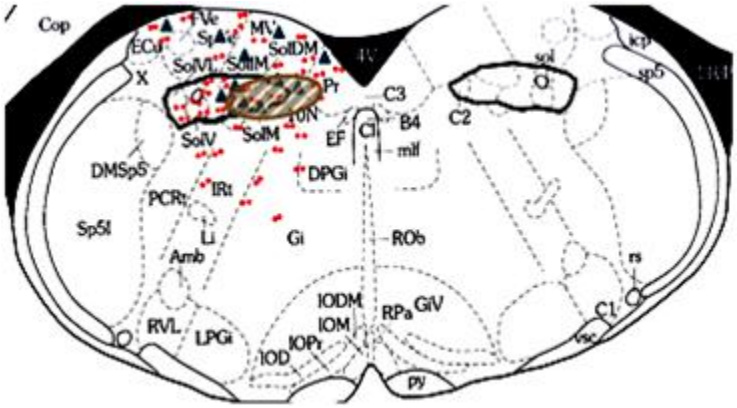


Figure 1-1: Site of injection center at 0.50mm rostral to obex

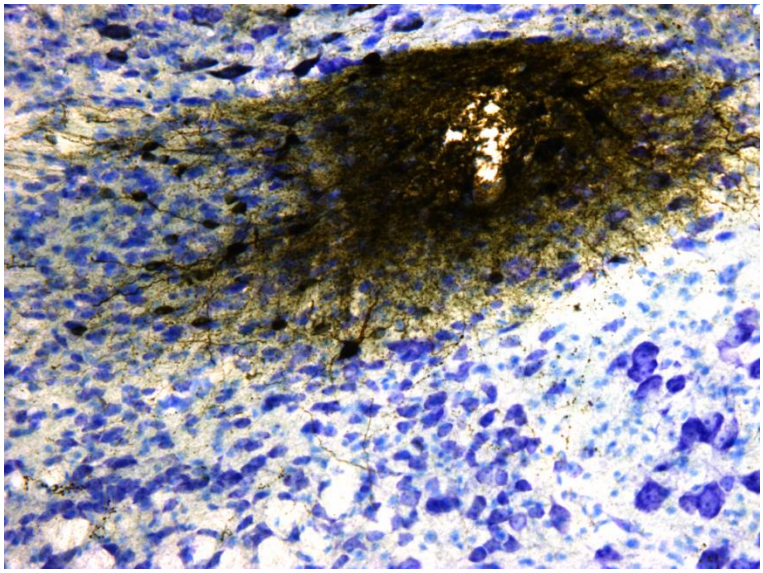
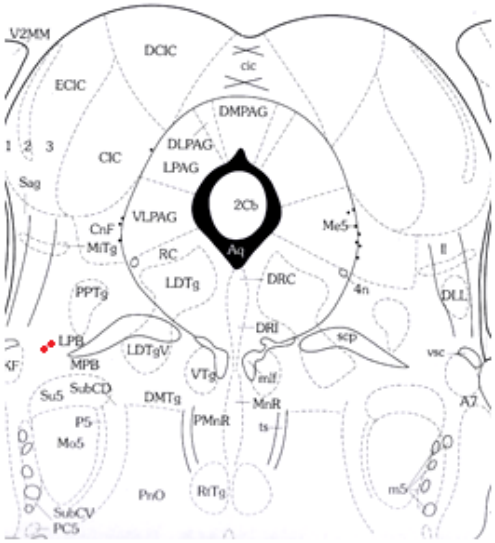


Figure 1-1A: Micrograph of injection center, 20X

A medium-sized injection was placed within the medial subdivision of the rostral NST. The injection was positioned 250µm rostral to the rostral pole of the area postrema, 275µm dorsoventrally, and 600µm lateral to the midline. Retrograde labeled cell bodies were present in within the medial, ventral, and rostral central subdivisions at the level of the

injection site. Anterograde labeled endings were present in these subdivisions as well as the rostral lateral portion of the NST at this level. Beyond the region of the NST, retrograde labeled cell bodies and anterograde endings were also visible in the prepositus nucleus (Pr), the parvocellular medial vestibular nucleus (MVePC), the spinal vestibular nucleus (SpVe), and the external cuneate nucleus (ECu). Within the reticular formation, anterograde endings were present in the gigantocellular nucleus (Gi), intermediate reticular nucleus (IRt), and the parvocellular nucleus (PCRt). Retrograde labeled cell bodies and anterograde labeled endings were also present in the dorsal motor nucleus of the vagus nerve (10N).

Coronal Series: Rostral to caudal sections of medulla containing label



<u>Figure 1-2</u>: Transported label 2.50mm rostral to the obex.

Anterograde labeled endings were detected within the lateral parabrachial nucleus (LPB). These data are consistent with previous research in the hamster by Joseph Travers in which labeled endings were detected within the parabrachial nucleus projecting from the anterior NST (1988). Additional retrograde studies on the connections between the parabrachial nucleus and the NST in the hamster confirmed the projections of regions of the

rostral and caudal NST to the parabrachial nucleus. Intermediately sized injections in the parabrachial nucleus resulted in retrograde labeled cell bodies at rostral and caudal levels of the NST (Whitehead, 1990).

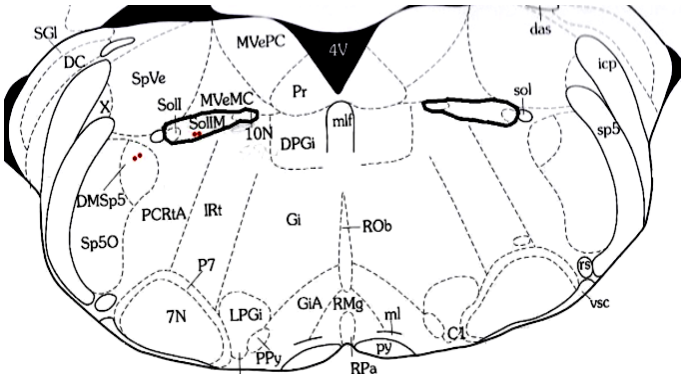


Figure 1-3: Transported label 1.0mm rostral to obex.

At the furthest rostral level of the NST, endings were present only in the ventral subdivision. Additional endings were present in regions of the spinal trigeminal nucleus (DMSp5).

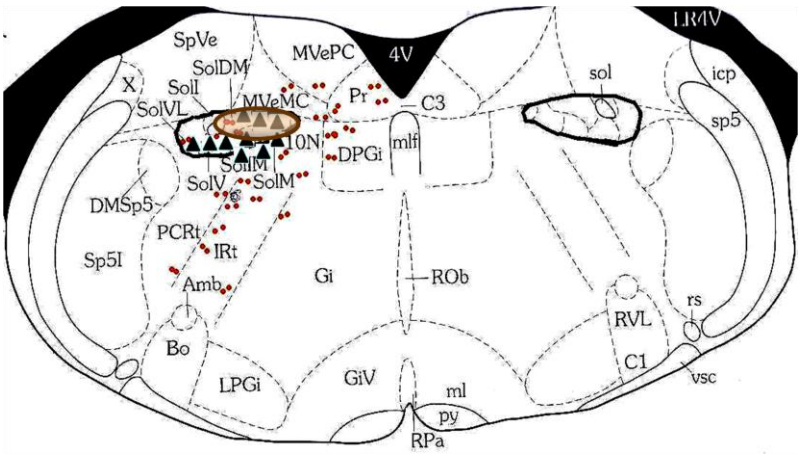


Figure 1-4: Transported label at edge of injection site 0.80mm rostral to obex.

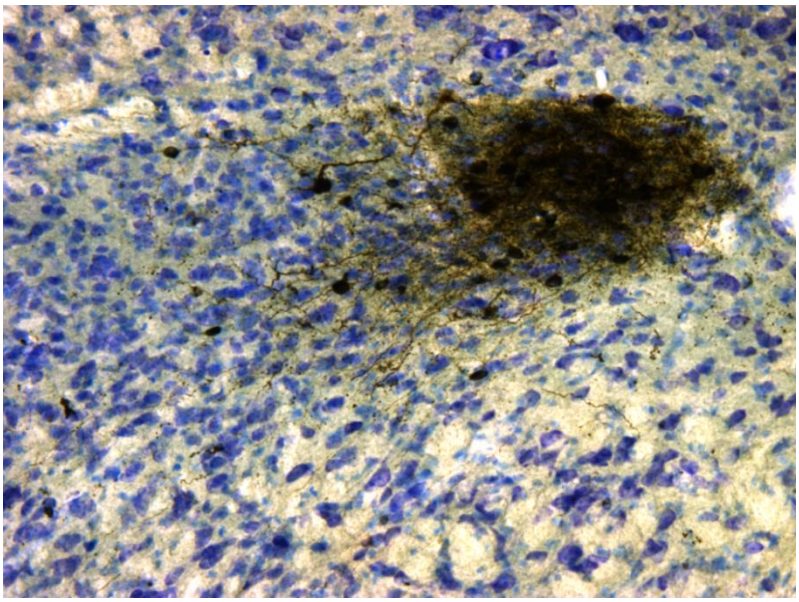


Figure 1-4A: Micrograph of labeling within rostral NST, 20X

Slightly rostral to the injection center, retrograde labeled cell bodies were especially visible in the medial, ventral, and rostral central subdivisions. Anterograde labeled endings were visible in these subdivisions as well. Beyond the region of the NST, anterograde labeled endings were visible in the prepositus nucleus (Pr) and the parvocellular medial vestibular nucleus (MVePC). In regions of the reticular formation, endings were visible in the gigantocellular nucleus (Gi), intermediate reticular nucleus (Irt), and parvocellular reticular

nucleus (PCRt). Labeled endings detected within the reticular formation are consistent with previous findings in the hamster by Beckman and Whitehead. Their use of horseradish peroxidase injections in the rostral NST resulted in anterograde labeled endings especially visible within the parvocellular reticular formation (1991).

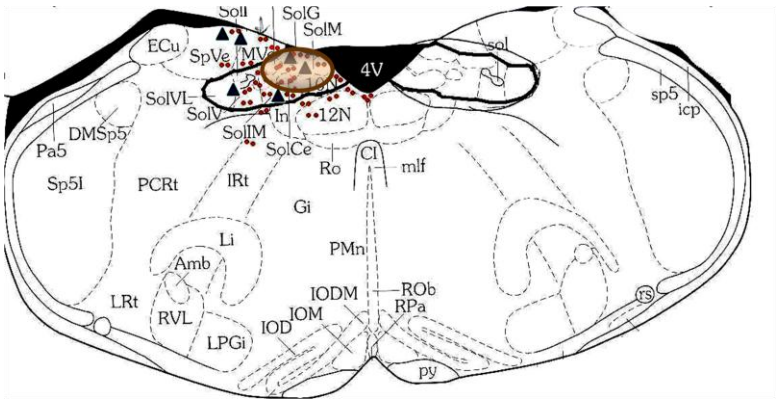


Figure 1-5: Transported label at edge of injection site 0.25mm rostral to obex

Slightly caudal to the center of the injection, retrograde labeled cell bodies remained present in the medial and ventral subdivisions, but were also present bordering the caudal lateral and ventral lateral subdivisions of the caudal NST. Anterograde labeled endings were present throughout the medial, ventral, and caudal central subdivisions. Beyond the region of the NST, retrograde labeled cell bodies and anterograde labeled endings were present in the parvocellular medial vestibular nucleus (MVePC) and the spinal vestibular nucleus (SpVe). Anterograde labeled endings were also present in the dorsal motor nucleus of the vagus nerve (10N) and the hypoglossal nucleus (12N). The anterograde labeling in the hypoglossal nucleus was consistent with previous studies by Travers and Norgren on the afferent projections to the hypoglossal nucleus in the rat nervous system. Their injections of the hypoglossal nucleus resulted in retrograde labeled cell bodies heavily within the reticular formation, as well as sparse labeling originating from the nucleus of the solitary tract. This suggested the possibility of connections between the hypoglossal nucleus and the reticular

formation, as well as direct projections between the NST and the hypoglossal nucleus (1983). This result was also consistent with later studies on injections of the anterior NST in the hamster by Travers (1988). The injections of rostral and caudal subdivisions of the NST in Cases 1-5 all resulted in anterograde labeled endings within various reticular nuclei in addition to labeled endings in the hypoglossal nucleus.

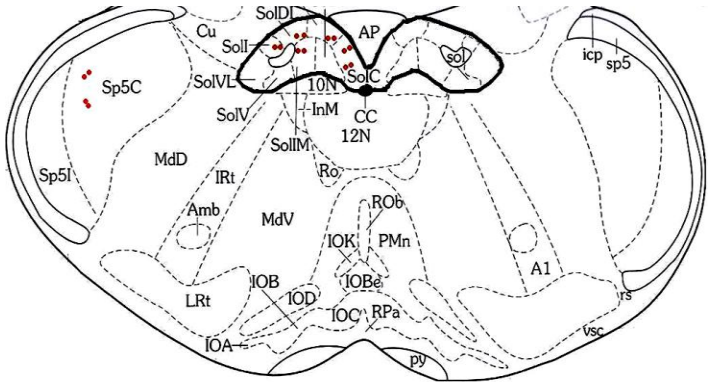


Figure 1-6: Transported label 0.12mm caudal to obex

At a further caudal level of the NST 0.12mm from the obex, retrograde labeled cell bodies were no longer present within the NST but endings were highly distributed in the commissural, medial, central caudal, and dorsal lateral subdivisions. Beyond the region of the NST, anterograde labeled endings were not visible in the reticular formation but were present within regions of the spinal trigeminal nucleus (Sp5).

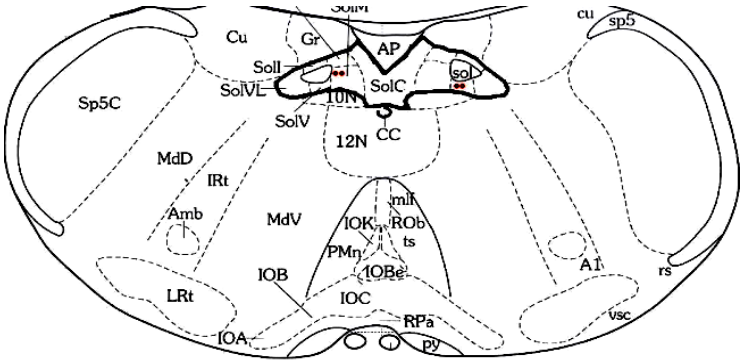


Figure 1-7: Transported label 0.48mm caudal to obex

Further caudally at 0.48mm from the obex, anterograde labeled endings were sparsely distributed in the central caudal and ventral subdivisions. Anterograde labeled endings were not visible in regions beyond the NST.

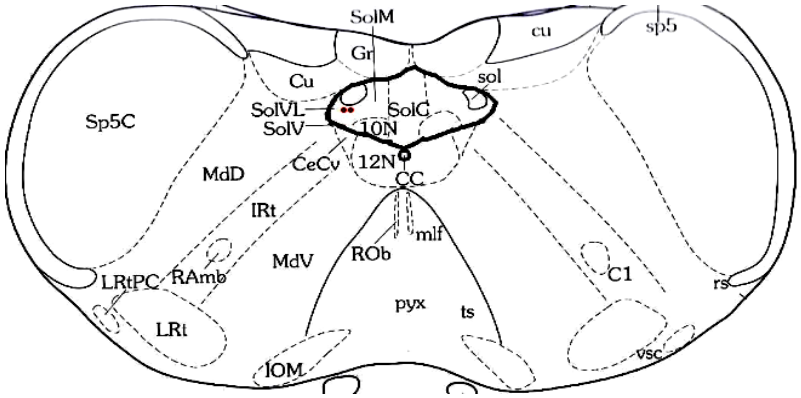
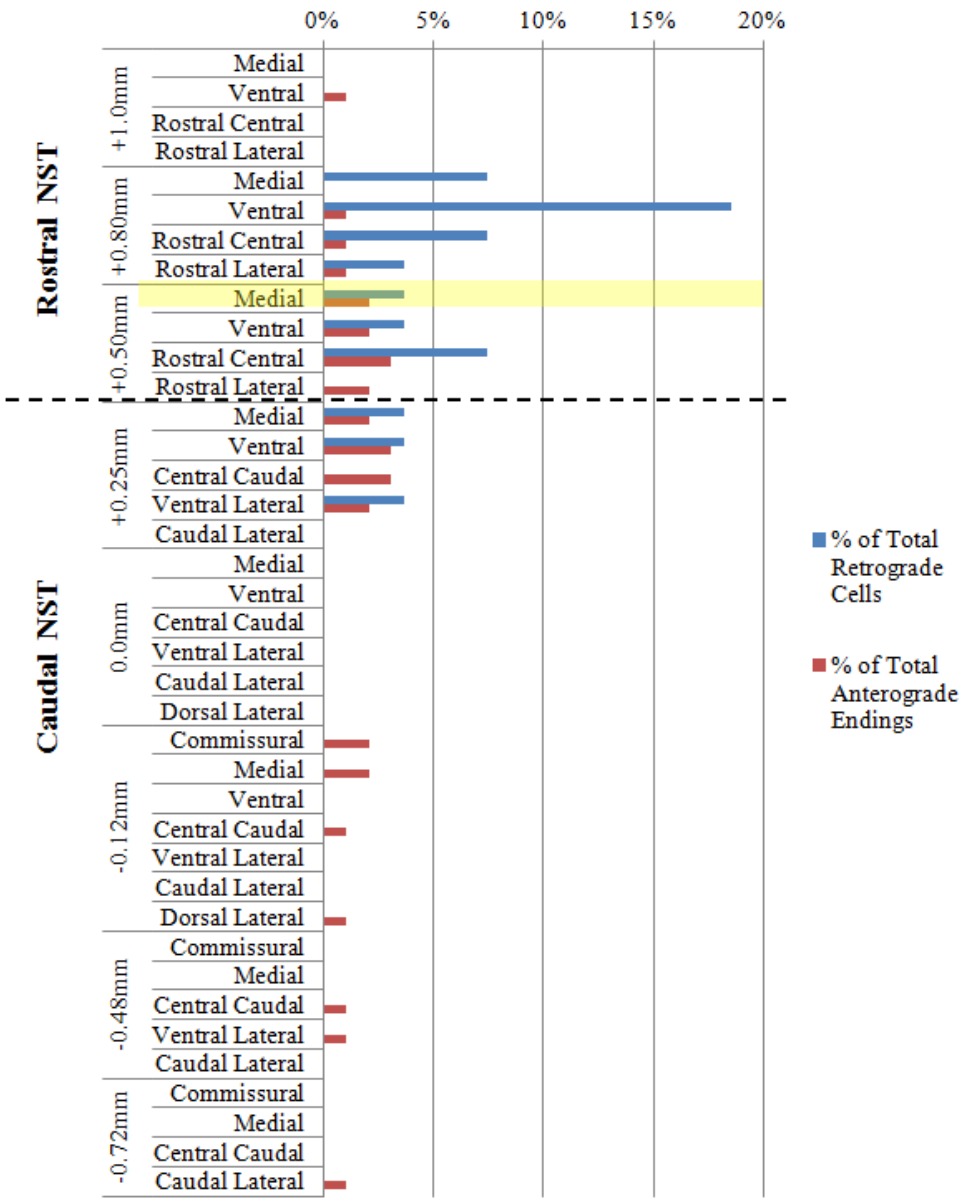


Figure 1-8: Transported label 0.72mm caudal to obex

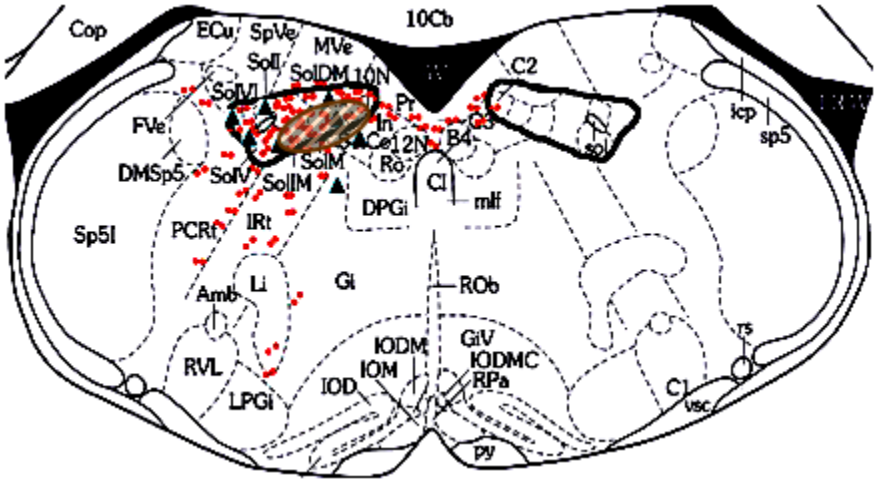
At the furthest caudal level of the NST at 0.72mm from the obex, anterograde labeled endings were present in the caudal lateral subdivision alone.

Graph 1: Summary of transported labeling in rostral and caudal NST, Case 1

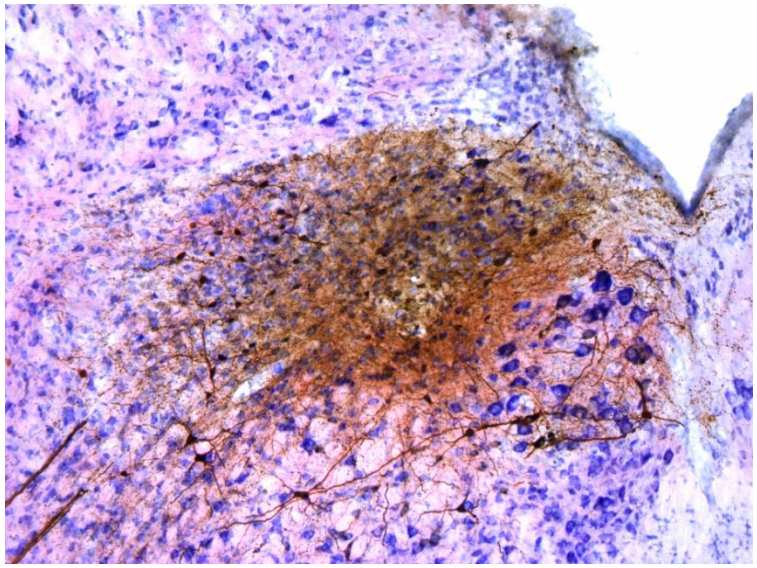


The figure above illustrates the total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

Case 2 – Center of injection in the ventral subdivision of the rostral NST



<u>Figure 2-1</u>: Site of injection center at 0.35mm rostral to obex

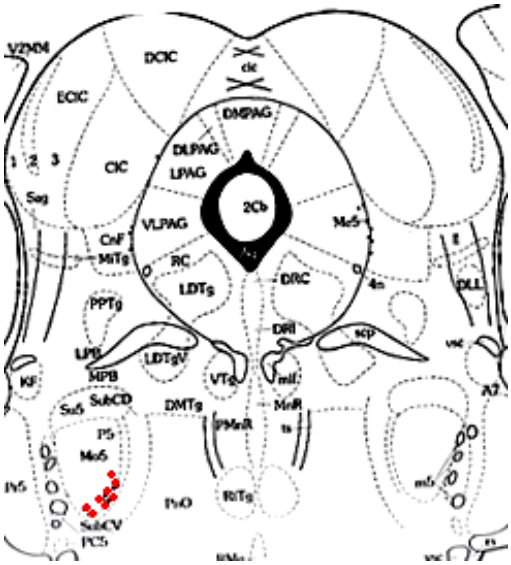


<u>Figure 2-1A</u>: Micrograph of injection center, 20X

A large injection was made in the ventral subdivision at the caudal portion of the rostral NST. The injection was positioned 250μm rostral to the rostral pole of the area postrema, 350μm dorsoventrally, and 600μm lateral to the midline. Retrograde labeled cell bodies were largely present in the ventral, rostral central, and rostral lateral subdivisions.

Anterograde labeled endings were visible in all subdivision of the rostral NST at this level. Outside the region of the NST, retrograde labeled cell bodies and anterograde labeled endings were visible in the gigantocellular nucleus (Gi). In other regions of the reticular formation, anterograde labeled endings were also visible in the intermediate reticular nucleus (Irt) and the parvocellular reticular nucleus (PCRt). Heavy anterograde labeling was also visible in the prepositus nucleus (Pr) and the medial vestibular nucleus (MVe). Labeled endings in the nucleus ambiguus (Amb) were not visible at this level; however endings were present in the linear nucleus (Li). Labeled endings were also visible in the dorsal motor nucleus of the vagus nerve (10N) and the hypoglossal nucleus (12N).

Coronal Series: Rostral to caudal sections of medulla containing label



<u>Figure 2-2</u>: Transported label 2.50mm rostral to obex

Anterograde labeled endings were distributed furthest rostrally in the trigeminal motor nucleus (Mo5). This result was consistent with studies by Norgren on the NST in the rat in which a projection from the anterior NST to the reticular formation was noted to eventually reach the trigeminal motor nucleus (1978). This result was not observed in the other cases

involving injections of the NST, and this may be a consequence of the injection size specific to Case 2.

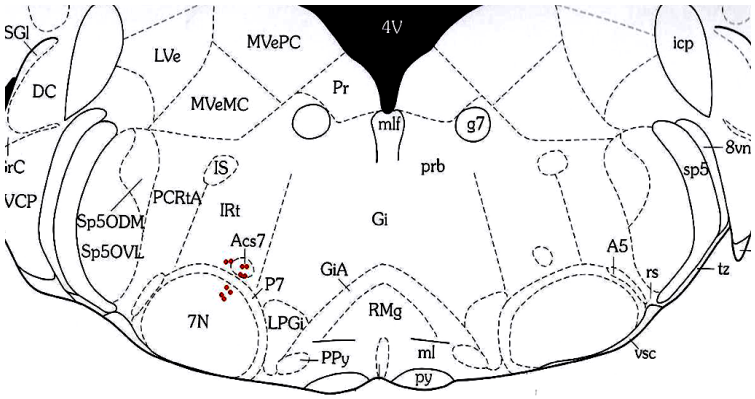


Figure 2-3: Transported label 1.50mm rostral to the obex

Slightly further caudal, anterograde labeled endings were visible in the facial motor nucleus (7N) as well as the accessory facial nucleus (Acs7). Within the reticular formation at this level, few endings were also visible in the intermediate reticular nucleus (Irt).

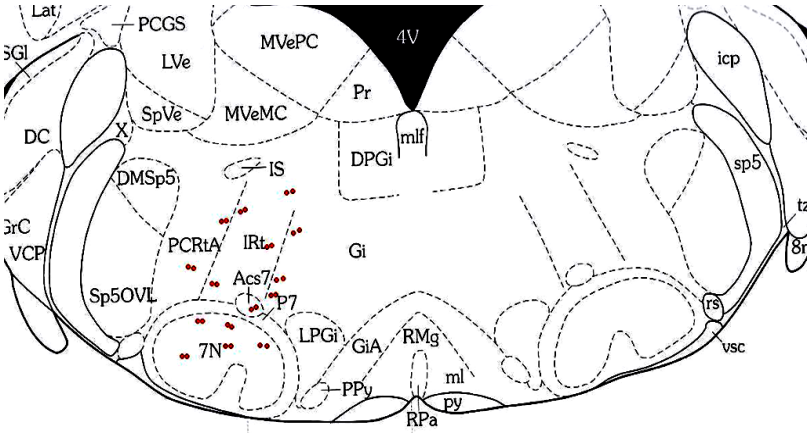


Figure 2-4: Transported label 1.25mm rostral to obex

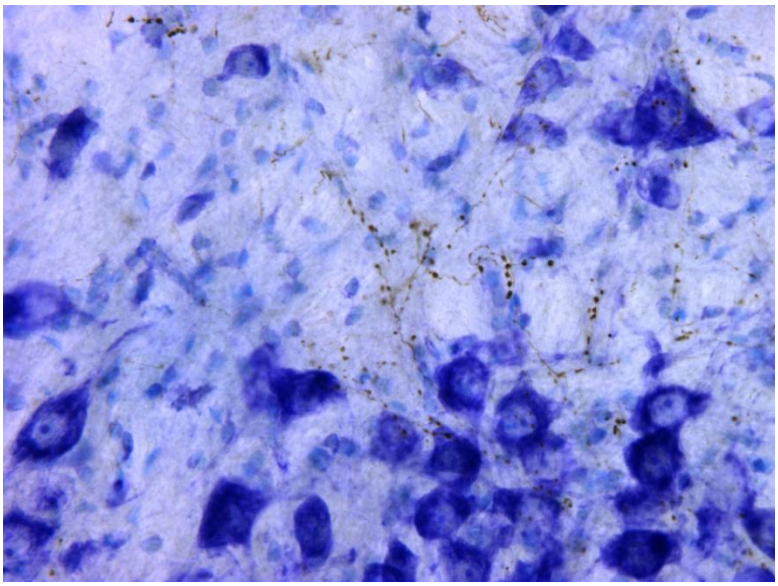


Figure 2-4A: Anterograde labeled endings in facial motor nucleus, 40X

Further caudally, endings remained visible in the facial motor nucleus (7N) as well as the accessory facial nucleus (Acs7). Heavier anterograde labeling of endings became visible throughout regions of the reticular formation, including the gigantocellular nucleus (Gi), the intermediate reticular nucleus (Irt), and the parvocellular reticular nucleus (PCRt).

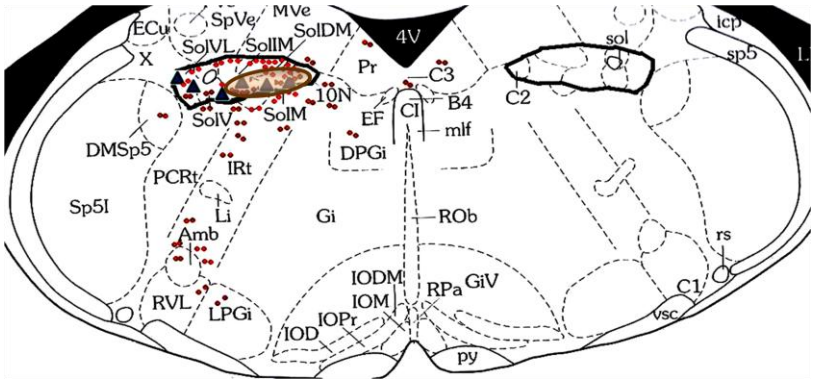


Figure 2-5: Transported label 0.50mm rostral to obex

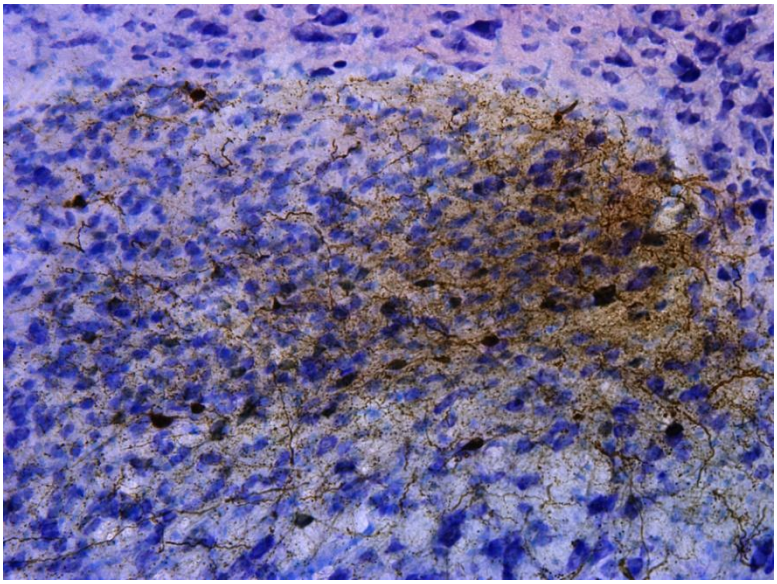


Figure 2-5A: Transported label throughout rostral NST, 40X

At the level of the rostral NST, anterograde labeled endings and retrograde labeled cell bodies were present in the medial, ventral, and sparsely in the rostral central and rostral lateral subdivisions. Beyond the region of the NST, anterograde-labeled endings were also present in the nucleus ambiguus (Amb) as well as the dorsal motor nucleus of the vagus nerve (10N) and regions of the spinal trigeminal nucleus (Sp5). Endings were also visible in the prepositus nucleus (Pr) bilaterally. Throughout regions of the reticular formation, anterograde labeled endings were visible in the gigantocellular nucleus (Gi) and the intermediate reticular nucleus (Irt).

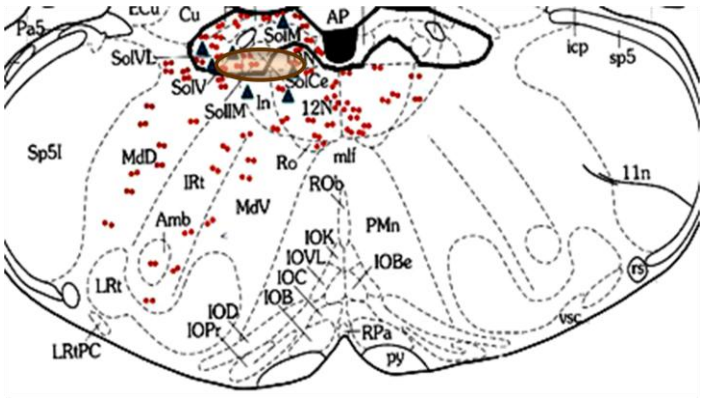


Figure 2-6: Transported label at the level of the obex

Slightly caudal to the injection site, retrograde labeled cell bodies were present in the medial, ventral, ventral lateral, and caudal lateral subdivisions of the NST. Anterograde labeled endings were visible in all subdivisions of the caudal NST at this level. The hypoglossal nucleus (12N) contained an especially high distribution of retrograde labeled cell bodies and anterograde labeled endings at this level. Anterograde labeled endings were also present in the dorsal motor nucleus of the vagus nerve (10N) as well as the nucleus ambiguus (Amb). Throughout regions of the reticular formation, anterograde labeled endings were present in the dorsal and ventral medullary reticular nuclei (MdD and MdV) as well as the intermediate reticular nucleus (Irt).

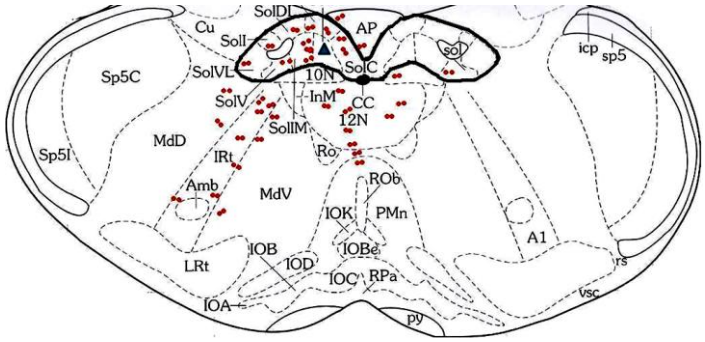


Figure 2-7: Transported label 0.12mm caudal to obex

Further caudal to the injection center, anterograde labeled endings were present within all subdivisions of the caudal NST, including the commissural, medial, central caudal, ventral,

ventral lateral, caudal lateral, and dorsal lateral subdivisions. However, bilateral labeling of axonal endings in the NST was only visible in the ventral lateral subdivision. Retrograde labeled cell bodies were also visible in the central caudal subdivision. Anterograde labeled endings were also present in the area postrema (AP) at this level. Anterograde labeling remained visible in the hypoglossal nucleus (12) as well. Labeled endings were also visible in the dorsal motor nucleus of the vagus nerve (10N) on the side contralateral to the injection site. Throughout the reticular formation, anterograde labeled endings were again present throughout the dorsal and ventral medullary reticular nuclei (MdD and MdV) as well as the intermediate reticular nucleus (Irt).

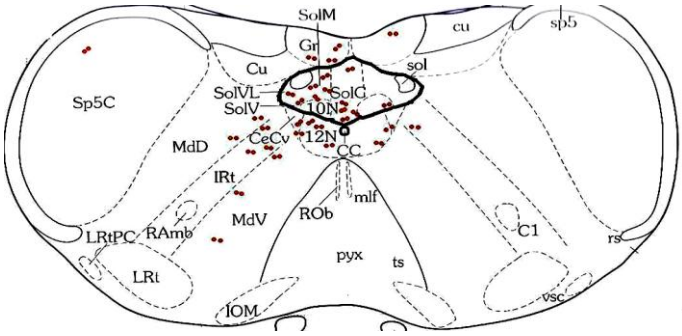


Figure 2-8: Transported label 0.72mm caudal to obex

At levels further caudal to the injection site, heavy anterograde labeling remained visible in the commissural, medial, central caudal, and caudal lateral subdivisions of the caudal NST. Labeled endings also remained in the dorsal motor nucleus of the vagus (10N) and the hypoglossal nucleus (12N). Additional anterograde labeled endings were visible in the gracile (Gr) nucleus as well as regions of the spinal trigeminal nucleus (Sp5). Throughout the reticular formation, anterograde labeled endings were present in the dorsal and ventral medullary reticular nuclei (MdD and MdV) as well as the intermediate reticular nucleus (Irt).

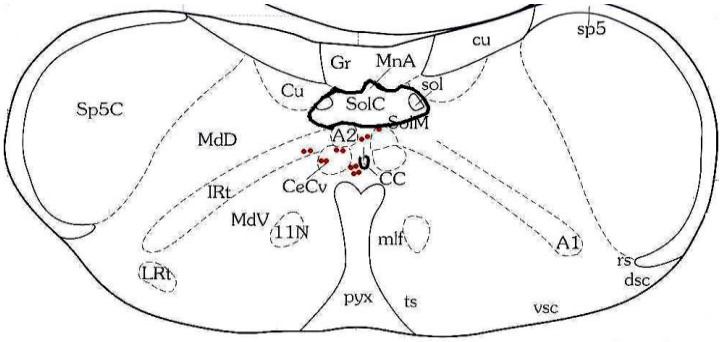
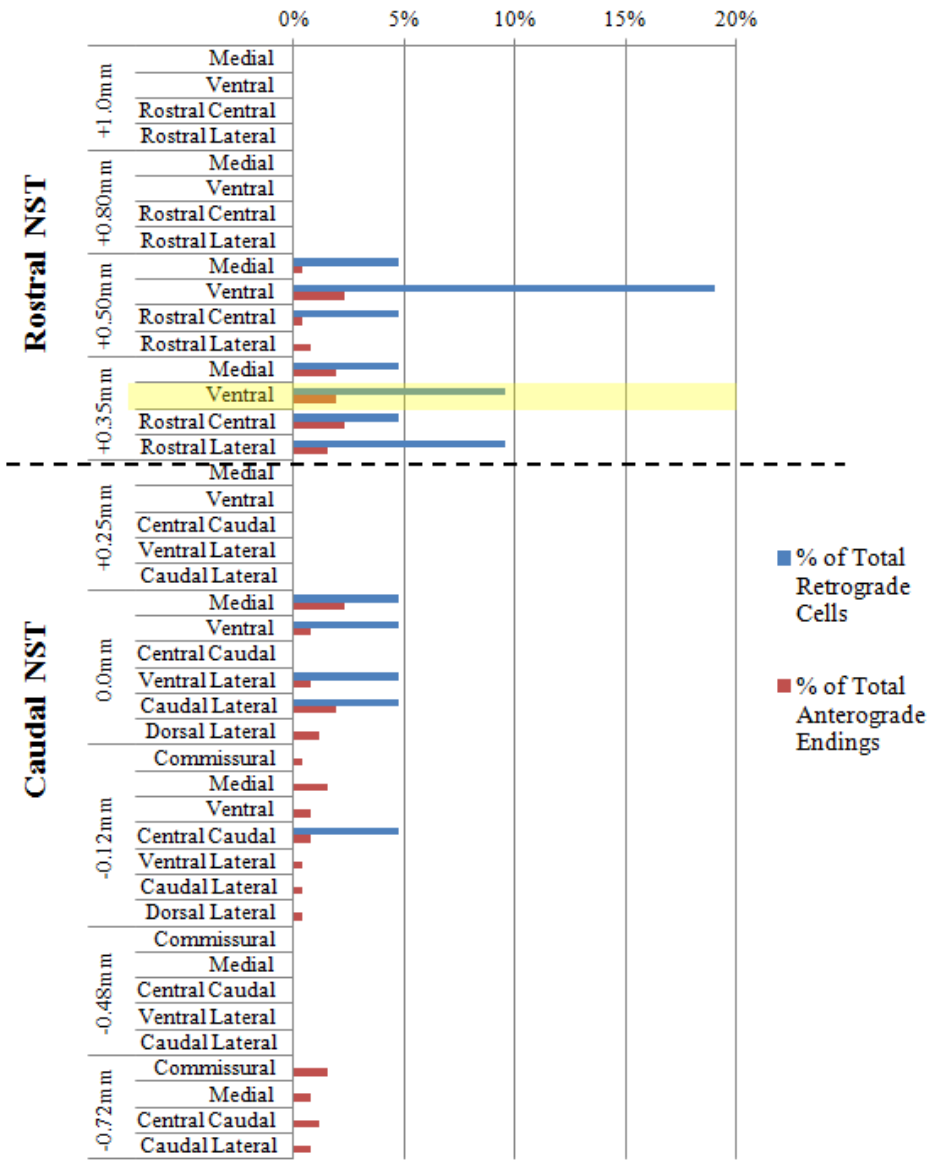


Figure 2-9: Transported label 0.90mm caudal to obex

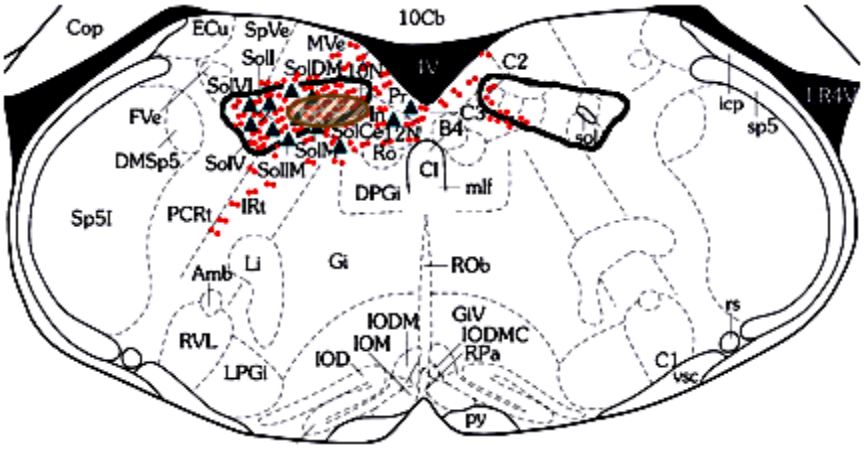
At the level furthest caudal to the injection site, anterograde labeled endings were no longer visible in the subdivisions of the caudal NST. However, labeled endings remained visible in the intermediate reticular nucleus of the reticular formation (Irt).

<u>Graph 2</u>: Summary of transported labeling in rostral and caudal NST, Case 2



The total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions is shown. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

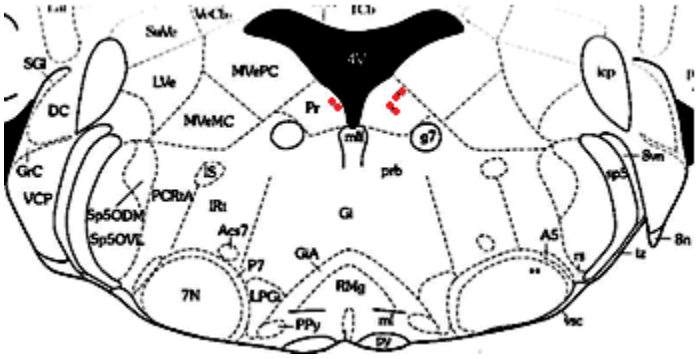
Case 3 – Center of injection in the medial and ventral subdivisions of the rostral NST



<u>Figure 3-1</u>: Site of injection center at 0.35mm rostral to obex

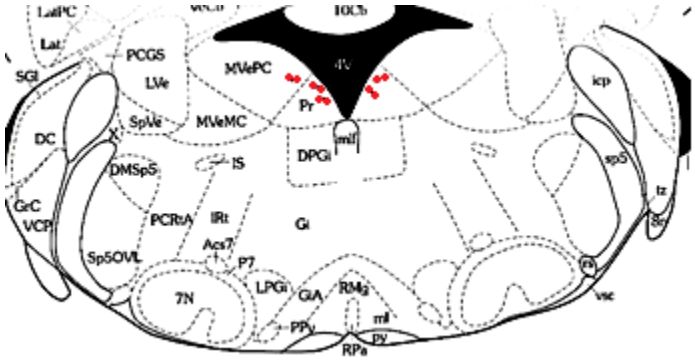
A small injection was centered in the medial and ventral subdivisions at the caudal end of the rostral NST. The injection was positioned 1mm rostral to the rostral pole of the area postrema, 250μm dorsoventrally, and 400μm lateral to the midline. Retrograde labeled cells were prominent within all subdivisions of the rostral NST but were especially heavy in the ventral, central caudal, and ventral lateral subdivisions. Anterograde labeled terminals were also present in all subdivisions of the NST and were most highly distributed in the medial subdivision. Sparsely distributed bilateral anterograde labeled endings were visible in the medial and ventral subdivisions of the NST. Beyond the region of the NST, retrograde labeled cell bodies and anterograde labeled endings continued to be visible in the dorsal motor nucleus of the vagus nerve (10N) as well as within the hypoglossal nucleus (12N). A few labeled cell bodies and axonal endings were present in the gigantocellular nucleus (Gi), and labeled endings were prominent within the intermediate reticular nucleus (Irt) of the reticular formation. Bilateral labeled endings in the prepositus nucleus (Pr) continued to be visible at this level, and endings also continued to be visible in the medial vestibular nucleus (MVe).

Coronal Series: Rostral to caudal sections of the medulla containing label



<u>Figure 3-2</u>: Transported label 1.50mm rostral to obex

Labeled endings were only detected within the prepositus nucleus (Pr) bilaterally.



<u>Figure 3-3</u>: Transported label 1.25mm rostral to obex

Slightly further caudal, endings were visible in the parvocellular medial vestibular nucleus (MVePC) and continued to be observed bilaterally in the prepositus nucleus (Pr).

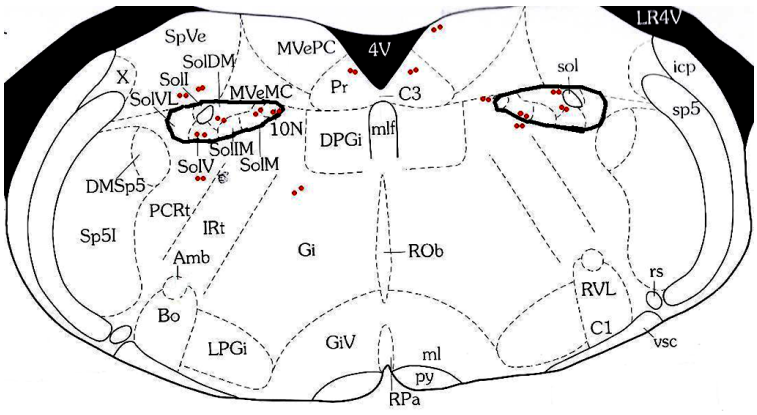


Figure 3-4: Transported label 0.80mm rostral to obex

Further caudally, endings continued to be observed bilaterally in the prepositus nucleus as well as the MVePC and the spinal vestibular nucleus (SpVe). In subdivisions of the rostral NST, anterograde labeled endings were visible in the medial, ventral, and rostral central subdivisions and were present bilaterally. Anterograde labeled endings were visible within the dorsal motor nucleus of the vagus nerve (10N). Endings also appeared in regions of the reticular formation within the gigantocellular (Gi) nucleus and the parvocellular reticular nucleus (PCRt).

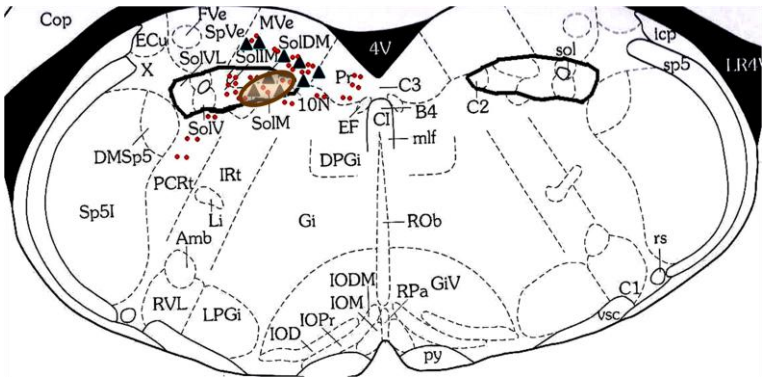


Figure 3-5: Transported label 0.50mm rostral to obex

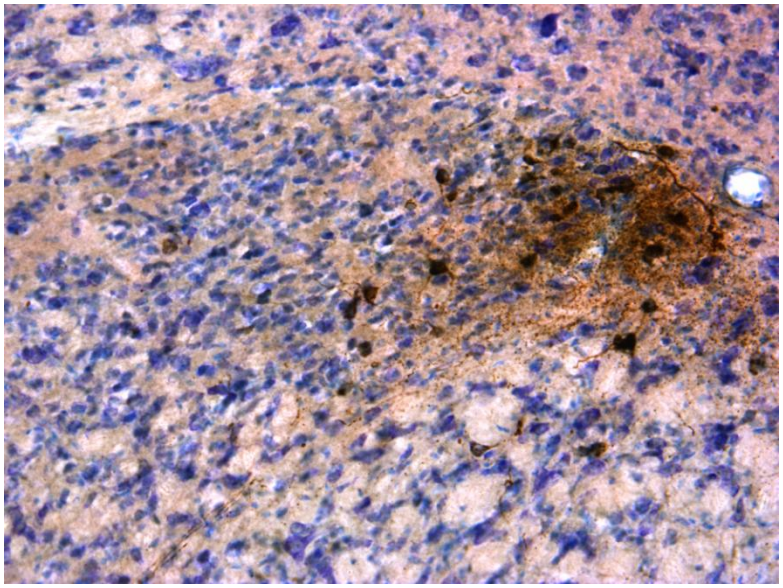


Figure 3-5A: Transported label throughout rostral NST, 20X

At a further caudal level, retrograde labeled cell bodies and anterograde labeled endings were present in the medial, ventral, and rostral central subdivisions of the rostral NST. The dorsal motor nucleus of the vagus nerve (10N) also contained retrograde labeled cells and anterograde labeled endings. Cell bodies and labeled endings were also visible in the prepositus nucleus (Pr) however were no longer present bilaterally. Endings and cell bodies were also observed in the medial vestibular nucleus (MVe), as well as the dorsal motor nucleus of the vagus nerve (10N). In the reticular formation, endings were only observed in the parvocellular reticular nucleus (PCRt).

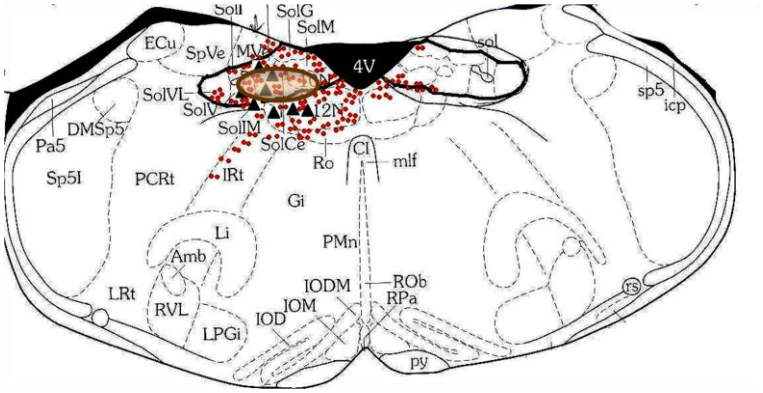


Figure 3-6: Transported label 0.25mm rostral to obex

Slightly caudal to the injection center, retrograde labeled cell bodies were visible within the ventral, central caudal, and ventral lateral subdivisions of the caudal NST, and anterograde labeled endings were visible in all subdivisions. Bilateral labeling of endings was visible within the medial and ventral subdivisions. Beyond the region of the NST, bilaterally labeled endings were also present in the dorsal motor nucleus of the vagus nerve (10N). Retrograde labeled cell bodies and heavy labeling of endings were also visible within the hypoglossal nucleus (12N). Within the reticular formation, the intermediate reticular nucleus (Irt) received anterograde labeled endings. Sparse anterograde labeling was present within the medial vestibular nucleus (MVe).

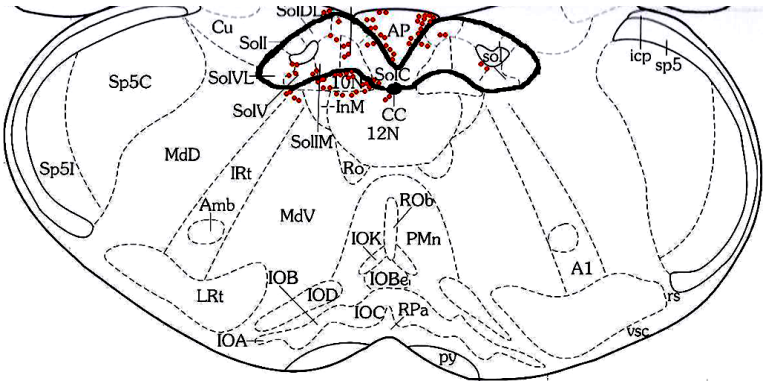


Figure 3-7: Transported label 0.12mm caudal to obex

Further caudal to the injection site, anterograde labeled endings continued to be visible within the commissural, medial, caudal central, ventral, and ventral lateral subdivisions of the caudal NST. Bilateral anterograde labeled endings were visible in the medial and ventral subdivisions. Heavy labeling of endings was also visible in the dorsal motor nucleus of the vagus nerve (10N), as well as the area postrema (AP). Sparse labeled endings were detected in the hypoglossal nucleus (12N), as well as the intermediate reticular nucleus (Irt) of the reticular formation.

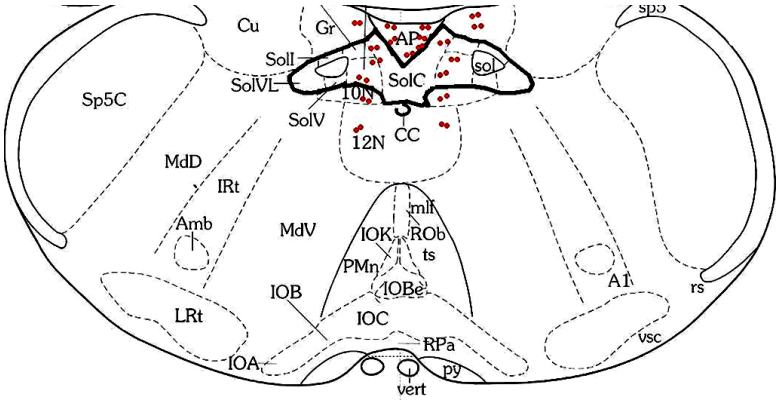


Figure 3-8: Transported label 0.48mm caudal to obex

At a further caudal level, bilateral anterograde labeled endings were present within the medial and central caudal subdivisions of the caudal NST. Heavier labeling of endings was visible within the area postrema (AP). Bilateral anterograde labeled endings were also visible

in the gracile nucleus (Gr) as well as the dorsal motor nucleus of the vagus nerve (10N). Anterograde labeled endings also became visible in the region of the hypoglossal nucleus (12N).

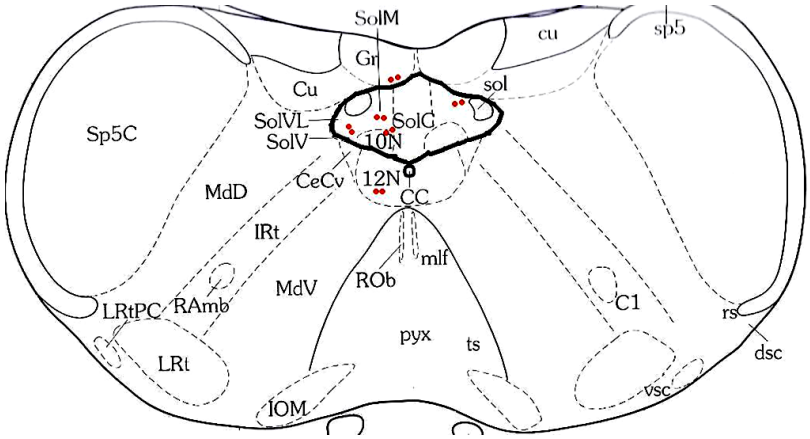


Figure 3-9: Transported label 0.72mm caudal to obex

Further caudally to the injection site, anterograde labeled endings were visible in the central caudal and caudal lateral subdivisions of the caudal NST. The labeled endings in the caudal lateral subdivision were present bilaterally. Anterograde labeled endings were also visible in the gracile nucleus (Gr), the dorsal motor nucleus of the vagus nerve (10N), and the hypoglossal nucleus (12N).

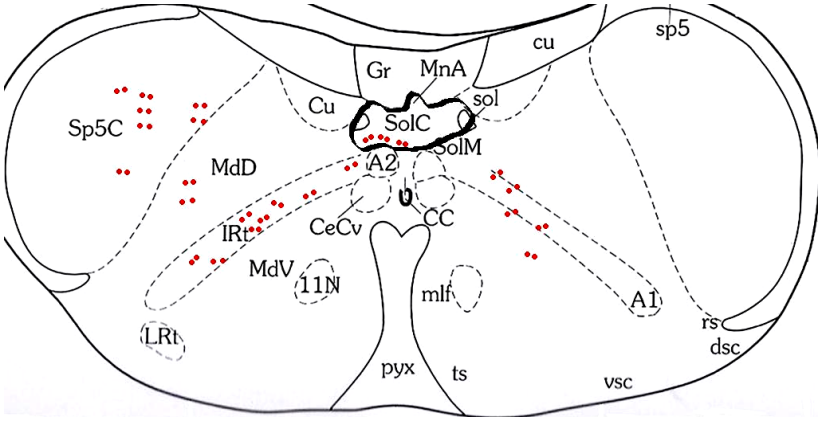
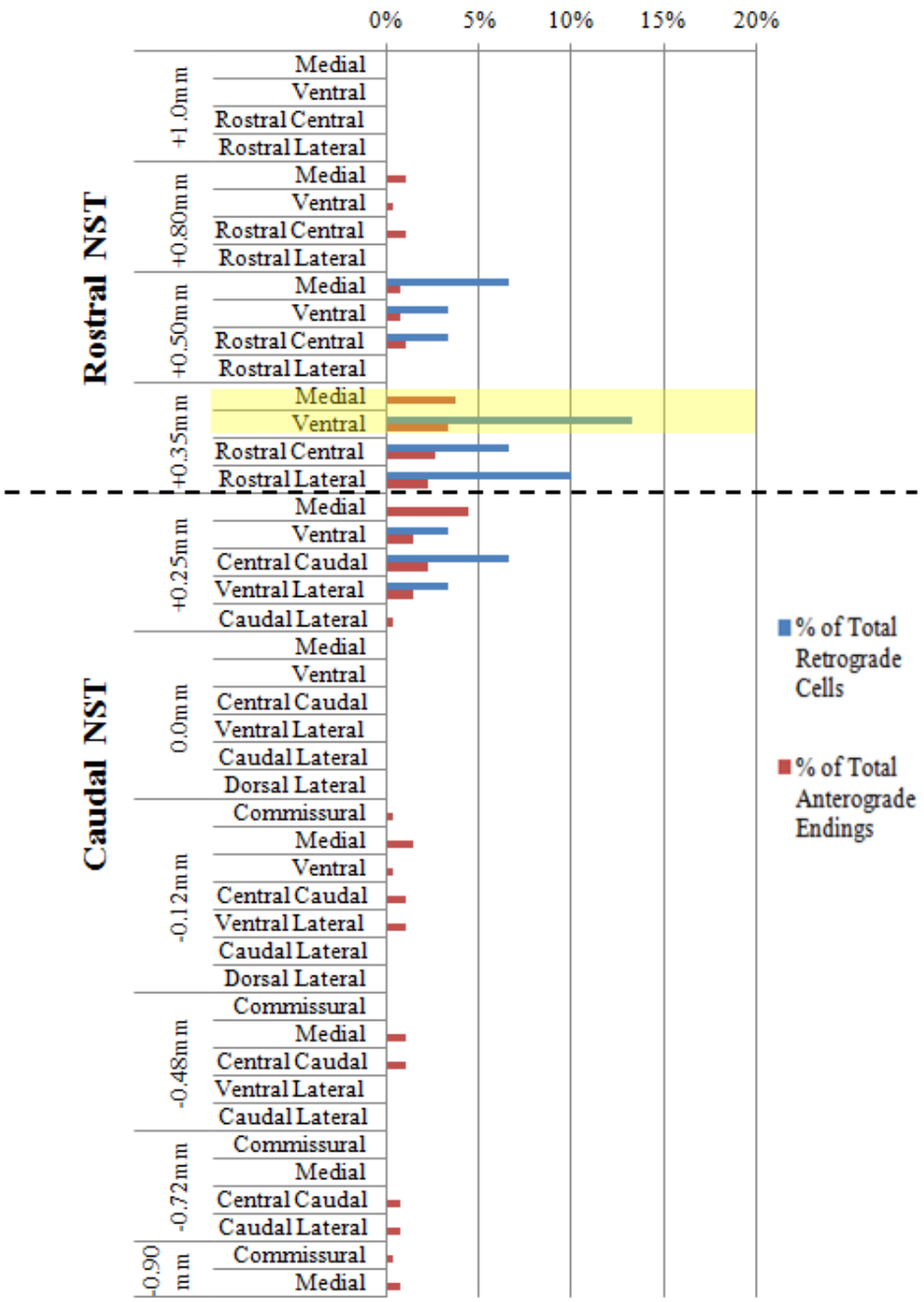


Figure 3-10: Transported label 0.90mm caudal to obex

At the furthest caudal level, anterograde labeled endings were still visible in the commissural, central caudal, and caudal lateral subdivisions of the caudal NST. Within the reticular formation, labeled endings continued to be visible within the intermediate reticular nucleus (Irt). Anterograde labeling was also present within the dorsal and ventral medullary reticular nuclei (MdD and MdV). Anterograde labeled endings were also present in the region of the spinal trigeminal nucleus (Sp5).

<u>Graph 3</u>: Summary of transported labeling in rostral and caudal NST, Case 3



The total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions is shown above. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla

Part B: Injections of the Caudal NST

Case 4 – Center of injection in the medial subdivision of the caudal NST

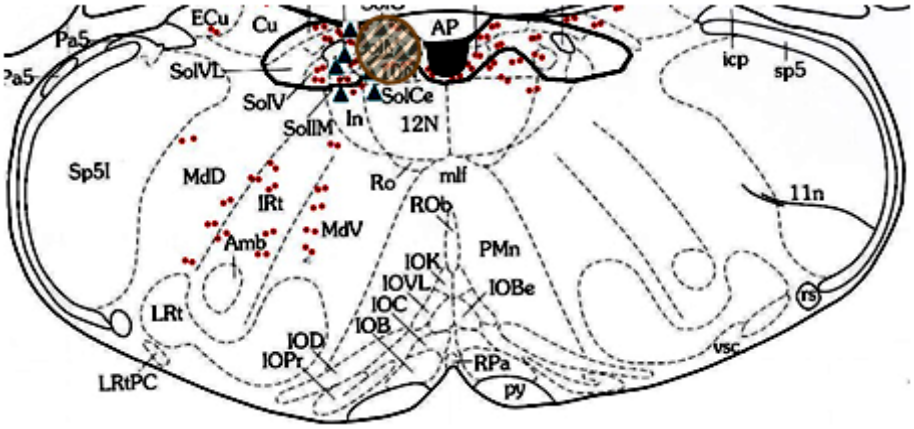
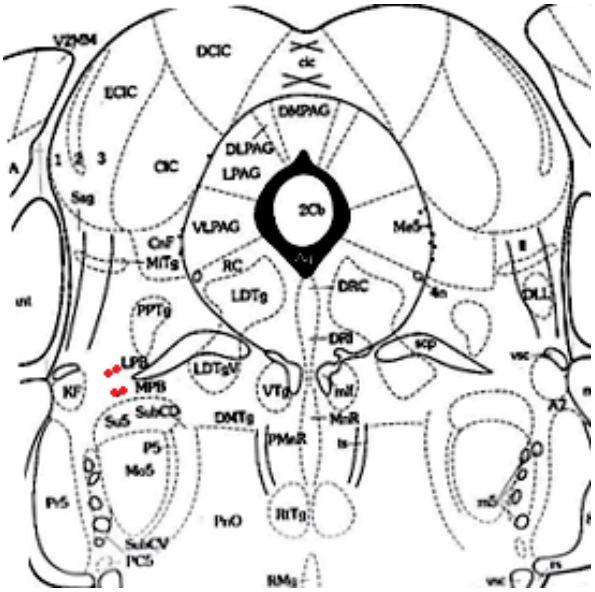


Figure 4-1: Site of injection center at the level of the obex

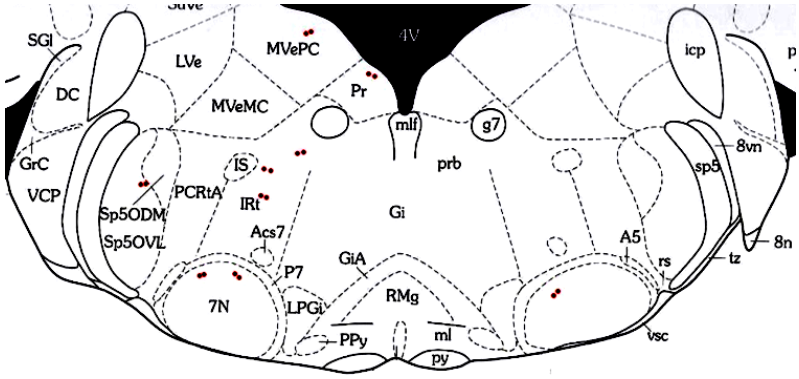
A large injection was made in the medial subdivision of the caudal NST and was positioned approximately 1mm rostral to the rostral pole of the area postrema, 250μm dorsoventrally, and 400μm lateral to the midline. At the center of the injection site, retrograde labeled cells and bilateral anterograde labeled endings were largely present in the medial, ventral, and central caudal subdivisions. Retrograde labeled cell bodies and sparse bilateral anterograde labeled endings were also present within the hypoglossal nucleus (12N) and the dorsal motor nucleus of the vagus (10N). The external cuneate nucleus (ECu) received few anterograde labeled endings. Regions of the reticular formation including the intermediate reticular nucleus (Irt) and the dorsal and ventral medullary reticular nuclei (MdD and MdV) also received anterograde labeled endings.

Coronal Series: Rostral to caudal sections of medulla containing label



<u>Figure 4-2</u>: Transport label 2.50mm rostral to the obex

Anterograde labeled endings were visible within the dorsolateral and ventromedial regions of the parabrachial nucleus (LPB and MPB).



<u>Figure 4-3</u>: Transported label 1.50mm rostral to obex

Further rostral to the level of the NST, bilateral anterograde labeled endings were visible in the facial motor nucleus (7N). Throughout the reticular formation, labeled endings were only visible within the intermediate reticular nucleus (Irt). Anterograde labeling was also visible within the prepositus nucleus (Pr) as well as the parvocellular medial vestibular

nucleus (MVePC). Regions of the spinal trigeminal nucleus also contained few anterograde labeled endings (Sp5).

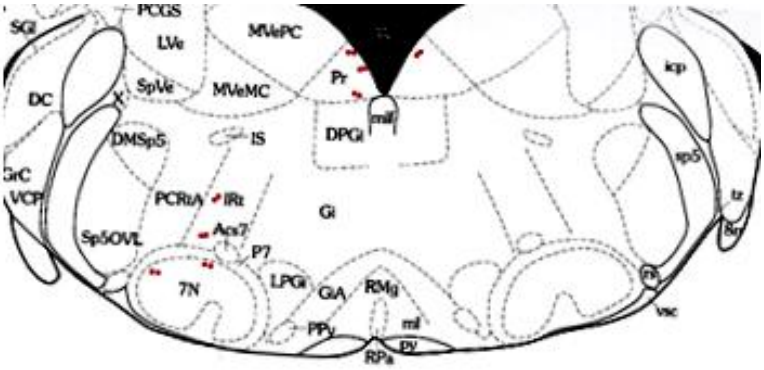


Figure 4-4: Transported label 1.25mm rostral to obex

Slightly further caudal, anterograde labeled endings remained visible in the facial nucleus (7N); however the labeling was no longer bilateral. Within the reticular formation, labeled endings were again visible within the intermediate reticular nucleus (Irt). Bilateral labeled endings were visible within the prepositus nucleus (Pr).

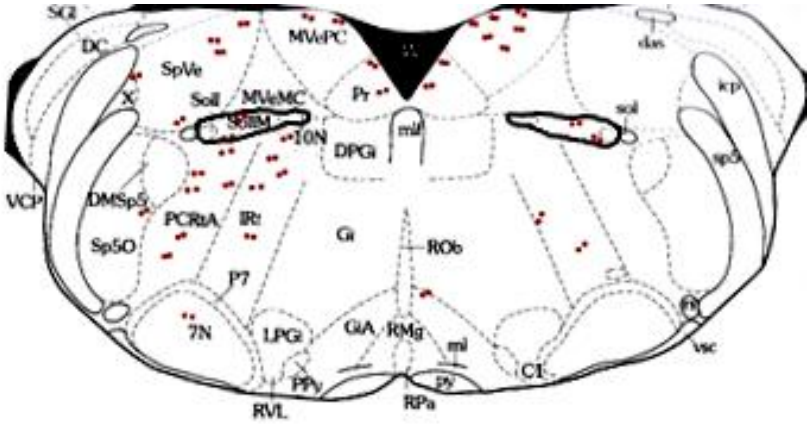


Figure 4-5: Transported label 1.0mm rostral to obex

At the level of the rostral NST, anterograde labeled endings were present bilaterally in the ventral and rostral central subdivisions. Heavy anterograde labeling was also present in

the reticular formation, within the parvocellular reticular nucleus (PCRt) and the intermediate reticular nucleus bilaterally (Irt). Bilateral anterograde labeled endings were also present in the prepositus nucleus (Pr), as well as the parvocellular medial vestibular nucleus (MVePC). Regions of the spinal vestibular nucleus (SpVe) and the spinal trigeminal nucleus (Sp5) also received anterograde labeled endings. Few anterograde labeled endings were also present in the facial motor nucleus (7N).

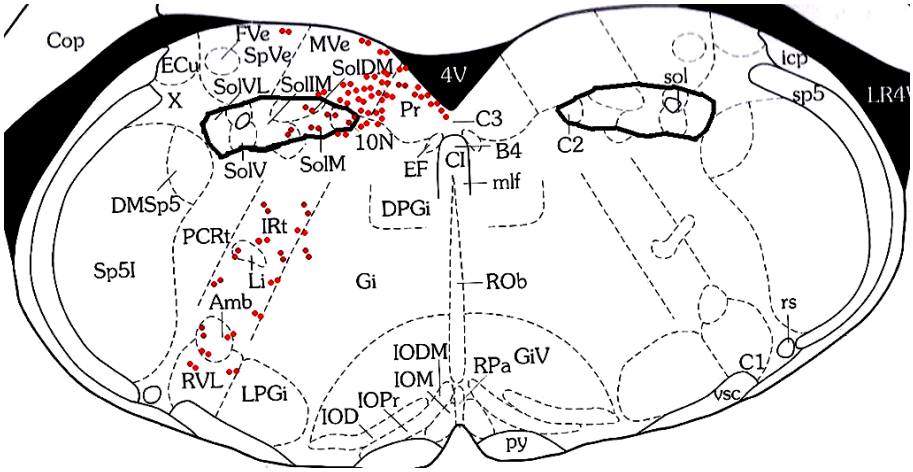


Figure 4-6: Transported label 0.50mm rostral to obex

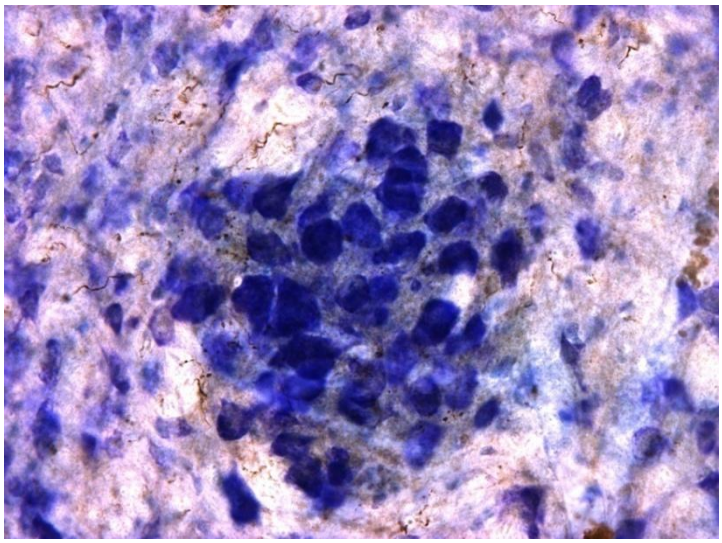


Figure 4-6A: Anterograde labeled endings in nucleus ambiguus, 40X

At a further caudal level, anterograde labeled endings were present within the medial, ventral, and rostral central subdivisions of the rostral NST. Heavy anterograde labeling was also visible within the prepositus and medial vestibular nuclei. Anterograde labeled endings were also present within the dorsal motor nucleus of the vagus nerve (10N), as well as the nucleus ambiguus (Amb). Sparsely labeled endings were present within the linear nucleus (Li) as well. Within the reticular formation, labeled endings were predominantly confined to

the intermediate reticular nucleus (Irt), and few were present within the gigantocellular nucleus (Gi).

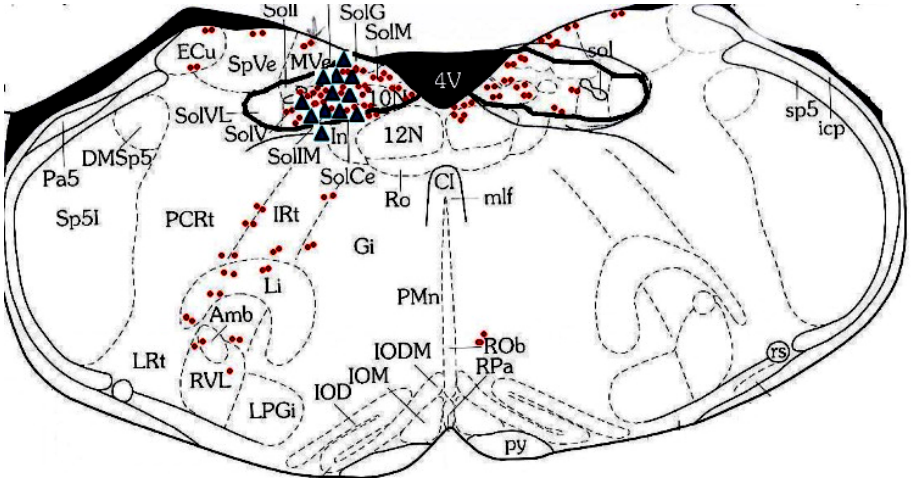
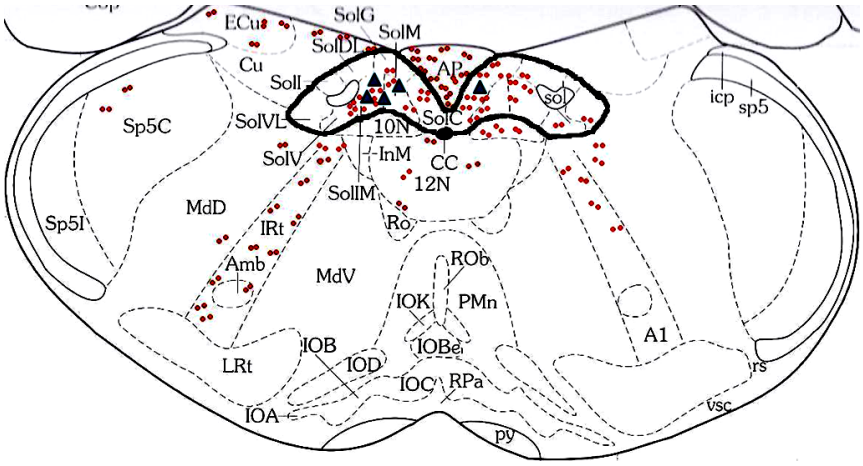


Figure 4-7: Transported label 0.25mm rostral to obex

Slightly rostral to the center of the injection site, heavy retrograde labeling of cell bodies as well as anterograde labeled endings were present within the medial, ventral, central caudal, and ventral lateral subdivisions of the caudal NST. Anterograde labeled endings were present bilaterally within the medial, ventral, and central caudal subdivisions. Outside the region of the NST, anterograde labeled endings were also found in the dorsal motor nucleus of the vagus nerve (10N), as well as the nucleus ambiguus (Amb) and the linear nucleus (Li). Anterograde labeled endings were also found bilaterally in regions of the vestibular nuclei as well as the external cuneate nucleus (ECu). Within the reticular formation, anterograde labeled endings were found along the border of the gigantocellular (Gi) nucleus and the intermediate reticular nucleus (Irt), as well as between the intermediate reticular nucleus and the parvocellular reticular nucleus (PCRt).



<u>Figure 4-8</u>: Transported label 0.12mm caudal to obex

Slightly caudal to the center of the injection site, retrograde labeled cell bodies were prominent in the medial, ventral, and central caudal subdivisions of the caudal NST. Labeled cell bodies were also present bilaterally in the medial subdivision. Anterograde labeled endings were visible in these subdivisions as well, and were also present bilaterally. Contralateral to the side of the injection site, few anterograde labeled endings were also visible in the ventral lateral and caudal lateral subdivisions. Beyond the region of the NST, anterograde labeled endings were also present within the cuneate nucleus (Cu) and the external cuneate nucleus (ECu). The area postrema (AP) also received heavy anterograde labeling of axonal endings. Contralateral to the injection site, anterograde labeled endings were present within the dorsal motor nucleus of the vagus nerve (10N). Sparse anterograde labeling of endings was also visible within the hypoglossal nucleus (12N), the nucleus ambiguus (Amb), and the spinal trigeminal nucleus (Sp5). Throughout the reticular formation, bilateral anterograde labeling of endings was visible within the intermediate reticular nucleus (Irt) as well as the dorsal medullary reticular nucleus (MdD).

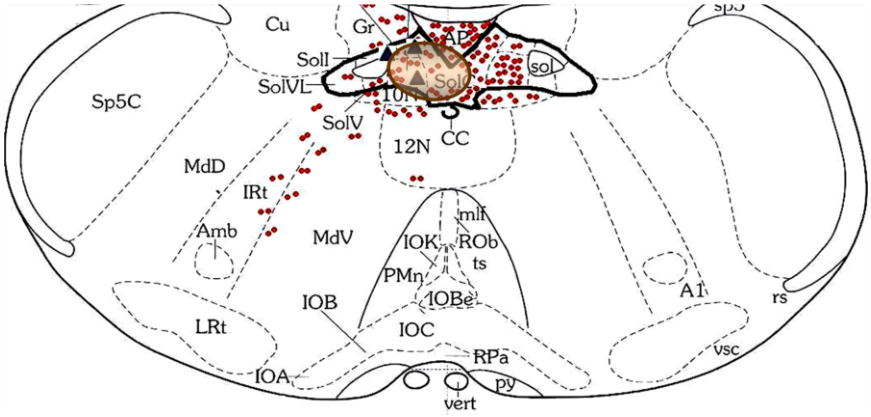


Figure 4-9: Transported label 0.48mm caudal to obex

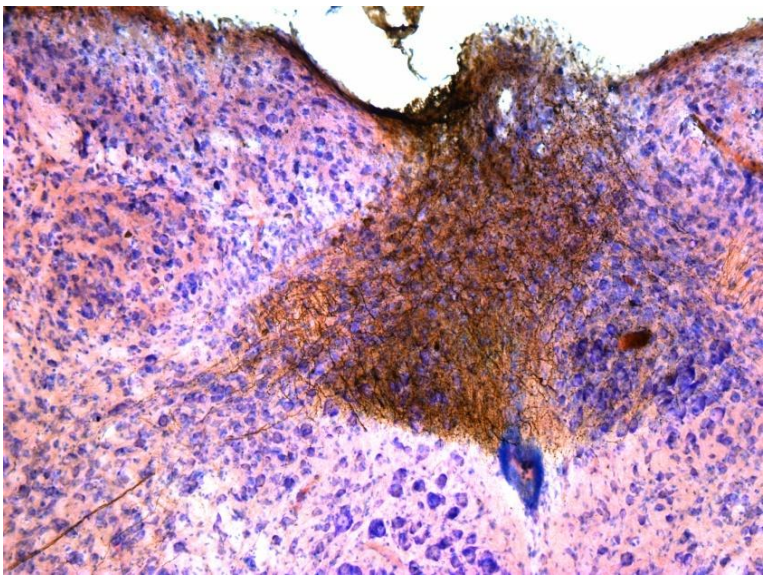


Figure 4-9A: Anterograde labeled endings in area postrema, 10X

Further caudal to the injection site, anterograde labeled endings were present within all subdivisions of the caudal NST and especially within the medial subdivision. Endings within the NST were labeled bilaterally throughout the commissural, medial, and central caudal subdivisions. Bilateral anterograde labeling of endings was also heavy in the dorsal motor nucleus of the vagus nerve (10N). Labeled endings were also present within the area postrema (AP), the hypoglossal nucleus (12N) and the gracile nucleus (Gr). Throughout the reticular formation, labeled endings were visible within the intermediate reticular nucleus (Irt)

and the ventral medullary reticular nucleus (MdV).

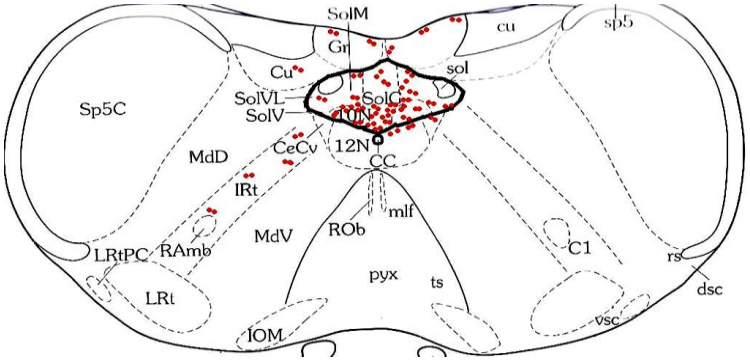


Figure 4-10: Transported label 0.72mm caudal to obex

At a slightly further caudal level, anterograde labeled endings continued to be visible within all subdivisions of the caudal NST, and especially within the commissural subdivision. The gracile (Gr) and cuneate (Cu) nuclei also received labeled endings, as well as the intermediate reticular nucleus (Irt) of the reticular formation.

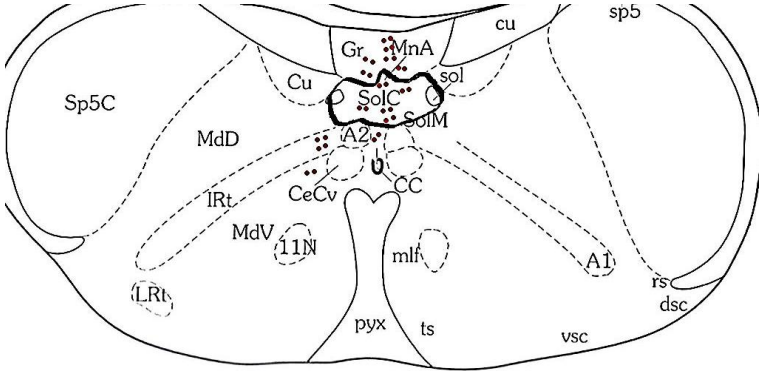
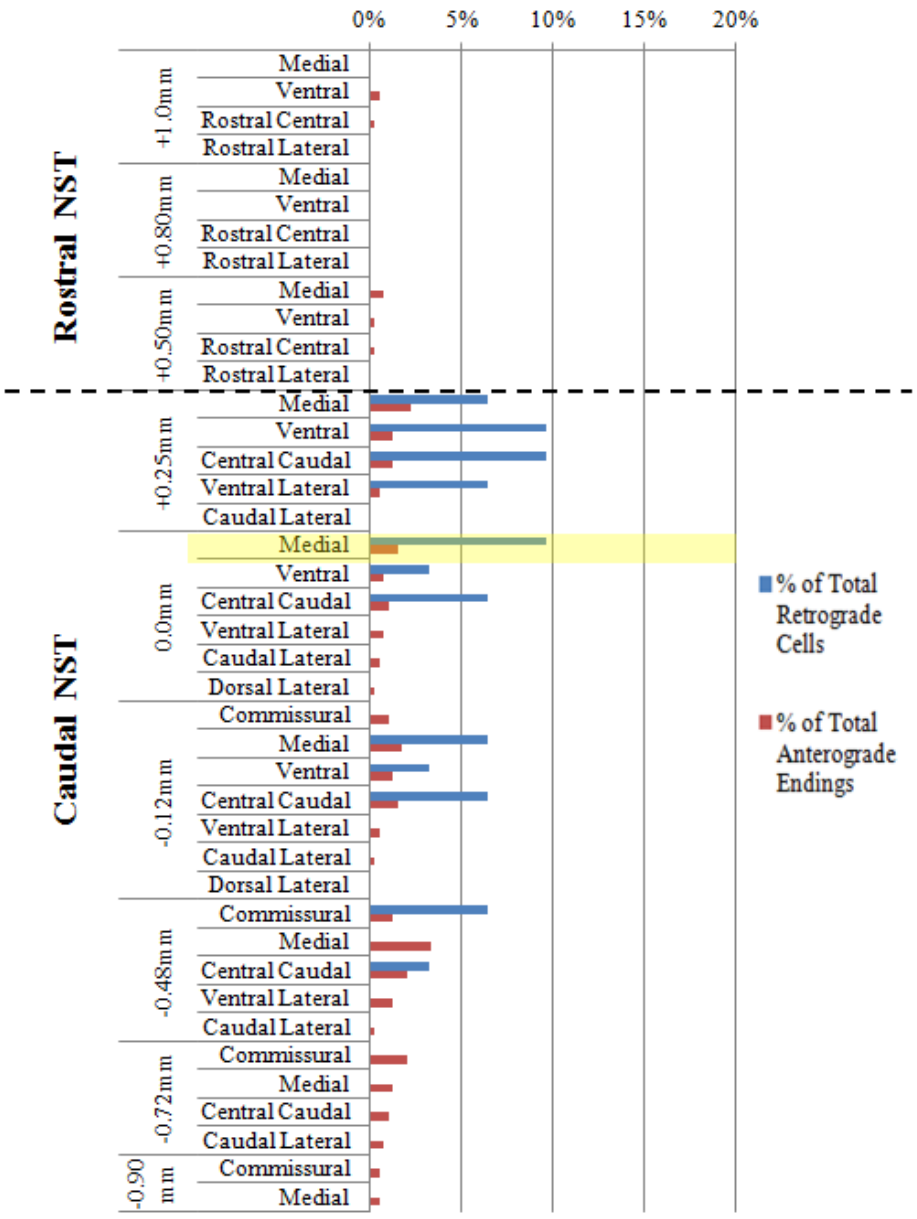


Figure 4-11: Transported label 0.90mm caudal to obex

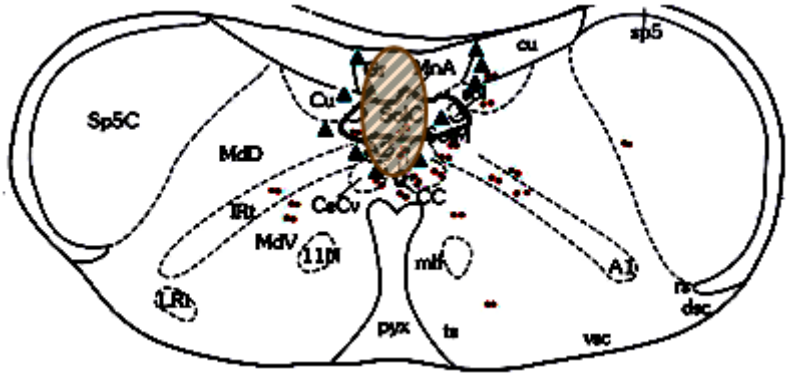
At the level furthest caudal to the injection site, anterograde labeled endings remained visible within the commissural and medial subdivisions of the NST. Labeled endings were also present within the gracile nucleus (Gr). The intermediate reticular nucleus (Irt) and the ventral medullary reticular nuclei (MdV) of the reticular formation also received anterograde labeled endings.

Graph 4: Summary of transported labeling in rostral and caudal NST, Case 4



The chart above illustrates the total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

Case 5 – Center of Injection in the commissural subdivision of the caudal NST



<u>Figure 5-1</u>: Site of injection center at 0.90mm caudal to obex

A large injection was made in the commissural subdivision of the caudal NST. The injection was positioned at the midline and 260μm dorsoventrally. Retrograde labeled cell bodies and anterograde labeled endings in the caudal NST were largely present within the commissural, medial, and caudal lateral subdivisions. Heavier retrograde labeling of cell bodies was present within the gracile nucleus (Gr) bilaterally. Retrograde labeled cell bodies in the gracile nucleus only were detected following the injection of the commissural subdivision of the caudal NST. Limited studies exist regarding possible projections from the NST to the gracile nucleus in the mouse model. Investigations of the gracile nucleus in the cat nervous system noted the existence of cytologically different subdivisions including rostral, middle, and caudal regions characteristic of the nucleus (Berkley and Hand, 1978). The lack of homogeneity in the gracile nucleus may also exist in the mouse nervous system, and this may explain the different labeling patterns resulting from injections of the NST. Retrograde labeled cell bodies and anterograde labeled endings continued to be visible in the intermediate and ventral medullary reticular nuclei of the reticular formation.

Coronal Series: Rostral to caudal sections of medulla containing label

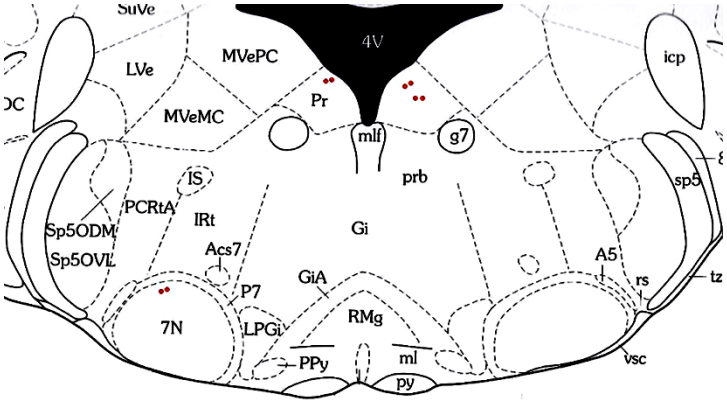


Figure 5-2: Transported label 1.50mm rostral to obex

Sparse endings within the facial motor nucleus (7N) were detected at a level further rostral to the injection center. Bilateral anterograde labeled endings were visible within the prepositus nucleus (Pr).

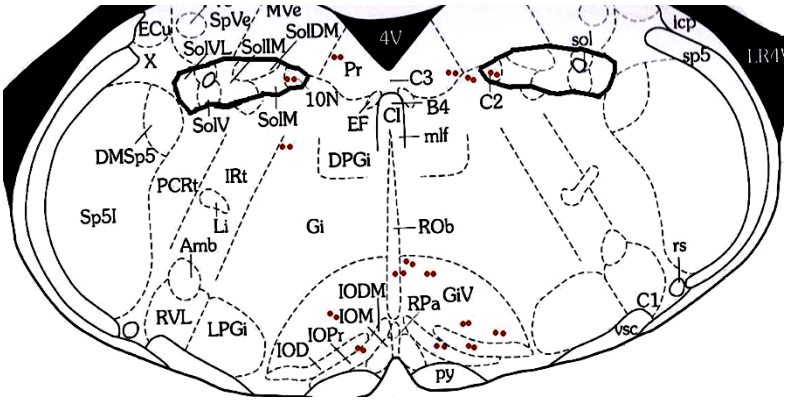


Figure 5-3: Transported label 0.50mm rostral to obex

At the level of the rostral NST, bilateral anterograde labeled endings were visible within the medial subdivision. Bilaterally labeled endings were also visible within the prepositus nucleus (Pr), as well as within the inferior olivary nucleus (IO). Sparse labeled endings were also visible bilaterally within the gigantocellular reticular nucleus (Gi).

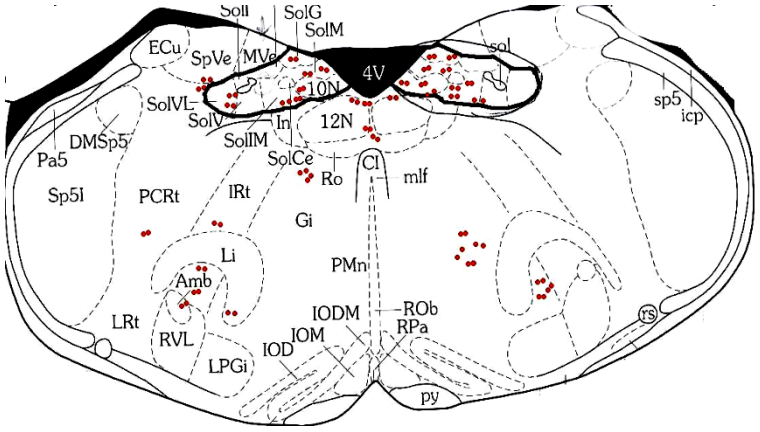


Figure 5-4: Transported label 0.25mm rostral to obex

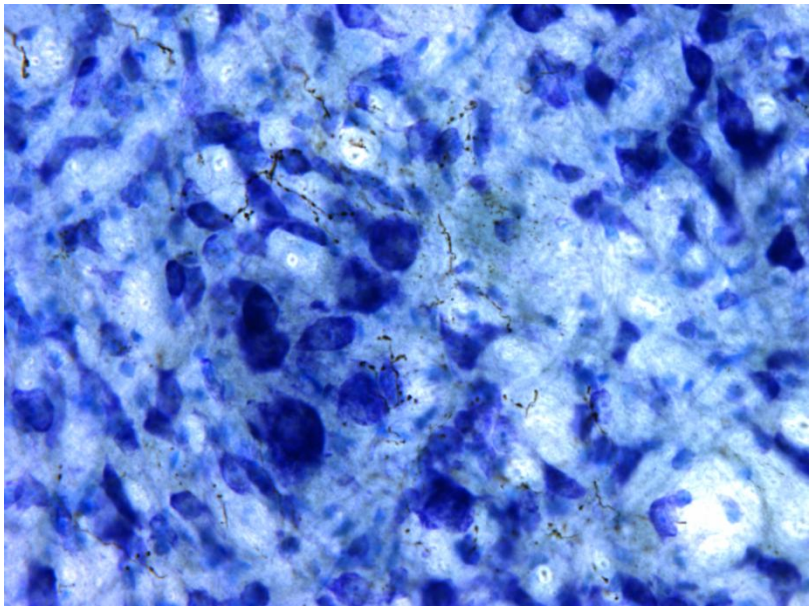


Figure 5-4A: Anterograde labeled endings in nucleus ambiguus, 40X

At a further caudal level, bilateral anterograde labeled endings were visible in all subdivisions of the caudal NST, as well as the dorsal motor nucleus of the vagus nerve (10N) and the hypoglossal nucleus (12N). Few anterograde labeled endings were also visible within the spinal vestibular nucleus (SpVe). Within the reticular formation, anterograde labeled endings were visible within the gigantocellular nucleus (Gi) bilaterally, as well as the intermediate reticular nucleus (Irt) and the parvocellular reticular nucleus (PCRt). Labeled endings were also visible within the linear nucleus (Li) and the nucleus ambiguus (Amb).

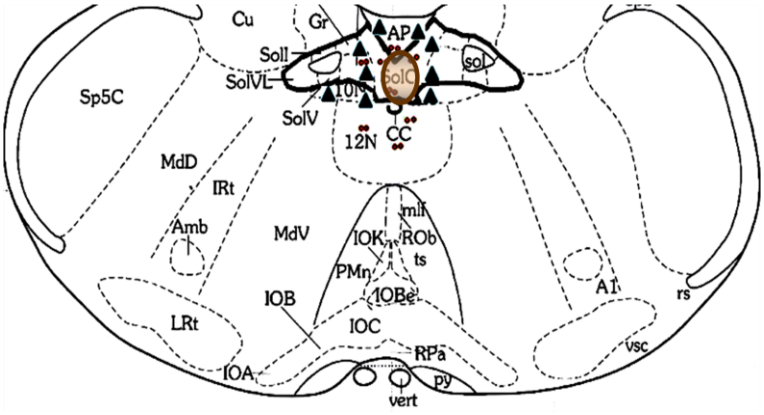


Figure 5-5: Transported label 0.48mm caudal to obex

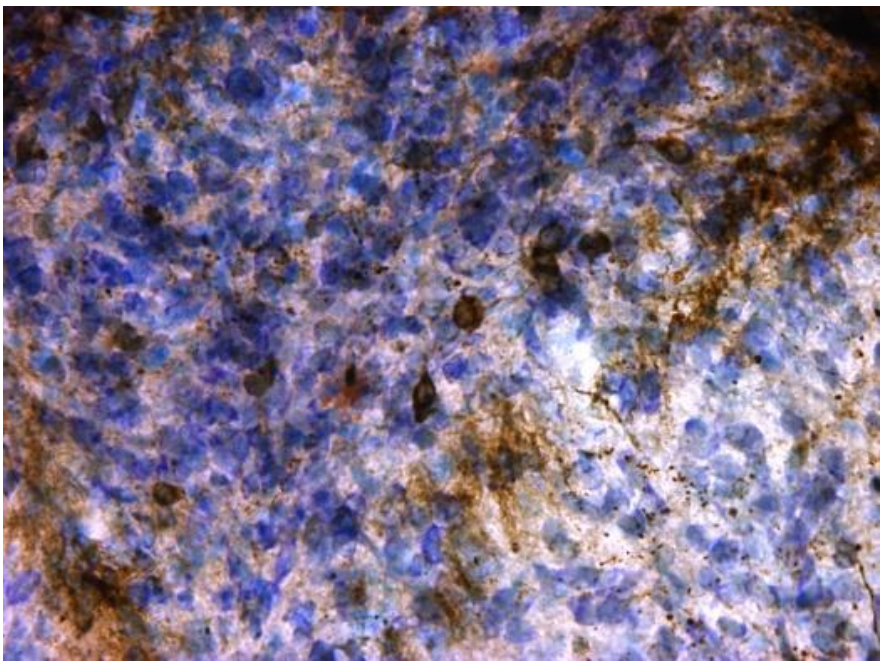


Figure 5-5A: Retrograde labeled cell bodies in area postrema, 40X

At a level further caudal, retrograde labeled cell bodies were present bilaterally within the commissural and medial subdivisions of the caudal NST. Anterograde labeled endings were present in the commissural, medial, and caudal central subdivisions. Retrograde cell bodies were also heavily labeled within the area postrema (AP) as well as the dorsal motor nucleus of the vagus nerve (10N) bilaterally. The hypoglossal nucleus (12N) also received anterograde labeled endings.

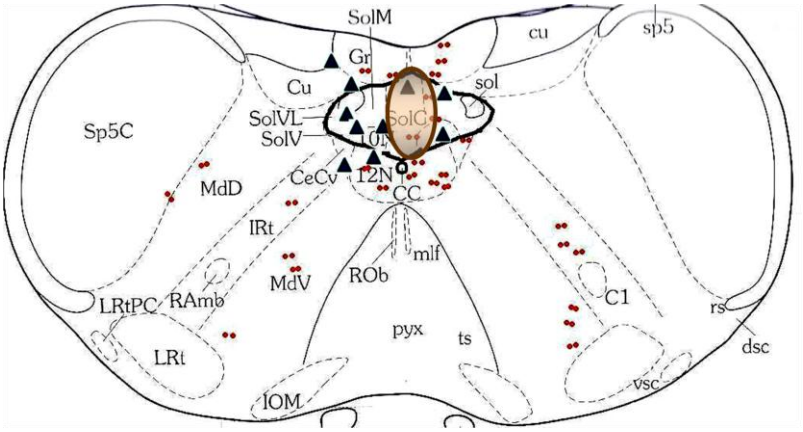
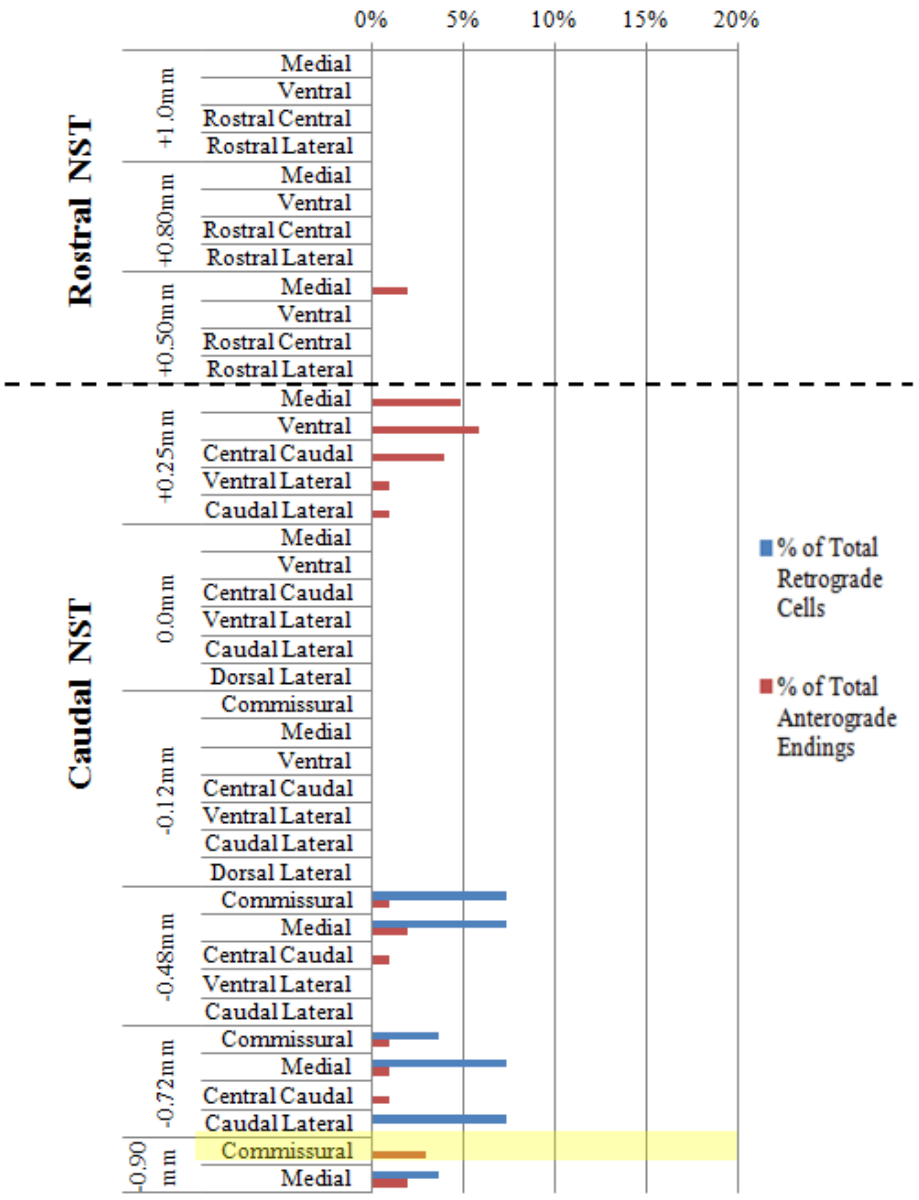


Figure 5-6: Transported label 0.72mm caudal to obex

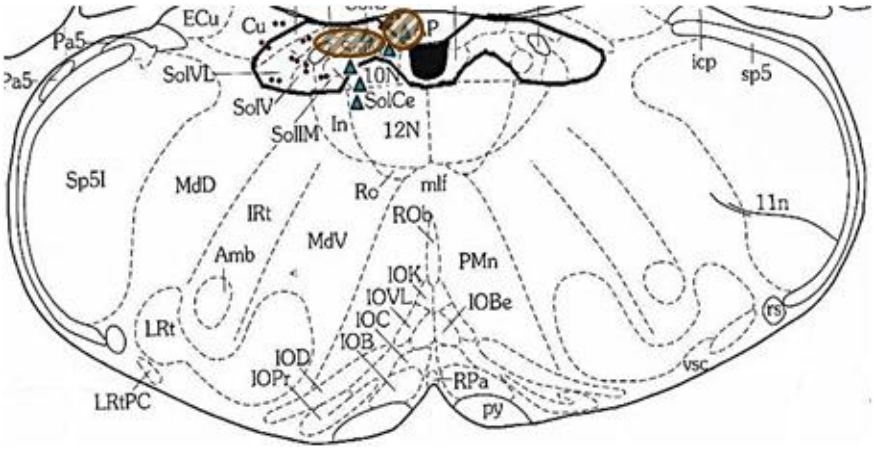
Slightly rostral to the injection center, retrograde labeled cell bodies and anterograde labeled endings were present within the commissural, medial, and caudal lateral subdivisions. Labeled cell bodies and endings were also visible within the dorsal motor nucleus of the vagus nerve (10N) and the hypoglossal nucleus (12N). The gracile (Gr) and cuneate (Cu) nuclei also received retrograde labeled cell bodies, and the gracile nucleus received anterograde labeled endings bilaterally. Within the reticular formation, anterograde labeled endings were present within the intermediate reticular nucleus (Irt) and the ventral medullary reticular nucleus (MdV) bilaterally as well as the dorsal medullary reticular nucleus (MdD).

Graph 5: Summary of transported labeling in rostral and caudal NST, Case 5



The total distributions of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions are displayed above. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

Case 6– Center of injection at the central caudal subdivision of the caudal NST and the area postrema partially



<u>Figure 6-1</u>: Site of injection center at the level of the obex

A small injection was made in the caudal NST and included regions of the area postrema. The injection was positioned approximately 1mm rostral to the rostral pole of the area postrema, 250μm lateral to the midline, and 250μm dorsoventrally. Anterograde labeled endings in the caudal NST were not extremely heavy but were present in all subdivisions. Retrograde labeled cell bodies were present within the medial and central caudal subdivisions. The dorsal motor nucleus of the vagus nerve (10N) also received retrogradely labeled cell bodies. The cuneate nucleus (Cu) also received anterograde labeled endings. The area postrema (AP) also received retrograde labeled cell bodies and anterograde labeled endings.

Coronal Series: Rostral to Caudal sections of medulla containing label

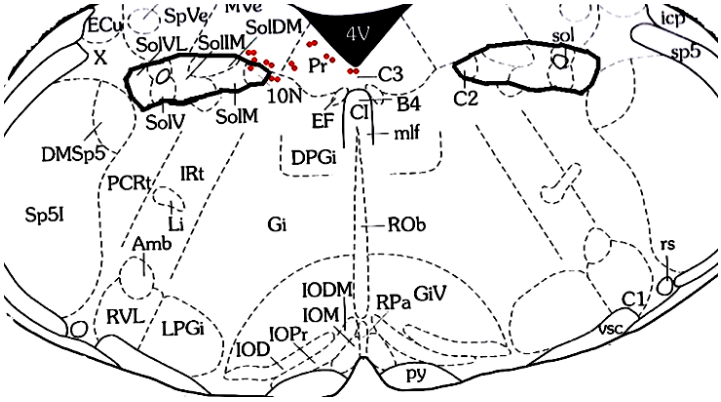


Figure 6-2: Transported label 0.50mm rostral to obex

At the level of the rostral NST, few anterograde labeled endings were present within the medial subdivision. Anterograde labeled endings were also visible in the dorsal motor nucleus of the vagus nerve (10N), the prepositus nucleus (Pr), and the medial vestibular nucleus (MdV).

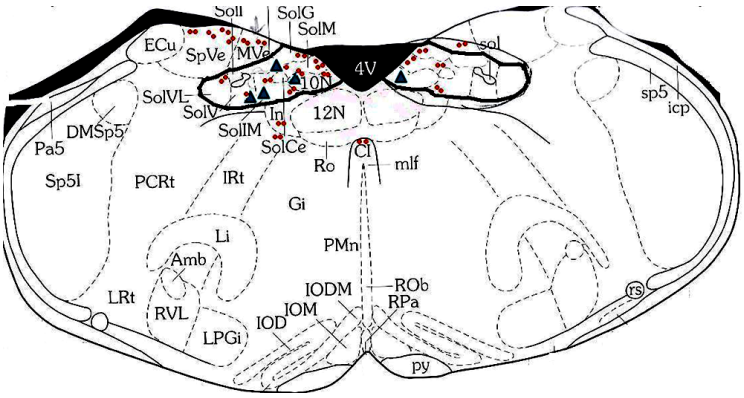


Figure 6-3: Transported label 0.25mm rostral to obex

Slightly further caudal, anterograde labeled endings were visible within all subdivisions of the caudal NST, and were present bilaterally within the medial, central caudal, and ventral subdivisions. Retrograde labeled cell bodies were present within the medial, ventral, central caudal, and ventral lateral subdivisions, and bilateral labeling of cell bodies was present only within the medial subdivision. Anterograde labeled endings were also

visible within the medial and spinal vestibular nuclei (MVe and SpVe), and sparsely distributed throughout the reticular formation.

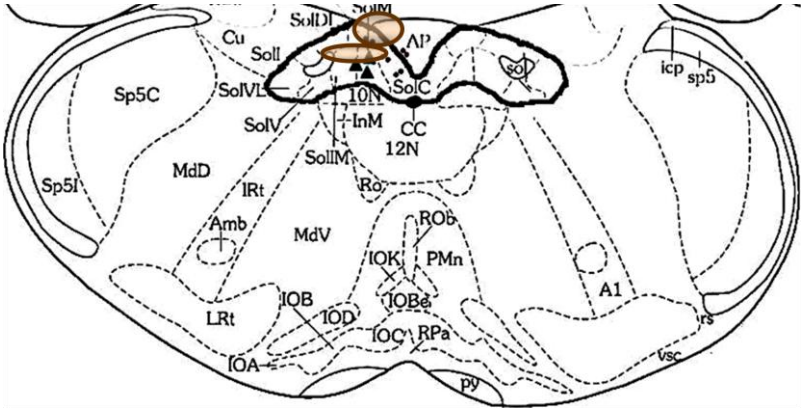


Figure 6-4: Transported label 0.12mm caudal to the obex

Further caudal to the injection site, retrograde labeled cell bodies were visible within the central caudal subdivision of the caudal NST, and anterograde labeled endings were visible within the medial subdivision. A few anterograde labeled endings were also visible in the area postrema (AP) at this level.

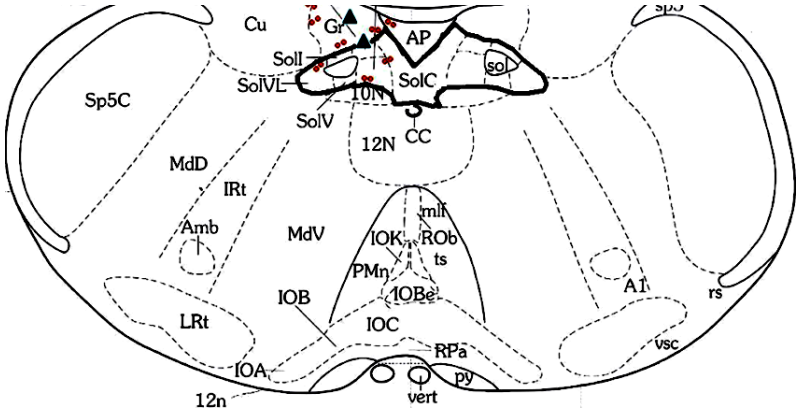


Figure 6-5: Transported label 0.48mm caudal to the obex

Further caudal to the injection site, anterograde labeled endings were present in the medial, central caudal, and caudal lateral subdivisions of the caudal NST. Retrograde labeled cell bodies and anterograde labeled endings were present in the gracile nucleus (Gr) at this

level. Anterograde labeled endings within the area postrema were also visible.

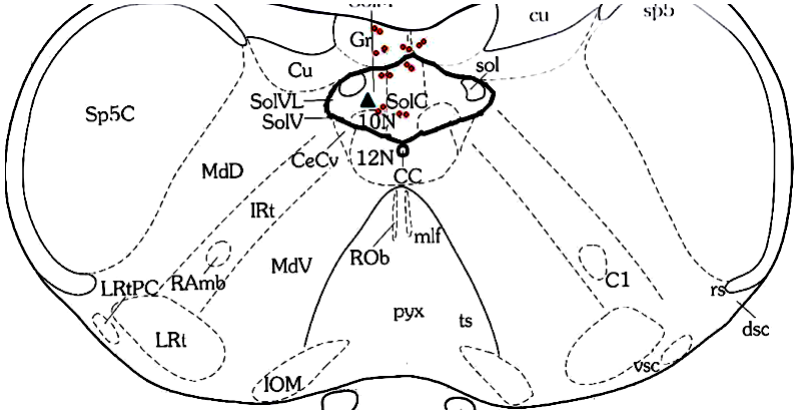
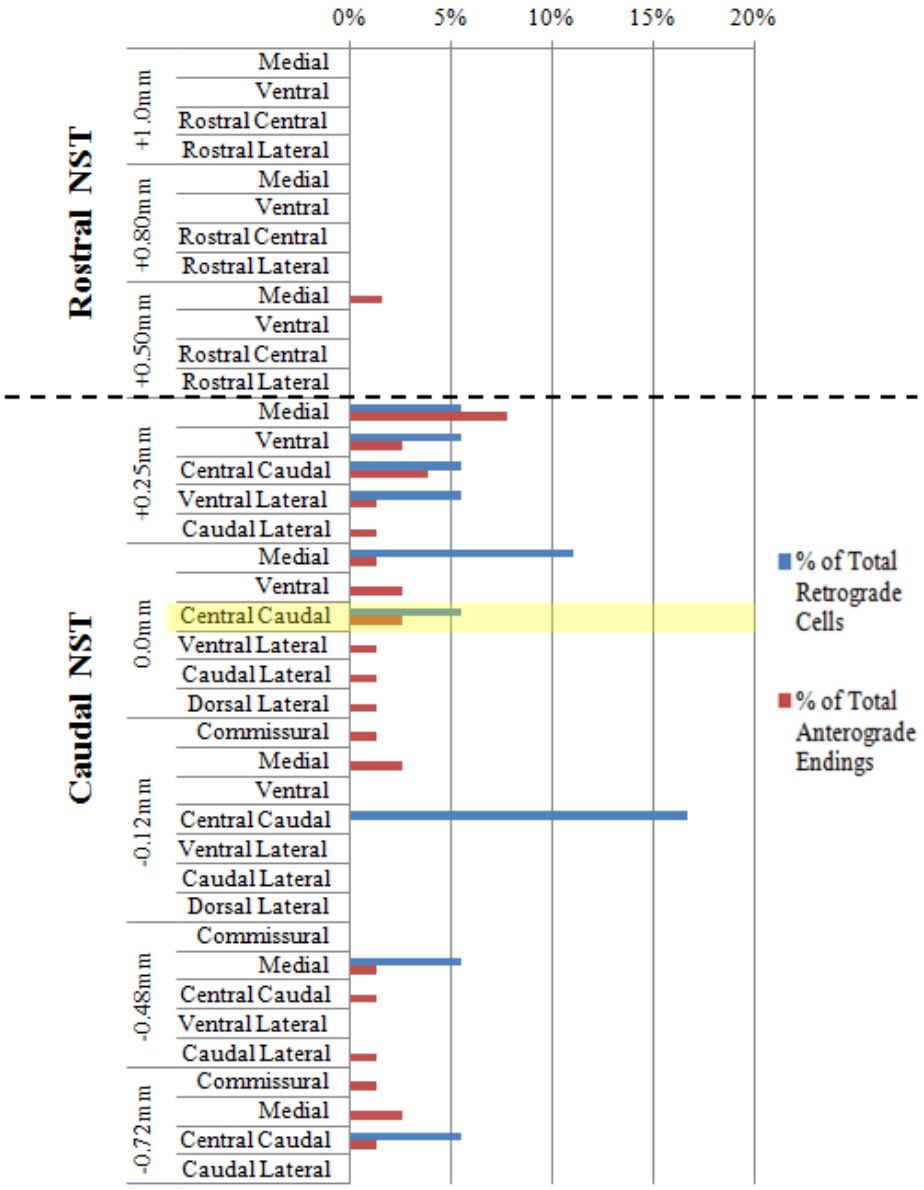


Figure 6-6: Transported label 0.72mm caudal to obex

At a further caudal level of the NST, anterograde labeled endings were present within the commissural, medial, and central caudal subdivisions. Retrograde labeled cell bodies were visible within the central caudal subdivision. The gracile nucleus (Gr) also received anterograde labeled endings.

Graph 6: Summary of transported labeling in rostral and caudal NST, Case 6



The figure above shows the total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

Part C: Injection of the Area Postrema

Case 7 – Center of injection at the area postrema

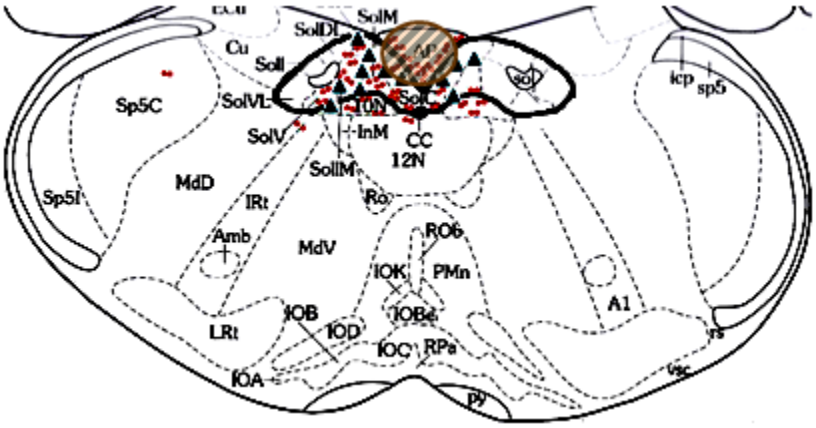


Figure 7-1: Site of injection center at 0.12mm caudal to obex

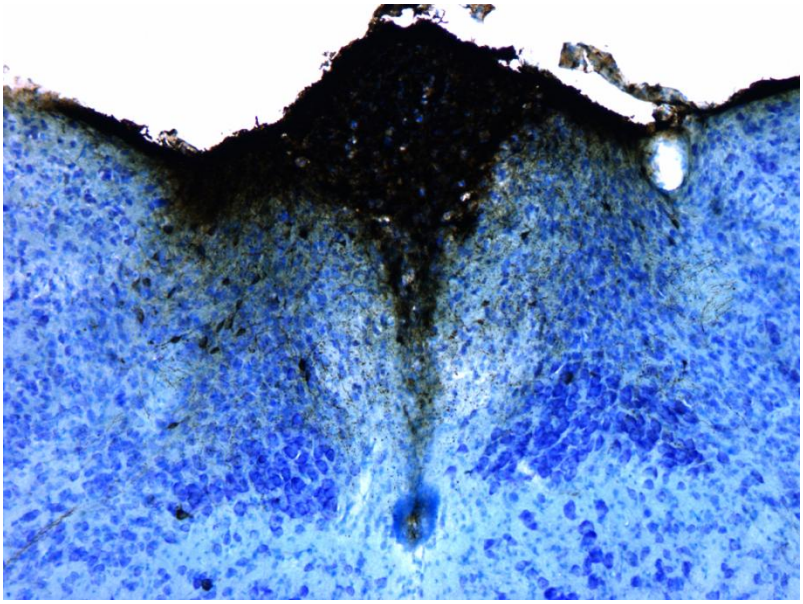
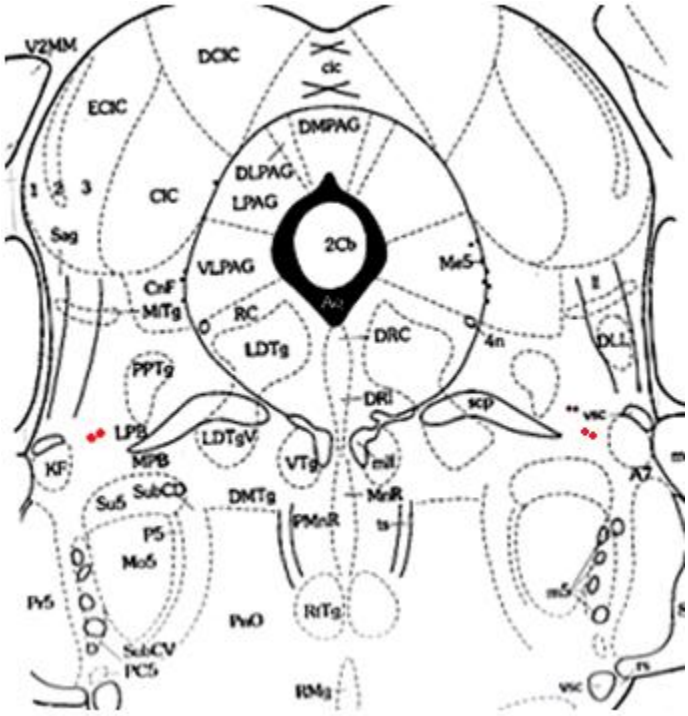


Figure 7-1A: Micrograph of center of injection site, 10X

A small injection was made into the area postrema on the midline and approximately 50µm dorsoventrally. Retrograde labeled cells and anterograde labeled endings in the NST were largely confined to the commissural, medial, central caudal, and ventral subdivisions of

the caudal NST. Bilateral labeling of cell bodies occurred in the commissural and medial subdivisions. Anterograde labeled endings were also highly visible within the dorsal motor nucleus of the vagus nerve (10N) and the spinal trigeminal nucleus. Sparsely labeled endings were visible in the dorsal medullary reticular nucleus (MdD) of the reticular formation.

Coronal Series: Rostral to caudal sections of medulla containing label



<u>Figure 7-2</u>: Transported label 2.50mm rostral to obex

Faint anterograde labeled endings were visible bilaterally within the dorsolateral region of the parabrachial nucleus.

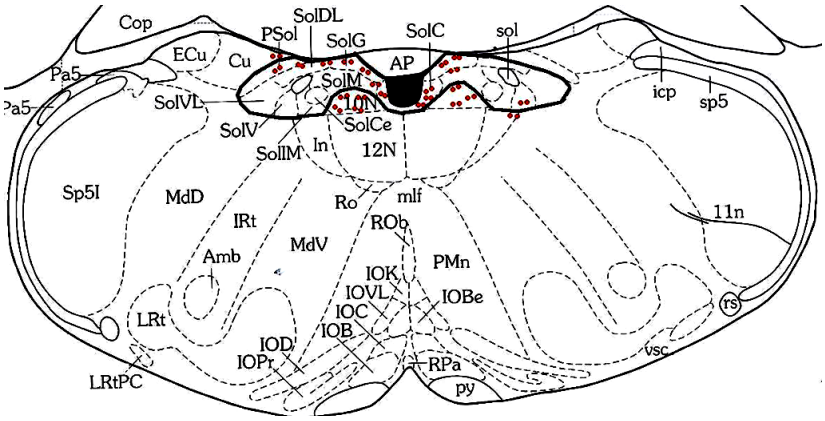


Figure 7-3: Transported label at level of obex

Slightly rostral to the center of the injection site, anterograde labeled endings were visible within the medial subdivision bilaterally, the caudal lateral and dorsal lateral subdivisions, and the ventral lateral subdivision. Labeled endings were also highly distributed throughout the dorsal motor nucleus of the vagus nerve (10N) bilaterally.

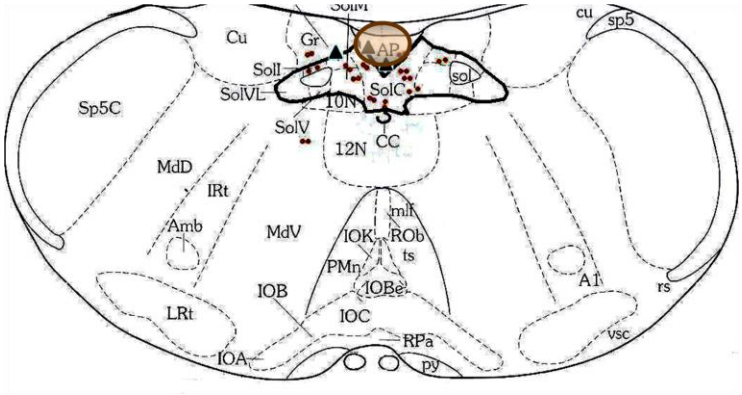


Figure 7-4: Transported label 0.48mm caudal to obex

Slightly caudal to the injection site, anterograde labeled endings remained visible in the commissural, medial, and central caudal subdivisions, but were also present within the caudal lateral subdivision of the caudal NST. Few retrograde labeled cell bodies were visible within the medial subdivision. The area postrema (AP) received retrograde labeled cell bodies as well. Few anterograde labeled endings were present in the gracile nucleus (Gr) and the ventral medullary nucleus (MdV) of the reticular formation.

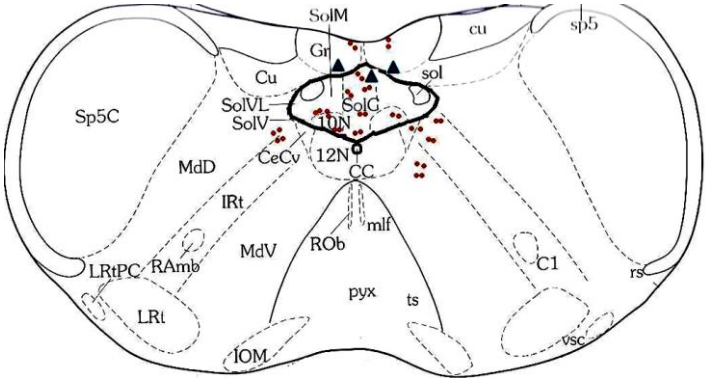


Figure 7-5: Transported label 0.72mm caudal to obex

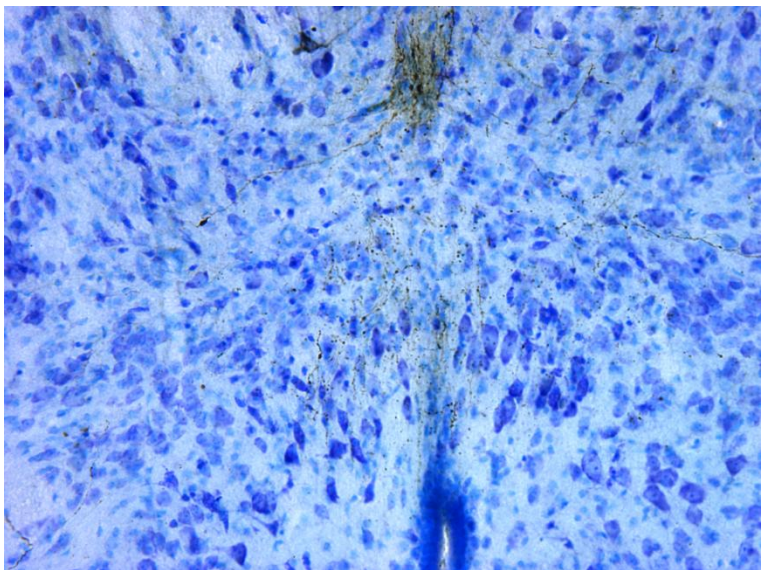


Figure 7-5A: Anterograde labeled endings in commissural NST

Further caudal to the center of the injection, few retrograde labeled cell bodies were visible in the commissural subdivision of the caudal NST. Anterograde labeled endings remained visible within the medial, commissural, and central caudal subdivisions. The dorsal motor nucleus of the vagus nerve (10N) also received anterograde labeled endings. Retrograde labeled cell bodies and anterograde labeled endings were present within the gracile nucleus (Gr) bilaterally. Within the reticular formation, labeled endings were visible within the ventral and dorsal medullary reticular nuclei (MdD and MdV) as well as the intermediate reticular nucleus bilaterally (Irt).

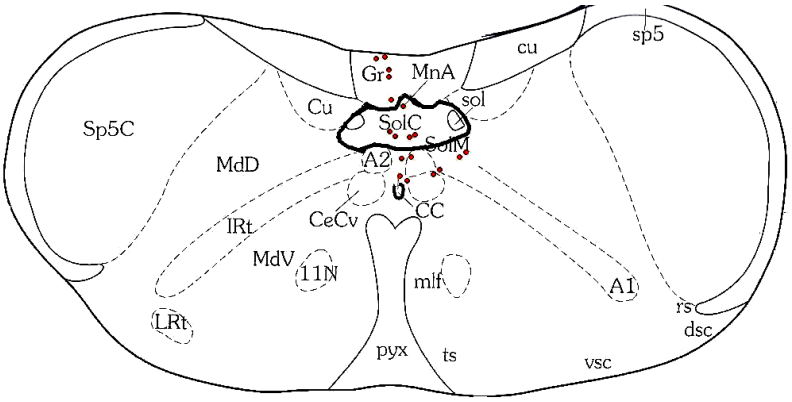
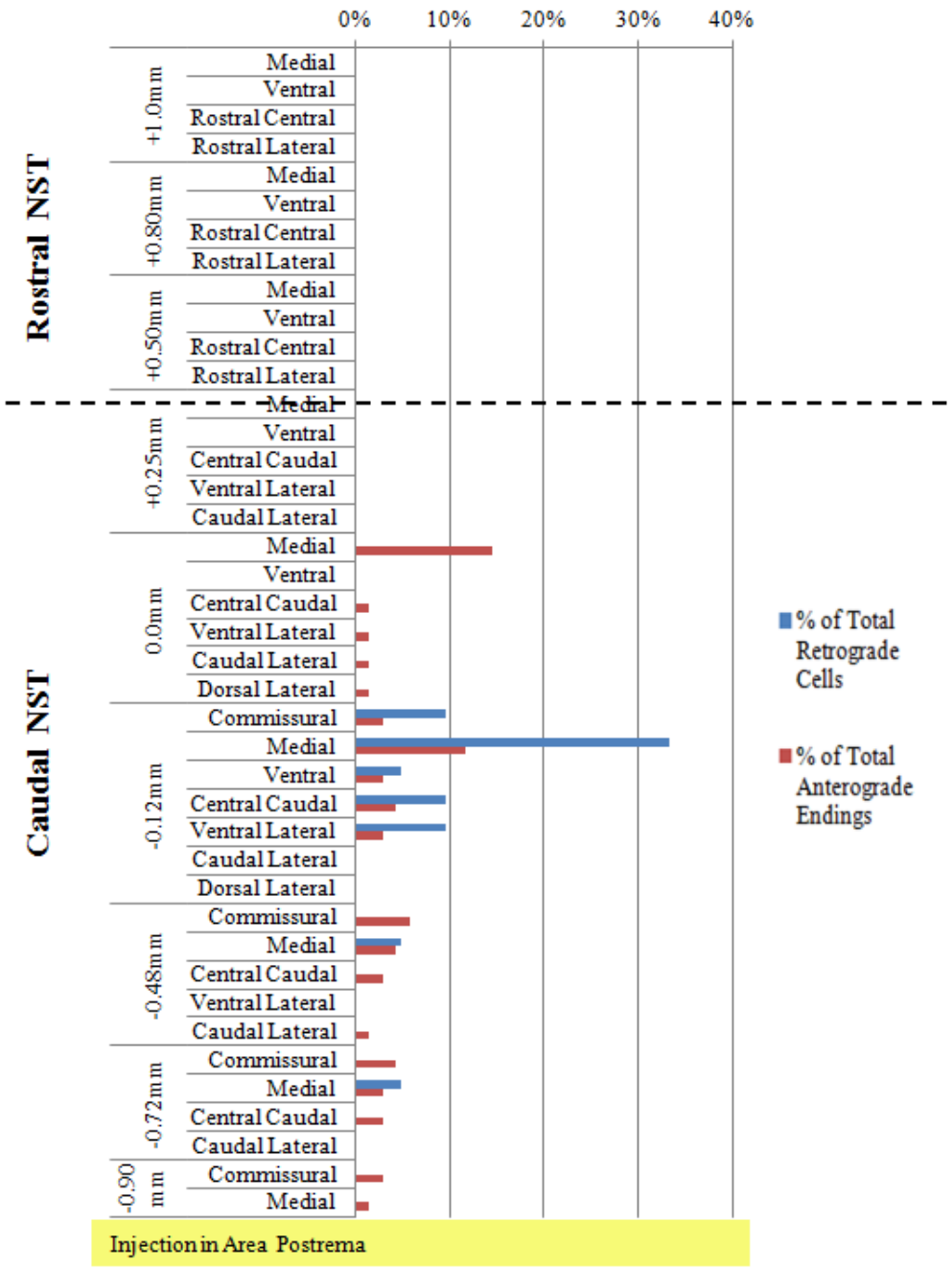


Figure 7-6: Transported label 0.90mm caudal to obex

At the furthest caudal level, the commissural subdivision received anterograde labeled endings within the caudal NST. Additional regions receiving labeled endings included the gracile nucleus (Gr) and the intermediate reticular nucleus (Irt).

Graph 7: Summary of transported labeling in rostral and caudal NST, Case 7



The chart above illustrates the total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

Part D: Injections of the Reticular Formation

Case 8 – Center of Injection in the Reticular Formation

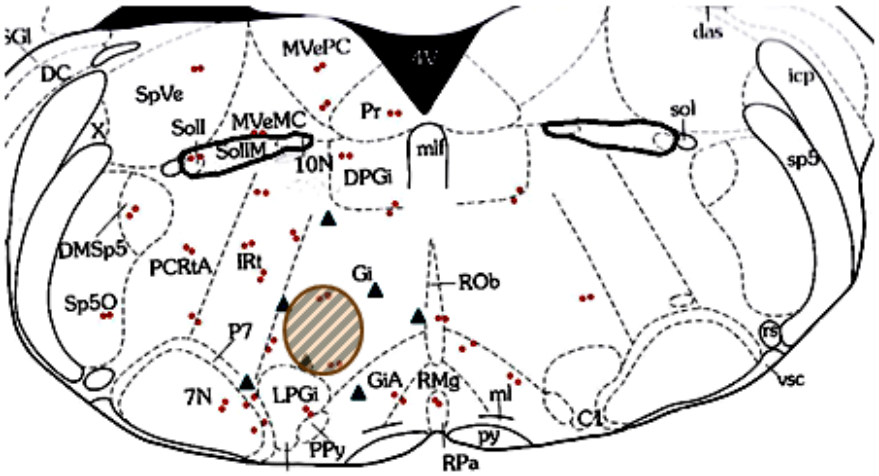
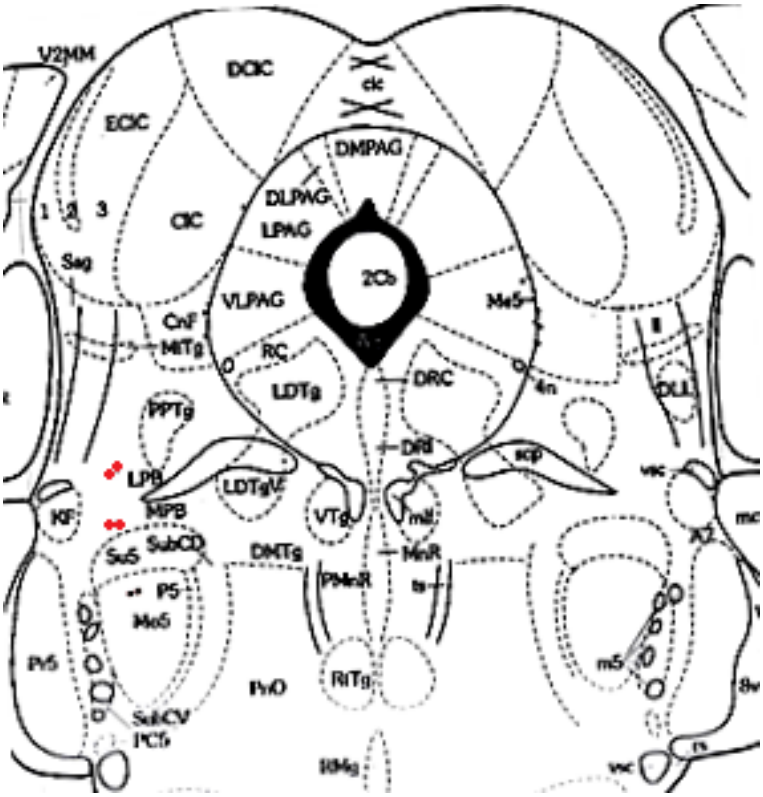


Figure 8-1: Site of injection center at 1.00mm rostral to the obex

A large injection was made in the gigantocellular nucleus (Gi) of the reticular formation. The injection was positioned 75µm rostral to the rostral pole of the area postrema, 50µm lateral to the midline, and 150µm dorsoventrally. Retrograde labeled cell bodies and bilateral anterograde labeled endings increased within the gigantocellular nucleus. The intermediate reticular nucleus (Irt) and the parvocellular reticular nucleus (PCRt) received greater levels of anterograde labeled endings as well. Retrograde labeled cell bodies were not visible in the subdivisions of the rostral NST, but endings were labeled in the rostral lateral subdivision. The facial motor nucleus (7N) continued to receive anterograde labeled endings and retrograde labeled cell bodies at this level as well. Other regions including the spinal trigeminal nucleus (Sp5), the prepositus nucleus (Pr) and the medial and spinal vestibular nuclei also continued to receive anterograde labeled endings.

Coronal Series: Rostral to caudal sections of medulla containing label



<u>Figure 8-2</u>: Transported label 2.50mm rostral to the obex

Anterograde labeling was visible in the later and medial parabrachial nuclei (LPB and MPB).

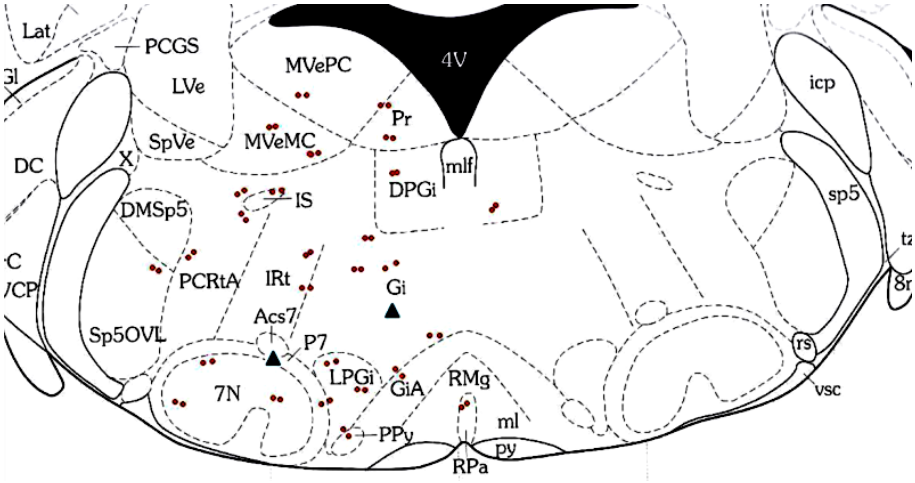


Figure 8-3: Transported label 1.25mm rostral to the obex

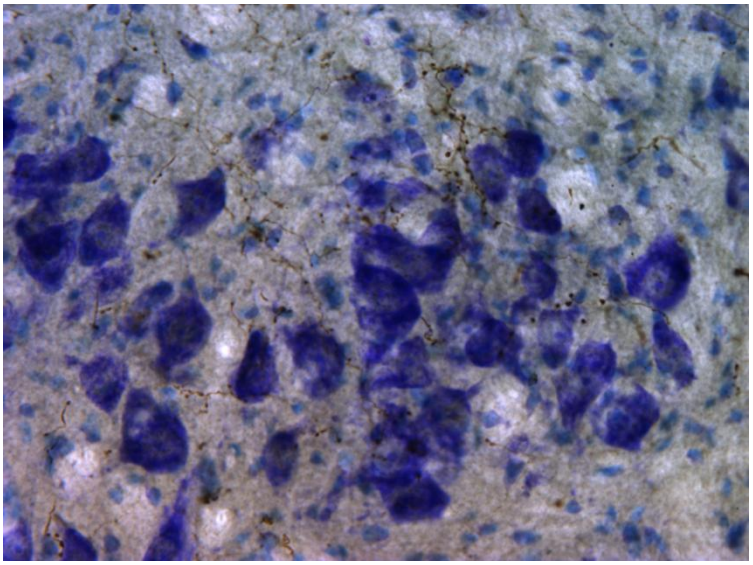


Figure 8-3A: Anterograde labeled endings in facial motor nucleus, 40X

Slightly caudal to the center of the injection, retrograde labeled cell bodies and anterograde labeled endings were present within the gigantocellular nucleus (Gi). Other regions of the reticular formation including the parvocellular reticular nucleus (PCRt) and the intermediate reticular nucleus (Irt) received anterograde labeled endings as well. Retrograde labeled cell bodies and anterograde labeled endings were also present in the facial motor nucleus (7N). The spinal trigeminal nucleus (Sp5), the prepositus nucleus (Pr) and the parvocellular (PCRt) and vestibular nuclei also received anterograde labeled endings.

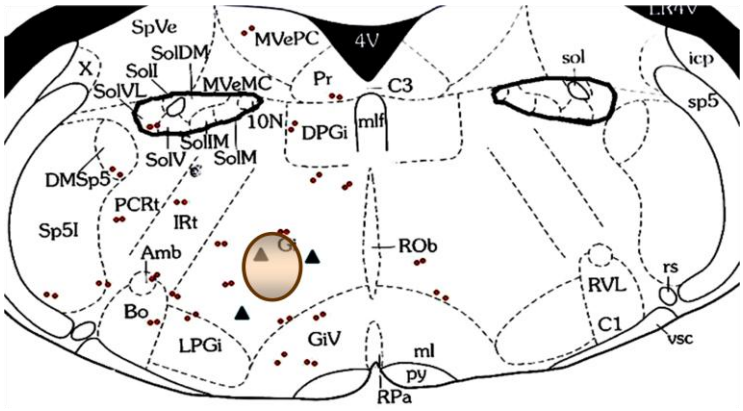


Figure 8-4: Transported label 0.80mm rostral to the obex

Slightly caudal to the injection site, retrograde labeled cell bodies and anterograde labeled endings remained present in the gigantocellular nucleus (Gi). Other regions of the reticular formation including the intermediate reticular nucleus (Irt) and the parvocellular reticular nucleus (PCRt) continued to receive anterograde labeled endings as well. Sparsely labeled endings were visible in the ventral subdivision of the rostral NST. Anterograde labeled endings were also detected in the nucleus ambiguus (Amb), the Bo nucleus, the spinal trigeminal nucleus (Sp5) region, the prepositus nucleus (Pr), and the parvocellular medial vestibular nucleus (MVePC).

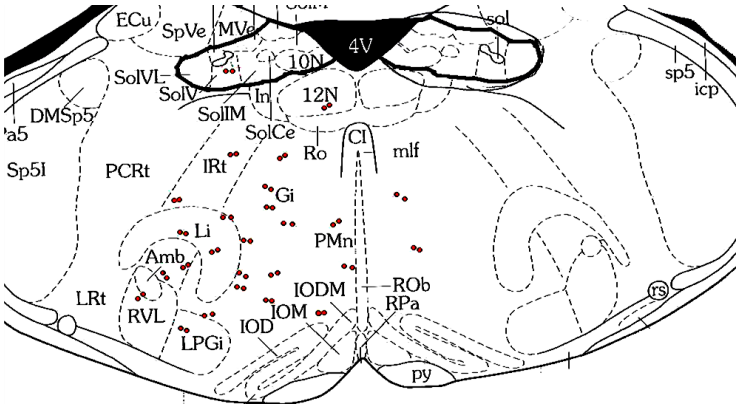


Figure 8-5: Transported label 0.25mm rostral to the obex

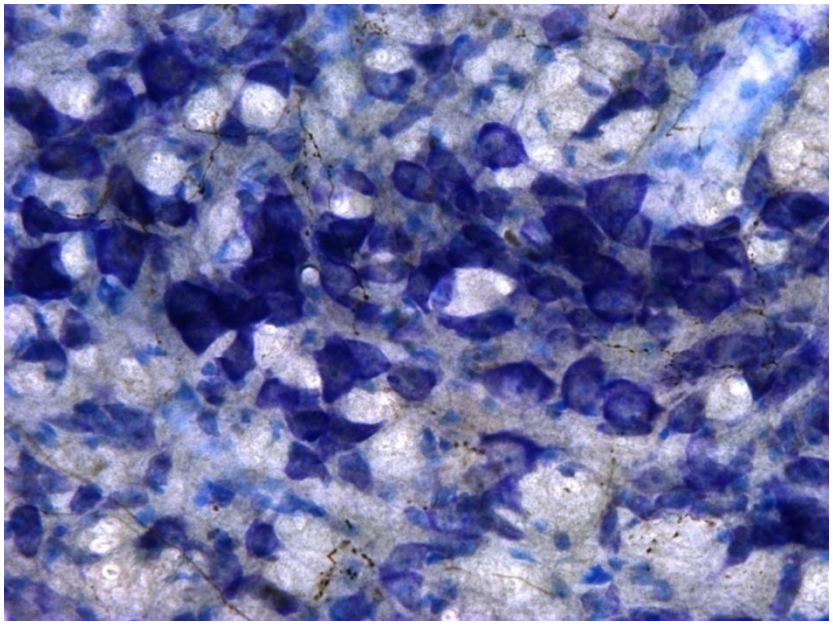


Figure 8-5A: Anterograde labeled endings in nucleus ambiguus, 40X

At a further caudal level, retrograde labeled cell bodies in the reticular formation were no longer visible. Anterograde labeled endings were distributed throughout the gigantocellular nucleus (Gi) bilaterally, as well as the intermediate reticular nucleus (Irt). Very few labeled endings were visible within the ventral subdivision of the caudal NST. Sparsely labeled endings were also visible within the hypoglossal nucleus (12N) at this level, as well as the nucleus ambiguus (Amb) and the linear nucleus (Li).

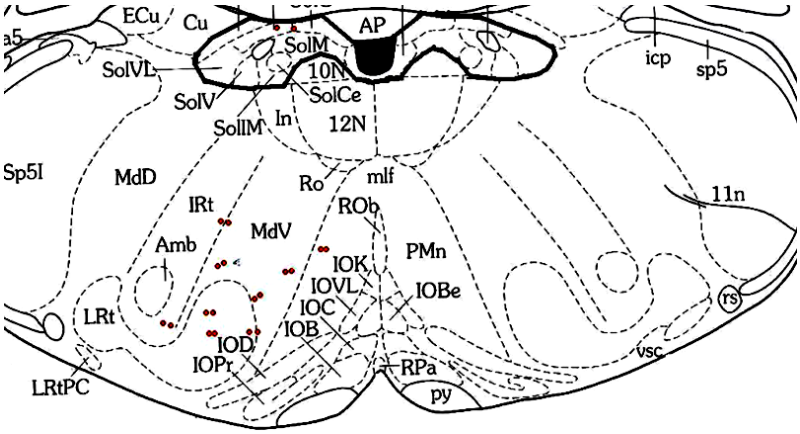


Figure 8-6: Transported label at the level of the obex

Anterograde labeled endings became less distributed further caudal to the injection site, but were still visible throughout the ventral medullary reticular nucleus, the lateral reticular nucleus (LRt), and the intermediate reticular nucleus (Irt). Very few anterograde labeled endings were visible in the caudal lateral subdivision of the caudal NST at this level.

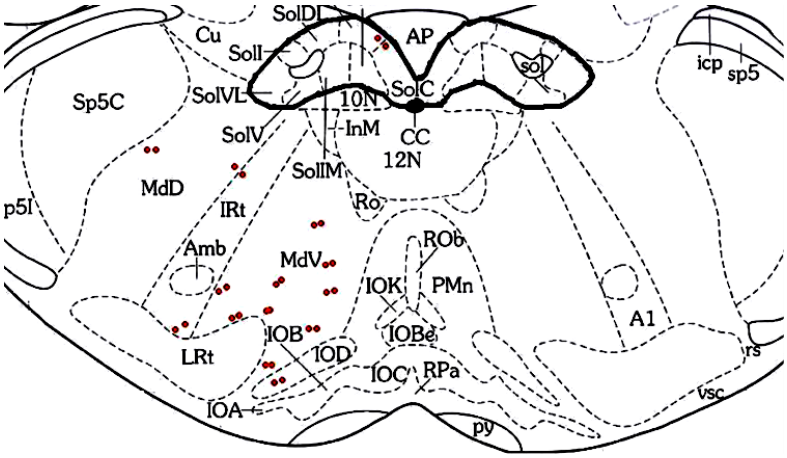
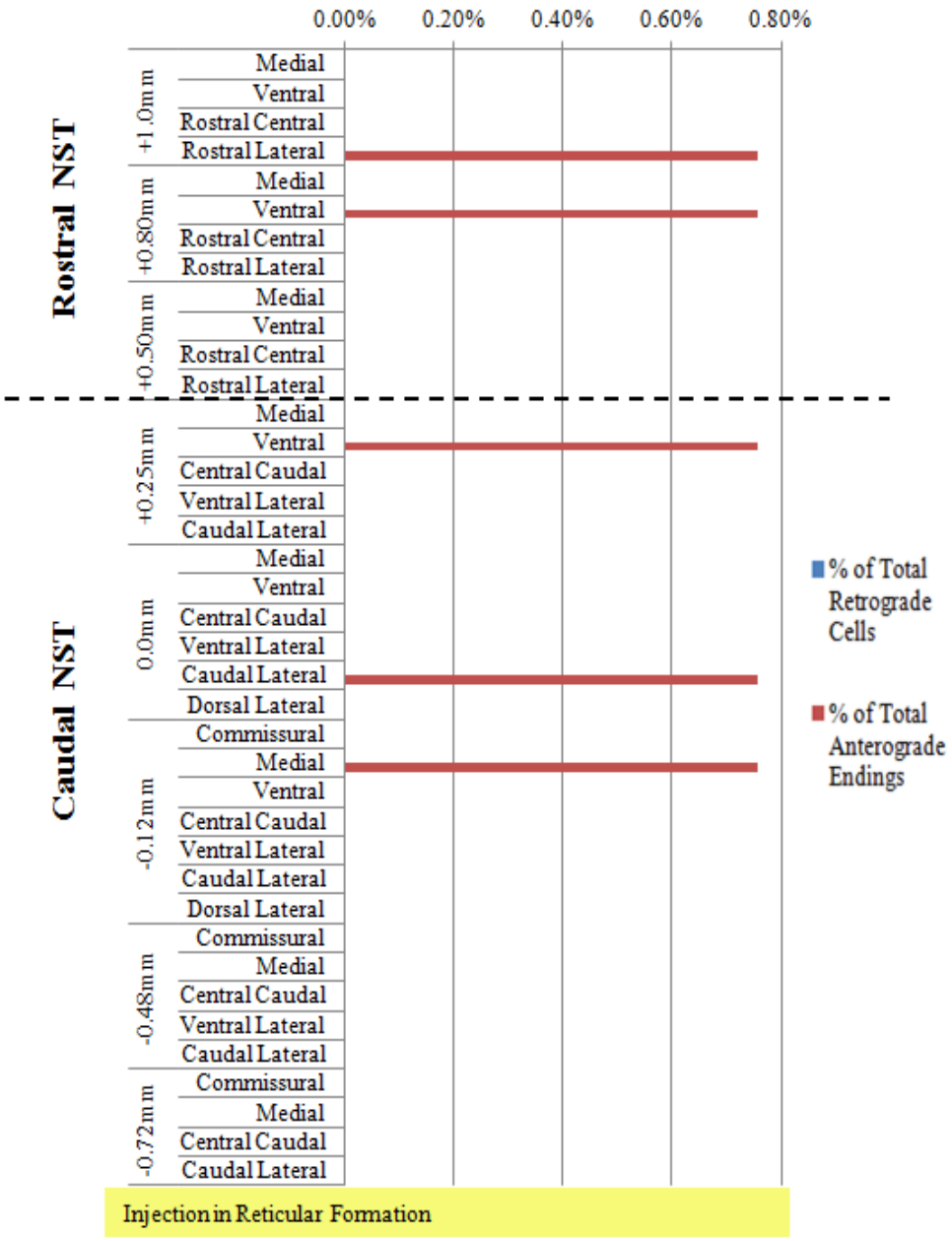


Figure 8-7: Transported label 0.12mm caudal to the obex

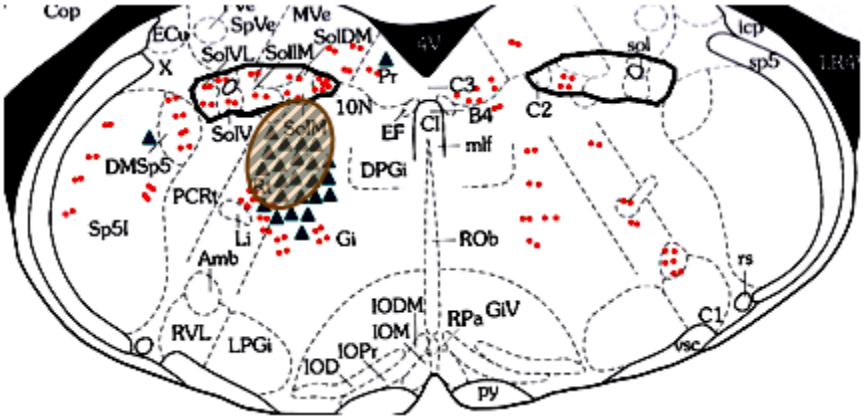
Further caudally, anterograde labeled endings continued to be visible within the ventral and dorsal medullary reticular nuclei (MdD and MdV) as well as the intermediate reticular nucleus (Irt). The medial subdivision of the caudal NST received few anterograde labeled endings.

Graph 8: Summary of transported labeling in rostral and caudal NST, Case 8



The total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions are illustrated above. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

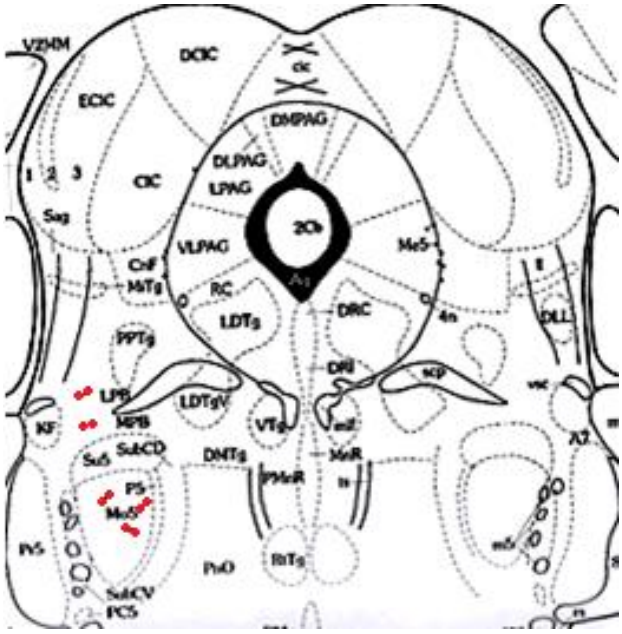
Case 9 – Center of injection in the Reticular Formation



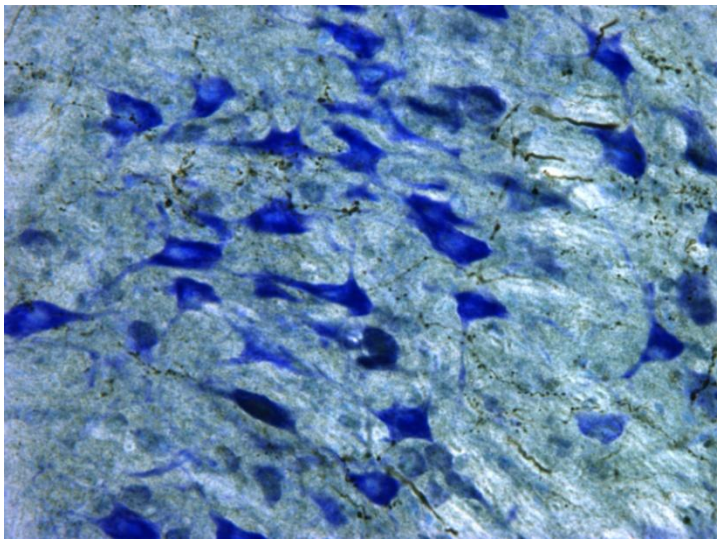
<u>Figure 9-1</u>: Site of injection center at 0.50mm rostral to the obex

A medium injection was made on the border between the intermediate reticular nucleus and the gigantocellular nucleus within the reticular formation. The injection was positioned 1mm rostral to the rostral pole of the area postrema, 1mm lateral to the midline, and 1mm dorsoventrally. Retrograde labeled cell bodies and bilaterally labeled endings were highly distributed throughout the gigantocellular (Gi) and intermediate reticular nuclei (Irt) of the reticular formation. Anterograde labeled endings were also distributed throughout all subdivisions of the rostral NST, and bilaterally within the medial subdivision. Retrograde labeled cell bodies and bilaterally distributed anterograde labeled endings were also visible within the prepositus nucleus (Pr) and the spinal trigeminal nucleus. The medial vestibular nucleus (MdV) also received bilateral anterograde labeled endings at this level. The dorsal motor nucleus of the vagus nerve (10N) also received anterograde labeled endings at this level. Additionally, labeled endings were visible within the nucleus ambiguus (Amb) contralateral to the injection site.

Coronal Series: Rostral to caudal sections of medulla containing label



<u>Figure 9-2</u>: Transported label 2.50mm rostral to the obex



<u>Figure 9-2A</u>: Anterograde labeled endings in trigeminal motor nucleus, 40X

At the furthest rostral level, anterograde labeled endings were visible in the trigeminal motor nucleus (Mo5) and within the lateral and medial divisions of the parabrachial nucleus (LPB and MPB).

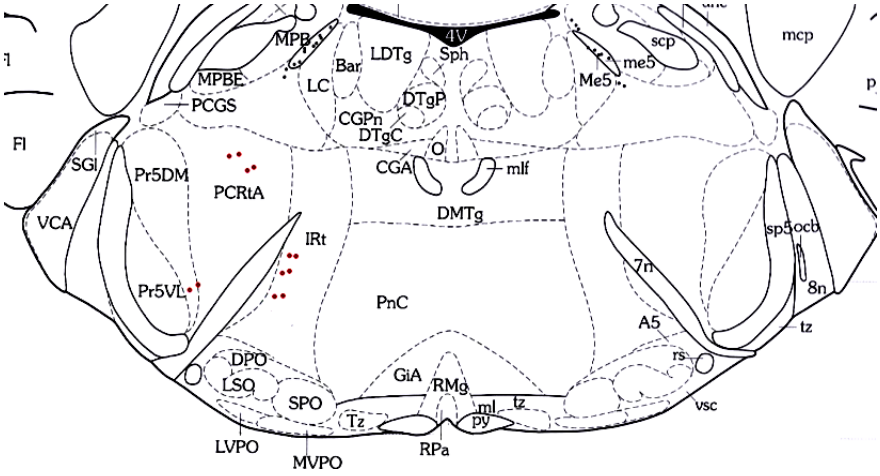


Figure 9-3: Transported label 2.0mm rostral to the obex

At a slightly further caudal level, anterograde labeled endings were only visible in regions of the reticular formation including the parvocellular reticular nucleus (PCRt) and the intermediate reticular nucleus (Irt).

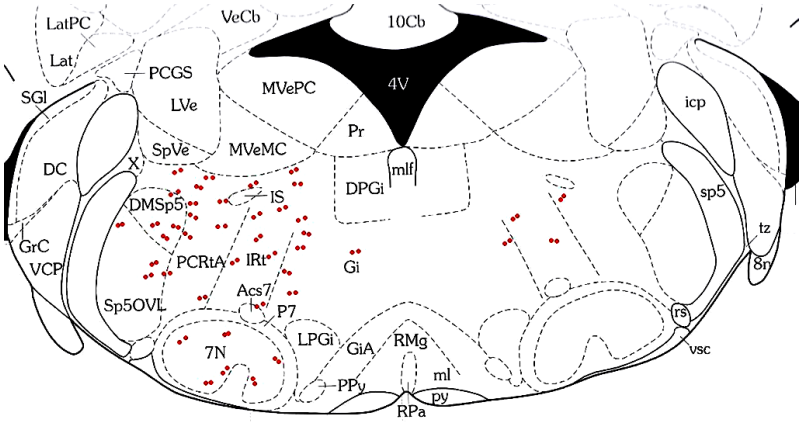


Figure 9-4: Transported label 1.25mm rostral to the obex

Further caudally, heavier anterograde labeling of endings was visible throughout regions of the reticular formation including the gigantocellular nucleus (Gi) bilaterally, the intermediate reticular nucleus (Irt), and the parvocellular reticular nucleus (PCRt). Anterograde labeled endings were also prominent throughout the facial motor nucleus (7N) and the accessory facial nucleus (Acs7), as well as the spinal trigeminal nucleus (Sp5) region.

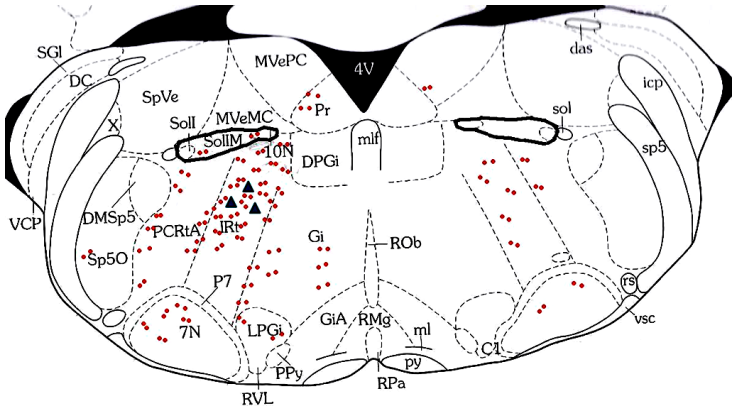


Figure 9-5: Transported label 1.0mm rostral to the obex

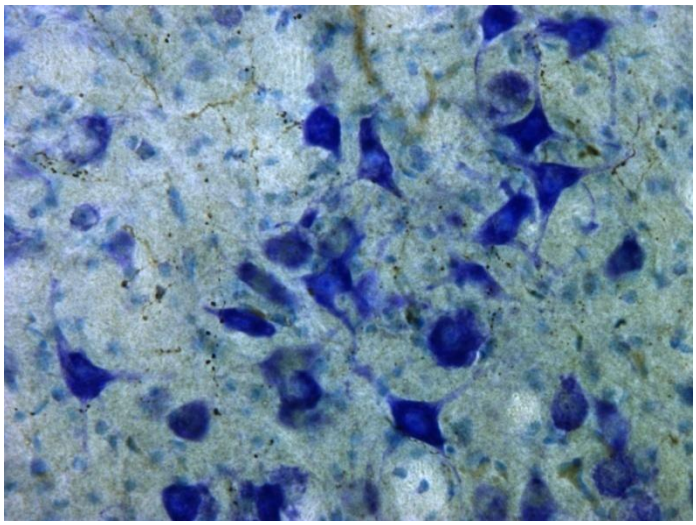


Figure 9-5A: Anterograde labeled endings in facial motor nucleus, 40X.

Further caudally, retrograde labeled cell bodies and bilateral anterograde labeled endings were visible within the intermediate reticular nucleus (Irt). Labeled endings also remained visible in the gigantocellular (Gi) and parvicellular reticular nuclei (PCRt) at this level. Within the rostral NST, anterograde labeled endings were also visible within the medial and ventral subdivisions. Anterograde labeled endings also remained distributed throughout the facial motor nucleus (7N) at this level. Other regions including the prepositus nucleus (Pr) and the spinal trigeminal nucleus (Sp5) region also contained anterograde labeled endings.

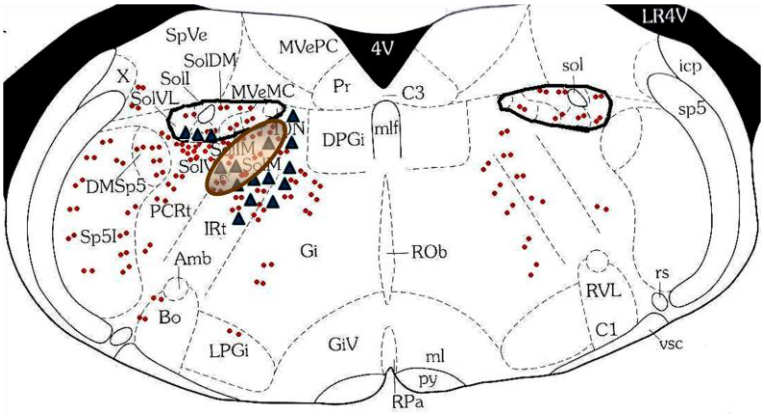


Figure 9-6: Transported label 0.80mm rostral to the obex

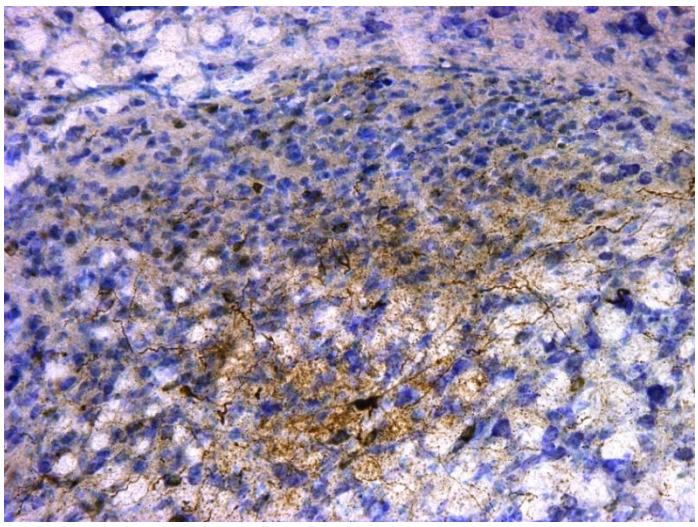


Figure 9-6A: Transported label throughout rostral NST, 20X

At a level slightly rostral to the center of the injection site, retrograde labeled cell bodies were heavily distributed throughout the intermediate reticular nucleus (Irt), parvocellular reticular nucleus (PCRt), and the gigantocellular reticular nucleus (Gi) of the reticular formation. Anterograde labeled endings were present within these regions as well and were distributed bilaterally. Anterograde labeled endings were also distributed throughout all subdivisions of the rostral NST bilaterally at this level. Additionally, labeled endings were especially distributed within the spinal trigeminal nucleus (Sp5).

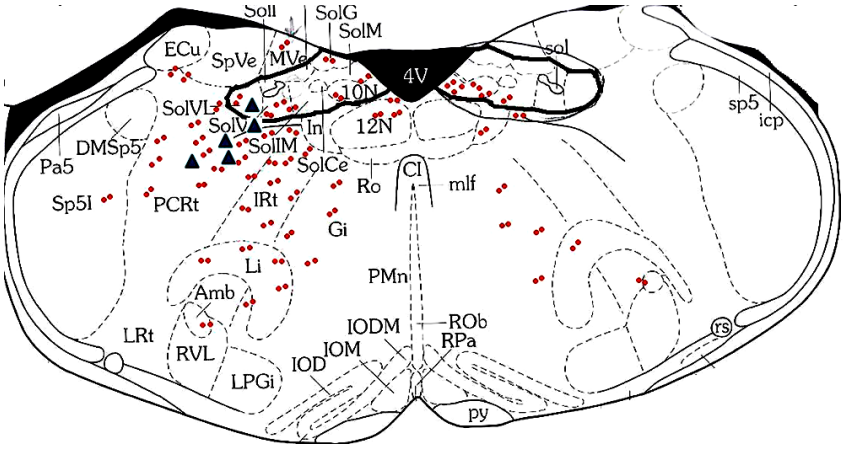


Figure 9-7: Transported label 0.25mm rostral to the obex

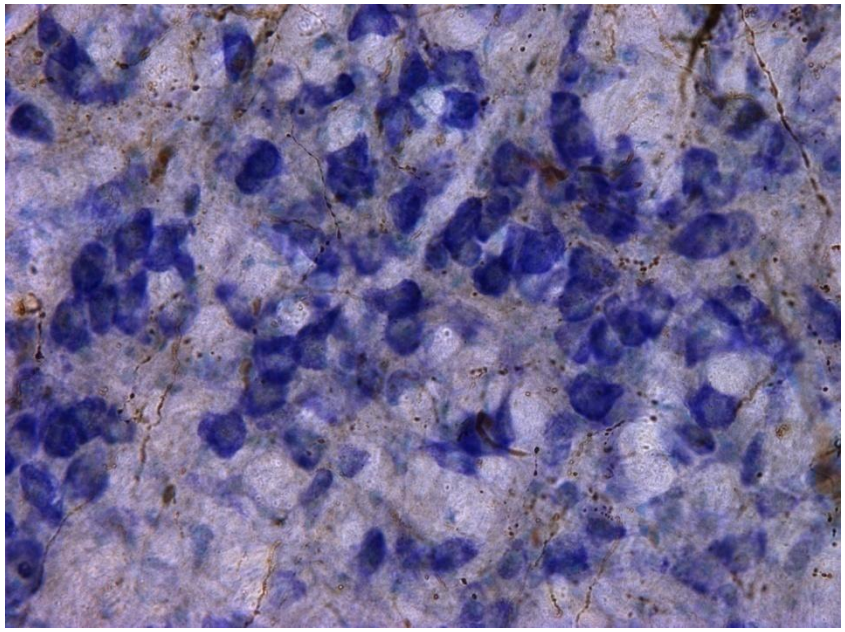


Figure 9-7A: Anterograde labeled endings in nucleus ambiguus, 40X

Slightly caudal to the center of the injection site, anterograde labeled endings and retrograde labeled cell bodies remained distributed throughout the parvocellular reticular nucleus (PCRt). Other regions including the intermediate reticular nucleus (Irt) and the gigantocellular nucleus (Gi) also contained a heavy distribution of anterograde labeled endings. Anterograde labeled endings and few labeled cell bodies were also present within the caudal lateral subdivision of the NST, as well as labeled endings in the medial, ventral, and

ventral lateral subdivisions at this level. Bilateral anterograde labeling of endings within the NST was present in the medial and ventral subdivisions. Sparse labeled endings were visible within the medial vestibular nucleus (MVe) as well as the spinal trigeminal nucleus (Sp5). The dorsal motor nucleus of the vagus nerve (10N) as well as the hypoglossal nucleus (12N) also received anterograde labeled endings. Bilaterally labeled endings were also present within the nucleus ambiguus (Amb) and the linear nucleus (Li) at this level.

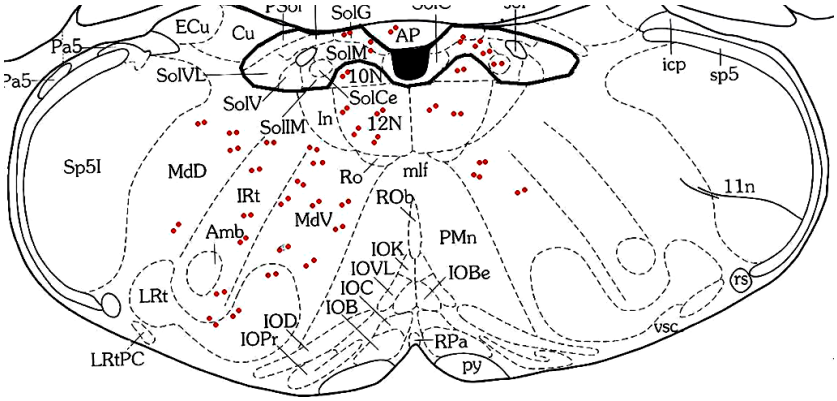


Figure 9-8: Transported label at the level of the obex

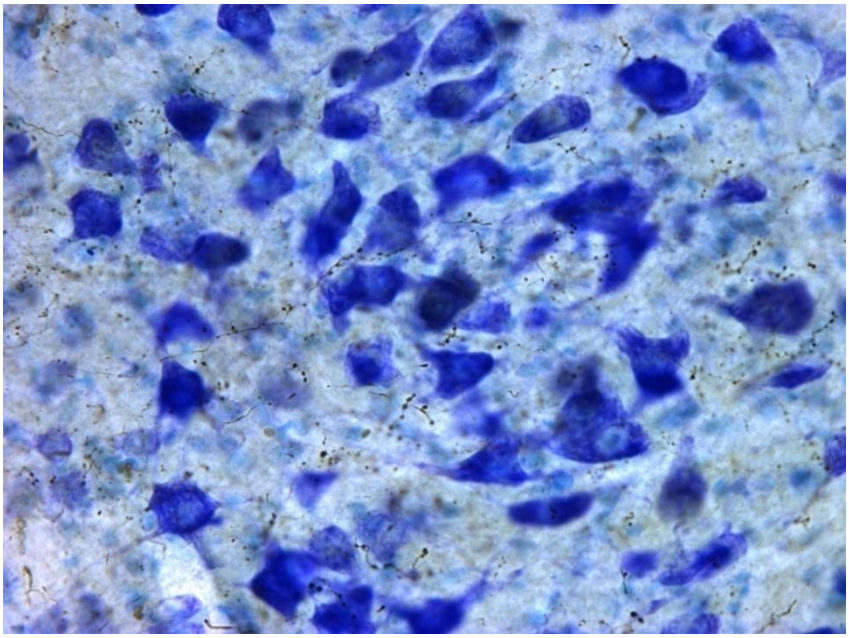


Figure 9-8A: Anterograde labeled endings in hypoglossal nucleus, 40X

Further caudally, anterograde labeled endings remained distributed throughout the reticular formation within the ventral and dorsal medullary reticular nuclei (MdD and MdV) and the intermediate reticular nucleus (Irt). Within the NST, labeled endings were visible throughout the medial subdivision ipsilateral to the injection side and throughout the ventral and central caudal subdivisions on the contralateral side. Additional anterograde labeled endings were visible within the dorsal motor nucleus of the vagus nerve (10N) and the

hypoglossal nucleus (12N). The area postrema (AP) received few anterograde labeled endings.

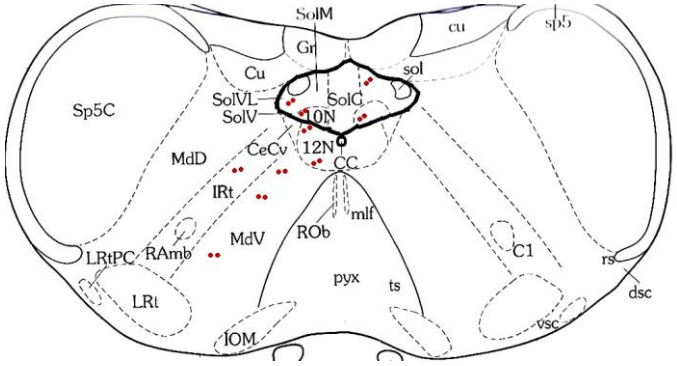


Figure 9-9: Transported label 0.72mm caudal to the obex

Further caudal to the injection site, anterograde labeled endings remained visible within the ventral medullary reticular nucleus (MdV) and the intermediate reticular nucleus (Irt). Within the NST, labeled endings were present within the medial subdivision contralaterally and the caudal lateral subdivision ipsilaterally. The dorsal motor nucleus of the vagus nerve (10N) also received bilaterally labeled endings at this level. Labeled endings were also visible in the hypoglossal nucleus (12N) as well.

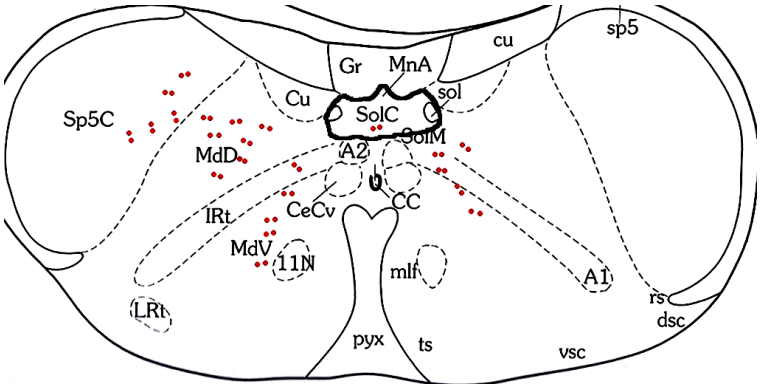
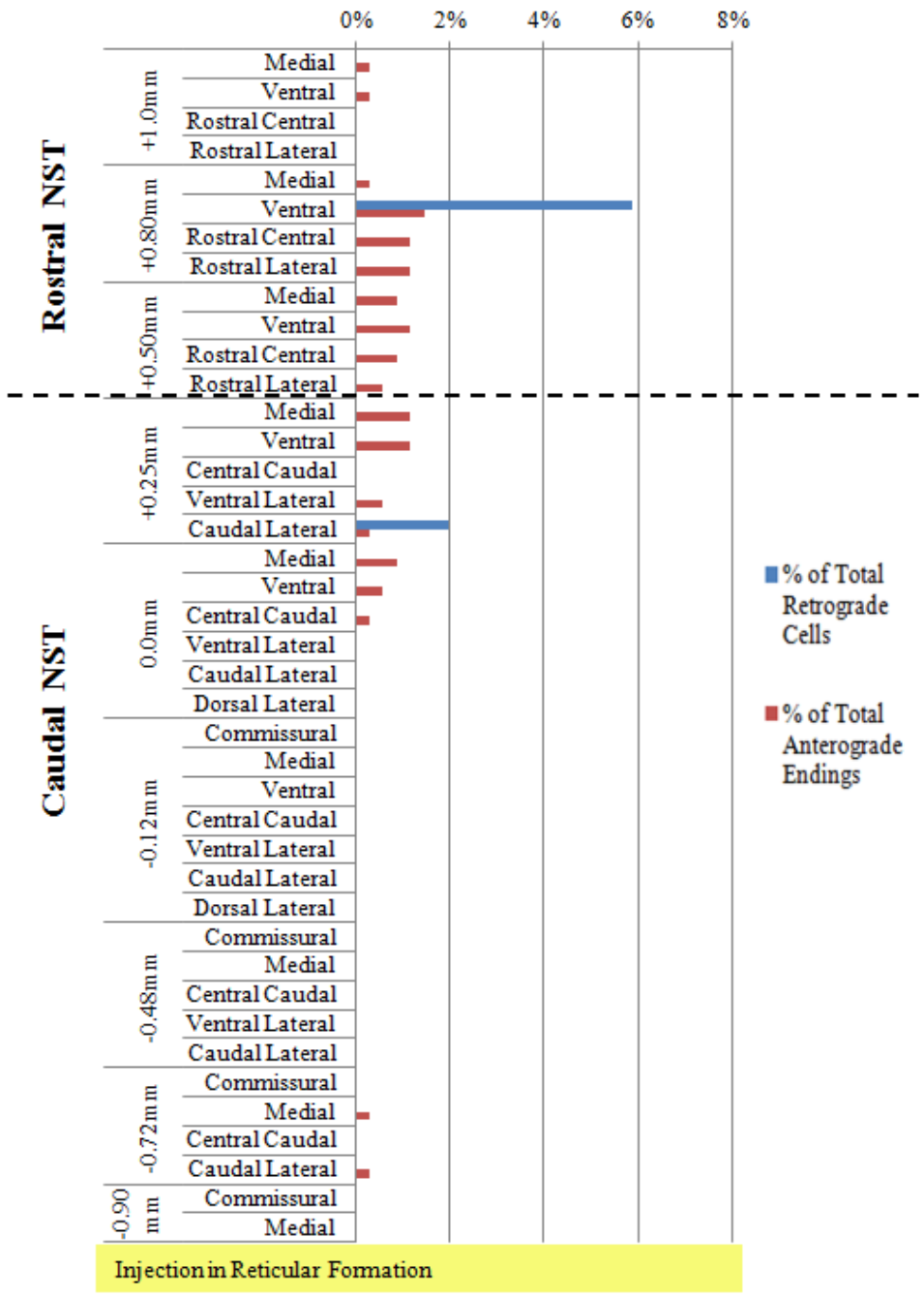


Figure 9-10: Transported label 0.90mm caudal to the obex

At the level furthest caudal to the injection site, labeled endings remained visible within the ventral and dorsal medullary reticular nuclei (MdD), as well as the intermediate

reticular nucleus (Irt). Only the commissural subdivision of the caudal NST received anterograde labeled endings at this level. The spinal trigeminal nucleus (Sp5) received anterograde labeled endings as well.

Graph 9: Summary of transported labeling in rostral and caudal NST, Case 9



The total distribution of labeled cell bodies and axon terminals visible throughout the rostral and caudal NST subdivisions are illustrated above. The percentages are based on the total labeled cell bodies and endings detected throughout all nuclei of the medulla.

Discussion

Investigating the NST in the mouse model with tract tracing techniques has revealed several conclusions regarding its connectivity between its rostral and caudal divisions, as well as its connections to other regions such as the area postrema and the surrounding medullary nuclei.

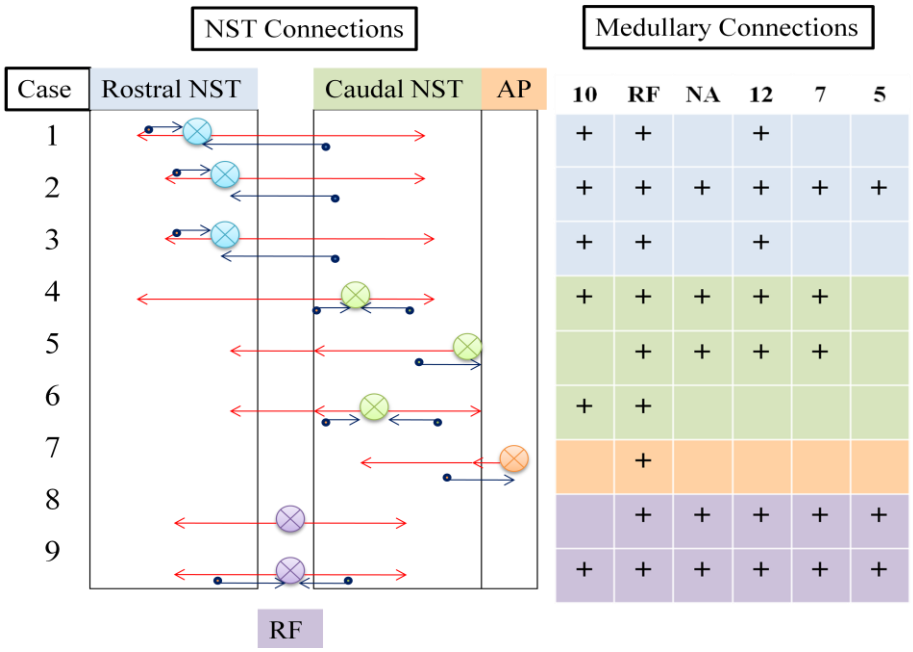


Figure 10: Projection Summaries, Cases 1-9

Table 2: Legend for NST connections

	Injection centered in **rostral NST**
	Injection centered in **caudal NST**
	Injection centered in **area postrema**
	Injection centered in **reticular formation**
←	Anterograde endings reaching the rostral NST
→	Anterograde endings reaching the caudal NST
→	Retrograde cell bodies

Table 3: Legend for medullary connections

10	Dorsal motor nucleus of the vagus nerve
RF	Reticular formation
NA	Nucleus ambiguus
12	Hypoglossal nucleus
7	Facial motor nucleus
5	Trigeminal motor nucleus
+	Anterograde endings

The pooled data among all cases illustrated in Figure 10 compares the projections resulting from injections of the rostral NST (Cases 1-3), injections of the caudal NST (Cases 4-6), injection of the area postrema (Case 7), and injections of the reticular formation (Cases 8-9). The injection centers of each category were positioned in Figure 10, and arrows were applied to each injection site in order to represent the extent of anterograde labeling observed in the rostral and caudal directions according the plotted coronal series for each injection case. Specific medullary projections from the injection sites of each case were also highlighted in Figure 10. Special attention was given to cases resulting in anterograde endings in the dorsal motor nucleus of the vagus nerve in the medulla. Previous research has established that endings from the vagus nerve (cranial nerve 10) reach caudal levels of the NST (Watson et al., 2012). Cases with labeled endings within the reticular formation were also noted due to the high level of connectivity of this region of the medulla. Additionally, cases resulting in anterograde labeled endings in the nucleus ambiguus (NA), hypoglossal (12), facial (7), and trigeminal (5) motor nuclei were also summarized in Figure 10 because they are classified as oral and pharyngeal motor nuclei (Travers, 1983). Figure 10 highlights five key points which will be discussed below.

Regions of the rostral NST project caudally and further rostrally

The injections of the rostral NST in Cases 1, 2, and 3 all resulted in anterograde labeled endings in regions of the caudal NST as well as further rostral regions of the NST. This suggests that connections exist between rostral levels of the NST and caudal levels of the NST, as well as between the rostral NST and further rostral levels of the NST. It is also interesting to note the presence of reciprocal connections between the rostral and caudal NST in Cases 2 and 3. This is suggested by the presence of retrograde labeled cell bodies in the rostral NST and anterograde labeled endings in the caudal NST as a result of injecting the rostral NST, and the presence of anterograde labeled endings in the rostral NST as a result of

injecting the caudal NST. Also, all three injections of the rostral NST resulted in anterograde labeling visible in the hypoglossal nucleus, however only Case 2 resulted in labeled endings in the other oral motor nuclei as well. Case 2 was also the only injection of the rostral NST resulting in anterograde labeled endings in the nucleus ambiguus. This may be due to the larger injection characteristic of Case 2, and additional injections would be necessary in order to determine if a specific subdivision of the NST played a role in this result.

Regions of the caudal NST project rostrally and further caudally

The injections of the caudal NST in Cases 4, 5, and 6 all resulted in anterograde labeled endings visible at rostral levels of the NST. This suggests possible connections existing between the caudal and rostral NST, and further supports the possibility of reciprocal connections between these regions. The connections link general viscerosensory (caudal NST) and gustatory (rostral NST) regions. Interestingly, none of the caudal NST injection cases resulted in anterograde labeled endings in the trigeminal motor nucleus. This suggests a lack of connectivity between non-gustatory regions of the caudal NST and the trigeminal motor nucleus, and also supports the idea that differential connections exist between the rostral and caudal regions of the NST.

The area postrema projects to the caudal NST

Following the injection of the area postrema in Case 7, anterograde labeled endings were detected at caudal levels of the NST. Retrograde labeled cell bodies detected within the area postrema following an injection of the caudal NST in Case 5 provides support for the existence of reciprocal connections between the area postrema and the caudal NST. Anterograde labeled endings were not detected in regions of the oral motor nuclei for this case.

The labeling resulting in this case is consistent with investigations by Shapiro and colleagues. In their study, it was reported that the area postrema projects to the NTS and the

dorsal motor nucleus of the vagus nerve at levels rostral as well as caudal to the obex (1985). The limited depth of the injection of the area postrema in this case perhaps influenced the lack of anterograde labeled endings visible within the dorsal motor nucleus of the vagus and the NST at further rostral levels. Case 7 also confirmed findings by Shapiro regarding the presence of anterograde labeled endings detected in the commissural subdivision of the NST (1985). Also in Case 7, labeled fibers in the solitary tract were observed due to the possible connection between the tenth sensory ganglion and the area postrema.

The rostral NST, the caudal NST, and the area postrema all project to the reticular formation

Although differences in anterograde and retrograde labeling of the oral motor nuclei and the NST varied between cases, all injections did label projections to various nuclei of the reticular formation. The injections of Cases 8 and 9 also resulted in labeling of projections between nuclei within the reticular formation, which supports the existence of differential connections within the reticular formation itself.

The reticular formation projects to the rostral NST, the caudal NST, the area postrema, and all oral motor nuclei

The vast connectivity of the reticular formation described by Jacobson and Marcus in 2011 is consistent with my data resulting from injections of this region. Injections of the reticular formation were utilized not only to investigate the connections unique to this region, but also to verify the anterograde labeled endings detected in the reticular formation resulting from injections of the NST in Cases 1-6. Injections of the reticular formation in Cases 8 and 9 resulted in anterograde labeled endings in the rostral and caudal NST, and these data support the existence of connections between the NST and the reticular formation. Transported label within the subdivisions of the rostral and caudal NST mainly consisted of anterograde endings, and only limited retrograde labeled cell bodies appeared in the ventral subdivision of

the rostral NST and the caudal lateral subdivision of the caudal NST in Case 8. This was only partially consistent with previous studies on the projections between the reticular formation and the NST in the rat nervous system by Halsell and colleagues. Injections confined to the parvocellular reticular nucleus in their study resulted in high levels of labeled neurons in the rostral NST (1996). The injections of the reticular formation in my study were generally confined to the gigantocellular reticular nucleus, and this location may have influenced the subsequent labeling produced. Future injections of different regions with emphasis on the parvocellular subnucleus of the reticular formation would be necessary in order to assess this result.

The injection of the reticular formation in Case 9 resulted in anterograde labeled endings in the area postrema as well, and these data verify the endings detected in the reticular formation resulting from the injection of the area postrema in Case 7. Additional anterograde labeled endings were also visible in other nuclei of the reticular formation surrounding the injection site, which supports the existence of projections among the specific nuclei of the reticular formation.

Future Directions

Future experiments will focus on defining, with even smaller CTb injections, the projections of the specific subdivisions of the rostral and the caudal NST in the mouse model. My studies have contributed to the understanding of the connectivity between the rostral and the caudal NST, as well as the connectivity of the rostral and caudal NST to other regions of the medulla such as the reticular formation, the area postrema, and the oral motor nuclei. Future injections will strive to specifically target individual subdivisions in order to study their unique connections.

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