NEUROCHEMICAL CYTOARCHITECTURE OF THE PRIMATE PARABRACHIAL NUCLEUS

A Thesis

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by

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Abstract

We describe the distribution of calbindin (CAL), cholecystokinin (CCK), dynorphin (DYN), galanin (GAL), neurotensin (NT), somatostatin (SOM), and substance P (SP) immunoreactive elements in the primate parabrachial nucleus (PbN), a pontine structure with a crucial role in autonomic control. This study follows earlier work using calcitonin gene-related peptide (CGRP) as a marker for ascending visceral pathways in the human brain (de Lacalle & Saper, 2000). Our observations were made on horizontal sections through the brainstem from four neurologically normal human individuals and five male Cebus monkeys. Tissue was processed for immunocytochemistry using commercially available antibodies. Which revealed areas of dense peptide immunoreactivity in fibers, as well as in cell bodies.

The extreme expansion of the superior cerebellar peduncle has altered the topographical organization of these pathways in the primate. However, using the peduncle as a marker, we found that similarities in the distribution of cell bodies and fibers remain conserved in some areas across species. The PbN of the Cebus monkey contained well defined external lateral and external medial subnuclei. The distribution of CAL-ir, CCK-ir, NT-ir, SOM-ir, and SP-ir elements were very similar in the human and monkey for the external lateral and external medial subnuclei, and DYN-ir elements were found in the external lateral PbN only in both species. GAL-ir elements were present in the central lateral PbN and dorsal medial PbN areas in both species.

Our results contribute to define a neurochemical identity for different regions in the primate PbN, thus providing support to the delineation of physiological roles for
the distinct subnuclei of this crucial visceral regulatory region.

*Keywords:* human, monkey, parabrachial nucleus, calbindin, cholecystokinin, dynorphin, galanin, neurotensin, somatostatin, substance P
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Introduction

The Anatomy of the Parabrachial Nucleus

The PbN is a pontine structure located in the brainstem, at the junction of the midbrain and the pons at the same level as the most caudal region of the locus coeruleus. In the primate brainstem, it surrounds the superior cerebellar peduncle as if it were its external shell. Imagining the brainstem as a vertical tube simplifies the visualization of the PbN area. Sections of the brainstem cut perpendicular to the long axis (Fig. 1A) of this structure shows the superior cerebellar peduncle as a kidney bean shaped structure oriented in the dorsal-ventral direction in the human. In the *Cebus* monkey, the peduncle is slightly more laterally skewed than in the human and is oriented in a medial-lateral direction, especially at caudal levels.

The extreme expansion of the peduncle in the human is one anatomically significant difference between humans and other species, and has stretched and distorted the PbN making cross species comparisons of the area difficult. This is most evident at caudal levels of the PbN where the peduncle expands against the border of the brainstem displacing the area that would contain the lateral PbN. The sagittal section through the peduncle in Figure 1A presents a visually clear demonstration of this. In the monkey the peduncle does not expand against the border of the brainstem and the lateral PbN is present in its entirety. This is also true of the rat.

The PbN consists of three major nuclei and as many as 12 subnuclei in the rat. The three nuclei are the lateral PbN, the medial PbN, and the Kölliker-Fuse nucleus
(KF nucleus) identified in a human cross-section in Figure 1B. Two subnuclei identified in the rat of significant relevance to this study were the external medial and external lateral nuclei as they were also prominent in the *Cebus* monkey. The KF nucleus is considerably more expansive in the human than in the rat (Lavezzi et al.,
Figure 1. (A) Camera lucida drawing of a sagittal section through the human brainstem. This section is approximately 8.5 mm from the midline. Each dotted line defines the approximate levels of analyses for each stain. Landmarks for each level
are as follows: the rostral level is found at the decussation of the trochlear nerve and the lateral lemniscus; the mid level is defined by the presence of the locus coeruleus and the raphe nucleus; the caudal level contains the tract of the trigeminal nerve and the superior medullary velum. (B) A microphotograph at the mid level showing the approximate locations of each nucleus of the human PbN area. (C) Schematic illustration of the subnuclei of the parabrachial in the rat (Krout & Loewy, 2000).

SCP: superior cerebellar peduncle; lPB: lateral parabrachial; mPB: medial parabrachial; KF: Kölliker-Fuse.

**Projections and Known Functions of the Parabrachial Nucleus**

Neuroanatomical and physiological studies have shown that the PbN serves as an integrative visceral relay center. It receives afferent and sends efferent signals to and from many higher brain structures. These widespread projections together with the diversity of neuropeptides present in them have contributed to elucidate the specific functions of the subnuclei of the PbN.

Most of the studies involving the investigation of visceral pathways through the PbN to the forebrain have been conducted in the rat. In order to ascertain the functions of the PbN, anatomical efferent and afferent connections must be established. Afferent projections to the external lateral PbN and KF nucleus from the spinal cord and trigeminal dorsal horn have been demonstrated. These projections may mediate the convergence of pain, chemosensory, and temperature sensibilities with
gustatory and cardiorespiratory systems in the PB (Cechetto & Standaert, 1985), as described below.

The nucleus raphe magnus (NRM) is located in the brainstem and also receives inputs from the spinal cord. Its main function is pain mediation (Fields & Anderson, 1978). Afferent connections to the ventral PbN and KF nucleus from the NRM have been established. Stimulation of these areas of the PbN resulted in inhibition of nociception (Holstege, 1988).

Afferent projections originating in the oral cavity and gastrointestinal tract relay through the nucleus of the solitary tract (NTS) and continue to the PbN. Cardiovascular and respiratory information also relay through these areas. The NTS is a region of the brainstem with nuclei arranged roughly by function. In the rat, the general visceral part of the NTS projects to the external lateral PbN, the respiratory portion of the NTS has reciprocal connection with the KF, and the gustatory part of the NTS projects primarily to the medial PbN (Herbert et al., 1990). Efferent outputs of the NTS also include the hypothalamus, amygdala and the locus coeruleus (LC).

Another major source of afferent projections to the PbN is the medullary reticular formation (MRF). The MRF is a loosely organized group of nuclei located in the brainstem. Neurons in the MRF innervate the same areas of the PbN as the NTS, such that the ambiguus region is reciprocally connected to the same areas of the PbN as the respiratory part of the NTS, the ventrolateral reticular nucleus is connected to the same areas of the PbN as the respiratory and general visceral NTS, and the
parvicellular reticular area is connected to the same areas of the PbN as the gustatory NTS (Herbert et al., 1990).

As a relay center, the PbN also sends afferent information to higher order brain structures, where it is processed and responsive efferent information is sent back through the brainstem, in what mostly seems to be reciprocal connection. Among these are the hypothalamus, amygdala, and thalamus.

Afferent projections from the PbN to the hypothalamus as well as efferent projections from the hypothalamus to the PbN have been demonstrated (Saper & Swanson, 1976). The hypothalamus, a part of the limbic system located just rostral to the brainstem, regulates many autonomic processes such as body temperature and hunger.

Another component of the limbic system, the amygdala, is located within the temporal lobes of the brain. It contains substantial afferent and efferent connections with the PbN (Fulwiler & Saper, 1984). Its primary function is to serve as an integrative center for emotions and motivation, such fear and aggression. The stria terminalis is the major output pathway of the amygdala. The neurons of the bed nucleus of the stria terminalis, which send efferents to the PbN, contain the same neurochemicals as the central amygdala (Moga et al., 1989).

Situated between the midbrain and the cerebral cortex, the thalamus is an important relay center between higher and lower brain structures. Regulation of consciousness, important sensory information, and motor signals are a few of its
primary functions. Reciprocal innervation of the PbN and the thalamus has been established in the rat (Krouth & Loewy, 2000).

Each of these areas works in conjunction with the PbN to modulate the appropriate physiological responses. For example, afferent projections originating from the PbN which travel to the lateral hypothalamus, central nucleus of the amygdala, and bed nucleus of the stria terminalis have been implicated in the modulation of taste activity in rats (Li et al., 2005). Taste responses in hamsters originated from two very specific regions in the PbN; one in the external medial nucleus, and the other in the external lateral PbN (Halsell & Frank, 1991). Stimulation of the lateral PbN resulted in an increased respiratory rate while stimulation of the KF nucleus and medial PbN resulted in a marked decrease in respiratory rate (Lara et al., 1994). These examples showcase the PbN’s role in a wide variety of physiological responses. In order to coordinate these diverse physiological effects, the PbN must organize information and send it to higher brain structures as well as relay the information it receives from them to the rest of the body which explains the many connections the PbN has with regions such as the NRM, MRF, hypothalamus, thalamus, and amygdala.

Roles and Locations of Neurochemicals

This study examines the presence of the following neuropeptides: cholecystokinin (CCK), calbindin (CAL), dynorphin (DYN), galanin (GAL), neurotensin (NT), somatostatin (SOM), and substance P (SP). Each of these has been
implicated in a wide variety of biological processes. They were chosen for this study in part due to their associated roles in visceral functions.

Calbindin-D28k, which was first observed in the intestine, is expressed in a number of neuronal and endocrine cells. For example, CCK/calbindin-containing GABAergic neurons have been identified in the hippocampal formation. In the brain Cal-D28k is involved in the translocation and sequestration of intracellular free calcium (Dutar et al., 1991). Cal-D28k and cal-D9k work in conjunction to maintain cell homeostasis via transepithelial calcium transport (Zheng et al., 2004). Acting as a calcium buffer, CAL protects neurons from apoptotic and necrotic cell death (Choi et al., 2008).

As a peptide hormone, CCK mediates satiety by acting on the CCK receptors distributed widely throughout the central nervous system (CNS), including the brainstem. It serves as a crucial signaling hormone both in the CNS as well as the gut. Among other actions, CCK’s stimulatory effects on the vagus nerve oppose those of ghrelin (Kobelt et al., 2005). CCK’s role as a satiety promoter has been demonstrated in the lateral PbN of the rat (Li & Rowland, 1994). CCK neurons in the lateral PbN also contribute to the inhibition of NaCl and water intake (Fratucci et al., 2001). Neurons in the PbN that express CCK and leptin receptors were found to play a crucial role in the counter regulatory response in hypoglycemic states (Flak et al., 2014).

DYN is a class of opioid peptide. It is found widely distributed throughout the CNS, where it is produced, with highest concentrations in the hypothalamus, medullopontes, midbrain, and spinal cord (Goldstein & Ghazarossian, 1980). As an opioid it
plays a role in analgesic response, however DYN’s specific role is still unclear. Depending on the region of release DYN can act as either a pain amplifier or pain-reliever (Ren et al., 1985). Corticotropin-releasing factor, activated during stress responses, provokes DYN release in the brain. This suggests a role of DYN in stress-induced responses (Land et al., 2008). DYN’s role in appetite and temperature regulation is interesting in the context of the PbN. After cerebrovascular injections of DYN$_{1-13}$, food ingestion increased in rats (Morley & Levine, 1981). In another experiment, hypothermia was induced in rats in proportional severity with the dose of DYN$_{1-17}$ administered (Xin et al., 1997). These roles indicate that DYN in the PbN may be involved in gustatory and temperature regulation.

GAL is a neuropeptide expressed widely in the brain and spinal cord with highest concentrations in the midbrain and hypothalamus. Interestingly it is also found in the gastrointestinal tract of mammals. The structure of GAL is extremely conserved among mammals, showing over 85% homology (Bersani et al., 1991). Not much is known about the specific functions of this neuropeptide other than the fact that it causes hyperpolarization and inhibits neurotransmitter release. Studies suggest that GAL may modulate feeding preference towards dietary fat (Leibowitz & Akabayashi, 1998). Endogenous GAL also inhibits neuronal growth in the spinal cord through the modulation of growth hormone (Wiesenfeld-Hallin & Langel, 1992). Evidence for a mechanistic relationship between amyloidosis and GAL over expression in Alzheimer’s Disease has also been explored (Mufson et al., 2005).
NT is a neuropeptide found in the gastrointestinal tract and distributed widely throughout the CNS with highest concentrations in the hypothalamus, amygdala and nucleus accumbens (Mustain & Rychahou, 2011). Anatomical interactions between endogenous NT, CCK, and secretin have been implicated in the physiologic regulation of pancreatic exocrine secretion (Sakamoto et al., 1984). Classically cited functions of NT include its role in pain modulation and temperature control. Depending on the site of administration, NT either enhanced pain response or produced profound analgesia (Dobner, 2006). Induced hypothermia and intolerance to cold occurred after intracisternal administration of NT (Quirion, 1983).

SOM is a peptide hormone commonly referred to as growth hormone inhibiting hormone. It contributes to the control of the endocrine system, neurotransmission, and cell proliferation. Among its other many other digestive related functions, SOM inhibits the secretion of stomach acid and suppresses the release of gastrointestinal and pancreatic hormones such as CCK and insulin (Boron & Boulpaep, 2012). This effectively slows down the digestive process. In the brain its main function is the inhibition of growth hormone release from the anterior pituitary (Bowen, 2002). Populations of SOM neurons have been established in the arcuate nucleus, the hippocampus, and the brainstem (Johansson et al., 1984). The particular roles of SOM in the PbN have yet to be demonstrated. However, SOM projections from the PbN to the central nucleus of the amygdala have been established indicating involvement in autonomic responses (Moga & Gray, 1985).
SP is a ubiquitous neuropeptide with an incredibly broad range of biological roles. Neurokinin 1 receptor (NK1R) is the receptor for the SP peptide (Gerard et al., 1991). Together, SP and NK1R modulate stress responses throughout the body. These responses include vasodilation, inflammation, nociception, anxiety, and vomiting (O'Connor et al., 2004). As of now, no conclusive behavioral studies involving the PbN and SP have been conducted. Numerous studies have demonstrated the presence of SP in the PbN and its extensive projections to the amygdala (Veening et al., 1984). This implicates PbN SP neurons in a number of autonomic processes.

**The Cebus Monkey**

The *Cebus* Monkey is a New World monkey native to Central and South America. The rainforest serves as its natural habitat, although it can easily adapt to areas colonized by humans. The *Cebus* is a species of monkey ranging anywhere from 12-22 inches and is characterized by its long tale and black, brown, and whitish fur. Small birds, fruits, and nuts serve as its primary food sources (Fragaszy et al., 2004).

The *Cebus* Monkey was chosen as the comparative species for this experiment for multiple reasons. First, *Cebus* monkey brainstem tissue was readily available as a gift from another laboratory. These tissue sections were stained against the same neuropeptides that we had used for the human brainstem. Second, the parabrachial region lacks any significant research on any species of monkey. The parabrachial area is highly conserved in most species. Due to the evolutionary proximity between the monkey and human, the monkey is likely to contain more anatomical conservation of
visceral pathways than any other species. This is important in establishing a foundation for future behavioral studies of PbN. The results from these studies can subsequently be applied to the human.

**Immunohistochemistry**

Immunohistochemistry (IHC) is a method of detecting antigens in biological tissues. This method exploits the specificity of the immune reaction so that antibodies will recognize specific antigens. In order to isolate primary antibodies, the protein of interest is injected into an animal to elicit an immune response. After the secondary immune response, the antibodies are isolated from the whole serum. Antibodies can be polyclonal so that they recognize several epitopes, or monoclonal so that they only recognize a single epitome.

Primary antibodies typically do not include a label. In order to visualize the areas where the primary antibodies bind, a secondary antibody and a reporter molecule are necessary. Secondary antibodies are raised against the immunoglobulins of the primary antibodies. The secondary antibodies are either directly attached to a reporter molecule or are attached to a linker molecule that recruits reporter molecules. For example, in many of our experiments we used as a primary antibody a rabbit IgG and therefore applied a secondary antibody that was a goat-anti-rabbit IgG coupled to horseradish peroxidase (HRP). HRP was reacted, and the precipitate in the tissue allowed visualization of the neurochemicals to which the primary and secondary
antibodies were bound. To improve visualization of the tagged tissue, counterstains can be added to darken and define cells as well as identify specific regions.

While most of the neuropeptides in this study are considerably conserved between different species, they may vary in sequence by a few amino acids. Because of this, the antiserum may not recognize all of the cells that contain the targeted neurochemicals. For our experiments this did not seem to pose a significant problem as staining in the human and monkey showed similar distributions. Just as the antiserum may not recognize every instance of a specific neurochemical, the opposite is also true. It is possible that this antiserum may recognize unidentified neuropeptides. Thus, the immunoreactivity detected in this study is more accurately described as neuropeptide-like immunoreactivity.

**Experimental Goals**

According to the literature, most of the studies on the PbN took place in the 1970’s through the 1990’s. During that time there was particular interest in establishing the afferent and efferent projections of the PbN in the rat, hamster, and cat. Since then very few studies have focused on this particular area of the brainstem in experimental animals, and even fewer in humans.

In order to establish the true physiological roles of each subnuclei in the PbN it is necessary to establish three things. First we can ask, what are the afferent and efferent connections between this area and other areas of the CNS? Establishing a map of the flow of information from one area of the CNS to the next is crucial in
ascertaining function. For example, the connections between the nucleus raphe magnus and KF nucleus helped to establish the role of the KF nucleus in pain modulation (Holstege, 1988). This determination of function can be enhanced further by narrowing flow of information to and from specific subnuclei and their connections to other precise locations within established brain structures.

Second, we can attempt to determine the presence or absence of specific neuropeptides in each subnuclei. Each neurochemical is involved in a variety of biological processes. The specific function of each neurochemical can vary somewhat widely depending on its location. However, its presence in a confined anatomical area provides invaluable insight to the possible functions of that area. Establishing the neurochemical cytoarchitecture of the PbN will ultimately narrow the possible physiological modulations that each subnuclei is responsible for.

Finally, what behavioral or physiological changes occur when the area in question is lesioned totally or partially? Lesion studies allow for loss-of-function analysis. In lesion studies a specific area of the brain is destroyed and the resulting changes in behavior and physiology changes are analyzed. This is how the role of the hippocampus in object recognition and object recency memory in rats was determined (Albasser et al., 2012). These studies can be difficult, especially when dealing with small areas such as the PbN. In the human, only naturally occurring lesions could be used, such as after a stroke. The likelihood of a stroke involving only the PbN is rare. In part due to its size, but also because of its location in the brainstem, an area of the brain that is highly vascularized. Electrical stimulation is another method that can be
used to interfere with the normal functioning of an area. For example, electrical stimulation of the PbN resulted in pain suppression in rats (DeSalles et al., 1985). This implicates the PbN in some level of nociceptive modulation. If electrical stimulation was focused to specific subnuclei, then the resulting physiological changes could be attributed to that area.

In summary, the purpose of this study was to identify the presence of the neuropeptides CCK, CAL, DYN, GAL, NT, SOM, and SP in the subnuclei of the PbN. Furthermore, we sought to establish similarities of the expression of these neuropeptides between the human and the monkey. These results contribute to provide support to the delineation of physiological roles for the distinct subnuclei of this crucial visceral regulatory region in the primate.
**Methods**

**Tissue Preparation**

*Cebus* monkey brainstem tissue was a gift from Dr. Jeff Kordower and was originally processed for use in other studies (Kordower et al., 1992). Human brainstem tissue was obtained at routine autopsy from 4 neurologically normal individuals (Table 1). Each brain was fixed by vascular perfusion with 2 l of a saline solution containing 20,000 IU of heparin, followed by 8 l of 4% neutral phosphate-buffered paraformaldehyde containing 1.6 g of picric acid and 216 g of NaCl, at 4°C. The brains were blocked either in the coronal or in the sagittal planes, and cryoprotected in a solution of 20% glycerol in 0.1 M phosphate buffer for two days. Frozen sections, 50 µm thick, were cut in a freezing microtome. Series of 1:24 sections were collected in 0.1 M phosphate buffer (pH 7.4) and 0.9% NaCl phosphate-buffered saline (PBS) with 0.02% sodium azide (Saper & de Lacalle, 2000). Alternating cross sectional series through the brainstem were processed for CCK, CAL, DYN, GAL, NT, SOM, and SP.

Our observations were made on tissue processed with the antibodies described in Table 2. *Cebus* monkey tissue and human tissue were processed in the same way. After preincubation in a solution of PBS with 0.25% Triton X-100 and 3% H2O2 for 0.5 h to block endogenous peroxidase, sections were washed in a dilution medium consisting of 5% non-fat dry milk in PBS with Triton-X for 1 h at room temperature. Sections were then exposed for 24 h at 4°C to the primary antibody. Sections were washed in several rinses of PBS and incubated in a goat-anti-rabbit IgG coupled to horseradish peroxidase (HRP; Vector) (1:50 in diluent) for 1 h at room temperature.
The sections were again washed in PBS and a peroxidase reaction was carried out with 0.05% 3-3′diaminobenzidine (DAB, Sigma) and 0.01% H2O2. Following immunostaining, the tissue was mounted on gelatinized slides, dehydrated in graded ethanols, cleared in xylene and coverslipped with DPX. A few selected immunostained sections were counterstained using a Giemsa dye24 to aid in the visualization of anatomical boundaries and darken immunocytochemical staining.

**Analysis & Data Collection**

Tissues from each case were examined using light microscopy. Sketch overlay drawings were created to aid in visualizing immunoreactive elements and anatomical features of the region. Photographs were taken using an Olympus Microfire camera and merged together in Photoshop CS6, version 13.0. We used the *Stereotaxic Atlas of the Brain of the Cebus Monkey* (Manocha et al., 1968) and the *Stereotaxic Atlas of the Human Brainstem and Cerebellar Nuclei* (Turner, 1979) to define anatomical landmarks.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>PMD</th>
<th>Primary diagnosis</th>
<th>Cause of death</th>
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<tbody>
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<td>38/Female</td>
<td>3</td>
<td>Uterine carcinoma</td>
<td>Cardiac arrest</td>
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<tr>
<td>H 98</td>
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<td>Hepatic tumor</td>
<td>Hepatic tumor</td>
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<tr>
<td>H 140</td>
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<td>Mesenteric thrombosis</td>
<td>Hypovolemic shock</td>
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<tr>
<td>H 141</td>
<td>83/Female</td>
<td>2:45</td>
<td>Pancreatic carcinoma</td>
<td>Carcinoma</td>
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<td>Monkey</td>
<td>Subject</td>
<td>Age</td>
<td>Sex</td>
<td></td>
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<tr>
<td></td>
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<td>C 5761</td>
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<td>C 5782</td>
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<td></td>
<td>C 5799</td>
<td>Young</td>
<td>Male</td>
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**Table 1.** Cebus monkey and human subject information.

<table>
<thead>
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<th>Neurochemical</th>
<th>Primary Antibody</th>
<th>Dilution</th>
<th>Source</th>
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<td>Peninsula</td>
</tr>
<tr>
<td>CCK</td>
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<td>1:1000</td>
<td>Incstar</td>
</tr>
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<td>DYN</td>
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<td>1:500</td>
<td>Stan Watson</td>
</tr>
<tr>
<td>GAL</td>
<td>Rabbit, polyclonal</td>
<td>1:1000</td>
<td>Peninsula</td>
</tr>
<tr>
<td>NT</td>
<td>Rabbit, polyclonal</td>
<td>1:500</td>
<td>Immunonuclear</td>
</tr>
<tr>
<td>SOM</td>
<td>Rabbit, polyclonal</td>
<td>1:500</td>
<td>Immunonuclear</td>
</tr>
<tr>
<td>SP</td>
<td>Rabbit, polyclonal</td>
<td>1:2000</td>
<td>J. Kenler</td>
</tr>
</tbody>
</table>

**Table 2.** Neuropeptide antibody information.
Results

The following illustrations and descriptions of the human and *Cebus* monkey brainstems were made on normal individuals (Table 1). Differences in *post mortem* delay and cause of death did not affect the distribution of neuropeptides in this area.

The PbN consists of 3 known subnuclei identified in Figure 1B. In the primate brainstem it surrounds the superior cerebellar peduncle on all sides similar to an external shell. The superior cerebellar peduncle is oriented almost directly vertically in the dorsal-ventral direction in the human. The *Cebus* monkey peduncle is oriented mostly in the medial-lateral direction, especially at caudal levels.

Our sections were sliced perpendicular to the longest axis through the brainstem. Neuropeptides were observed at 3 cross sectional levels in rostral to caudal distribution. These levels were determined approximately by the decussation of the trochlear nerve and the lateral lemniscus for the most rostral section, the mid portion of the locus coeruleus and the raphe nucleus for the mid section, and the tract of the trigeminal nerve and the superior medullary velum for the most caudal section (Fig. 1A).

Since each brainstem was cut in 1:24 series for each stain, the rostral, mid, and caudal levels may be shifted slightly caudally or rostrally. As a result, the slides for some stains do not meet the criteria for each level of observation described above. For example, *Cebus* NT-ir slides did not contain a rostral level. The slides for this level were too rostral, and therefore were not included.
**Human PbN**

*Calbindin*

CAL-ir cell bodies were found loosely distributed at the rostral level around the dorsal tip of the peduncle in the ventral lateral PbN and dorsal medial PbN (Fig. 2A). The cells in these areas were small or medium sized and were surrounded by a very loose network of fibers (Fig. 2D). They consisted of both ovoid and triangular shapes with two or three dendrites.

At the mid level two distinct cell and fiber groups emerged in the external lateral PbN and medial PbN. The medial PbN consisted of medium sized cells with at least two dendrites. CAL-ir cell bodies appeared directly central to the medial face of the peduncle and were scattered medially towards the locus coeruleus (Fig. 2E). The external lateral PbN contained a band of highly dense fibers in the dorsal-ventral direction (Fig. 2F). Far fewer cells were observable here compared to the medial PbN. Those present were obscured by surrounding fibers.

At the caudal level the external lateral PbN and external medial began to merge around the ventral portion of the peduncle (Fig. 2G). The external lateral PbN group moved ventrally and expanded into the KF nucleus (Fig. 2I). Most of the fibers and cell bodies in the external lateral PbN were contained in one dense area roughly in the shape of a bean. Cell bodies and fibers were scattered outward from this group in the dorsolateral and ventromedial directions. Cells in this area were a mix of ovoid and triangular shapes and were similar in size to those found at more rostral levels. The external medial PbN consisted of a dense assortment of cell bodies and fibers which
expanded into the medial PbN (Fig 2H). This group was elongated and contained a less dense fiber arrangement compared to the external lateral PbN. Traversing fibers were found projecting from the external medial PbN through the peduncle towards the external lateral PbN.
Figure 2. (A) Photoshop drawings of coronal sections through the human brainstem, at the level of the rostral tip of the SCP. Scattered CAL-ir cells and fibers surround the dorsorostral tip of the SCP. Box in A corresponds to the area of the photomicrograph in D. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. The drawings correspond approximately to the levels shown in Fig. 1A. (G,H,I) The PbN at the level shown in C, is illustrated at low magnification in G and higher magnification in H and I indicated by boxes.; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar (D): 100 nm. Scale bar (G-I): 300 nm.

Cholecystokinin

CCK-ir sections were characterized by cell bodies with clear cell profiles and moderate networks of fibers. At the rostral level cell bodies were evenly dispersed around the entirety of the rostral tip of the peduncle in the central lateral PbN, ventral lateral PbN, and medial PbN. The cell bodies at this level were small and had very short and wide dendrites (Fig. 3D).

Fibers and cells became more organized at the mid level. A small region of loosely organized fibers was present in the ventral lateral PbN. The central lateral PbN contained cell bodies and fibers in a streak parallel to the peduncle (Figs. 3E & 3F). There was moderate fiber density surrounding the cell bodies allowing the
identification of cell profiles. The cell bodies here were moderate in size and triangular shaped with two or three dendrites. In the medial PbN cell bodies and fibers were located along the entire border of the peduncle with most density in the dorsal medial PbN.

At the caudal level there were dense clusters cells in the medial PbN, external medial PbN, and external lateral PbN (Figs. 3G-3I). The cells in the external lateral PbN were triangular and enveloped in a moderately dense fiber field which formed an oval shape. The external medial PbN consisted of lesser fiber density than that of the external lateral PbN. The area was defined by moderately sized triangular cells. The third dense cluster of cells was located in the medial PbN (Fig. 3H). Smaller cells and fibers were bridging between this area and the external medial PbN, dispersed by the perpendicular fibers of the SCP.
Figure 3. (A) Photoshop drawings of coronal sections through the human brainstem, at the level of the rostral tip of the SCP. Scattered CCK-ir cells and fibers surround the rostral tip of the SCP. Box in A corresponds to the area of the photomicrograph in D. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. The drawings correspond approximately to the levels shown by the lines in Fig. 1A. (E,F) The lateral PbN, equivalent to the level shown in B, is illustrated at low magnification in E. A box specifies the area at higher magnification shown in F. (G,H,I) The ventrolateral and ventromedial PbN, equivalent to the level shown in C, is illustrated at low magnification in G. Boxes specify the area at higher magnification shown in H and I. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar (D): 100 nm. Scale bar (G-I): 300 nm

Dynorphin

Cell bodies and fibers were found in the DYN-ir human sections. At the rostral level fibers surrounded the dorsal tip of the peduncle in the central PbN, ventral lateral PbN, and dorsal medial PbN. Cell bodies were clearly visible around most of the peduncle’s perimeter at this level. A cell group was present in the central lateral PbN (Fig. 4E). This group consisted of triangular cells of moderate size in close proximity
with three dendrites. Neither the medial PbN or KF nucleus contained any significant groupings of cells but did contain cell bodies scattered throughout.

At the mid level the most notable DYN-ir group was in the external lateral PbN. It contained cell bodies and a moderate fiber field associated in a thin streak parallel with the peduncle (Fig. 4F). The medial PbN contained scattered DYN-ir elements along the entire border of the peduncle with the most fiber present in the dorsal medial PbN.

At the caudal level DYN-ir elements were most abundant in the medial PbN. The dorsal medial PbN was primarily fibers with some cell bodies scattered throughout. The medial PbN contained scattered cells throughout and a localized group of cells of varying shapes and sizes (Fig. 4G). This group was surrounded by a loose fiber field. The external lateral PbN contained a moderately dense region of fibers associated around a large blood vessel (Fig. 4H). Very few cells were present in this area.
**Figure 4.** (A) Photoshop drawings of coronal sections through the human brainstem, at the level of the rostral tip of the SCP. Scattered DYN-ir cells and fibers surround the rostral tip of the SCP. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. The drawings correspond approximately to the levels shown by the lines in Fig. 1A. (D,E) The lateral PbN, equivalent to the level shown in A, is illustrated at low magnification in D. A box specifies the area at higher magnification shown in E. Box in B corresponds to the area of the photomicrograph in F. (G,H) The ventrolateral and ventromedial PbN, equivalent to the level shown in C and specified by boxes. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar (E, F): 100 nm. Scale bar (D, G, H): 300 nm.

**Galanin**

GAL-ir elements were sparse in the PbN. No cell bodies were found at any rostral-caudal level. The rostral tip of the peduncle had a very loose fiber field at the dorsal portion of the peduncle which continued around to the lateral PbN.

At the mid level there was an increase in fiber presence in the ventral lateral PbN extending slightly into the central PbN. Loose fiber bundles originating from the ventral lateral PbN extended across the peduncle toward the medial PbN but do not make contact. The medial PbN lacks any observable fibers. Despite the clear presence
of the cell bodies in the locus coeruleus, there were no observable cell bodies in any region of the PbN.

At the caudal level two subnuclei contained GAL-ir fibers. In the external lateral PbN (Fig 5D &5E), the fiber field was moderately dense and confined to a small oval shaped area. This area was associated in a dorsal-ventral direction parallel to the lateral border of the brainstem. The second fiber group was loosely organized and extended across the dorsal medial PbN. It was not as dense as the group in the external lateral PbN but was larger. The rest of the medial PbN and the KF nucleus lacked any GAL-ir elements.
**Figure 5.** (A) GAL-ir photoshop drawings of coronal sections through the human brainstem, at the level of the rostral tip of the SCP. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. The drawings correspond approximately to the levels shown by the lines in Fig. 1A. (D,E) The lateral PbN, equivalent to the level shown in C, is illustrated at low magnification in D. A box specifies the area at higher magnification shown in E. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar: 300 nm

**Neurotensin**

We found no immunoreactive cell bodies at the rostral level, but NT-ir fibers were present in the central lateral PbN, ventral lateral PbN and dorsal tip of the peduncle.

At the mid level a dense and compact group of fibers were present in the external lateral PbN between the edge of the peduncle and the border of the brainstem (Figs. 6D & 6E). The fibers here were clustered around a large blood vessel. A small field of fibers was also located in the dorsal medial PbN. No NT-ir cells were present at this level.

At the caudal level there were two large fiber networks with scattered NT-ir cells embedded within. The first fiber group was located in the external lateral PbN
(Fig. 6I). This fiber group was extremely dense and was confined within a triangular-shaped area with well-defined borders. Any cell bodies that may have been present were obscured by the dense mesh of fibers. The entire medial PbN consisted of an extensive and moderately dense network of fibers with the most NT-ir prominence in the external medial PbN and medial PbN (Fig. 6F-6H). Embedded throughout the fibers were oval-shaped cells with two or three dendrites. Thin fibers traverse between the external medial PbN and the external lateral PbN.
Figure 6. (A) NT-ir Photoshop drawings of coronal sections through the human brainstem, at the rostral level. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. (D, E) The lateral PbN, equivalent to the level shown in B, is illustrated at low magnification in H and higher magnification in I. (F-I) The medial and lateral PbN at the most caudal level, equivalent to the level shown in C, is illustrated at low magnification in D. Boxes specify the area at higher magnification shown in G, H, and I. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar: 300 nm

Somatostatin

SOM-ir elements were most prominent around the dorsal half of the peduncle. At the rostral level cell bodies were found in two groups: one in the central lateral PbN (Figs. 7D & 7F) and another in the medial PbN (Figs. 7D & 7E). The group of cells in the central lateral PbN were restricted to a small region surrounded by a loose mesh of fibers. The cells were oval shaped and had very short dendrites which contrasted the distinct triangular cells with thin expansive dendrites found in the medial PbN. The cells in the medial PbN were spaced far away from each other. Other cell bodies and fibers were located sparsely along the rostral tip of the peduncle in the ventral lateral PbN and dorsal medial PbN.
At the mid level, fibers and cells were present in the central lateral PbN, dorsal lateral PbN, dorsal medial PbN, and medial PbN. The medial PbN cell group moved laterally, closer to the peduncle, and consisted of cells that were similar to those found at more rostral levels (Figs. 7G & 7H). The cells in the central lateral PbN became much less dense and expanded into the dorsal lateral PbN. Fibers appeared in the dorsal medial PbN at the tip of the peduncle. The fiber network in this area covered a wide area at more caudal regions.

At the caudal level many of the SOM-ir elements spread out, especially in the lateral PbN and medial PbN. The dorsal medial PbN contained a dense fiber field (Figs. 7I). These fibers extended ventrally and the area also contained a few scattered cells. A second fiber field of moderate density was observed in the external lateral PbN adjacent to a large blood vessel. The KF nucleus did not contain any significant SOM-ir elements at any level of the PbN.
Figure 7. (A) SOM-ir photoshop drawings of coronal sections through the human brainstem, at the level of the rostral tip of the SCP, as indicated in Fig. 1A. Two SOM-ir cell groups surround the rostral tip of the SCP. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. The drawings correspond approximately to the levels shown by the lines in Fig. 1A. (D, E, F) The ventromedial and central lateral PbN, equivalent to the level shown in A, is illustrated at low magnification in D. Boxes specify the area at higher magnification shown in E and F. (G, H) The ventromedial PbN, equivalent to the level shown in B, is illustrated at low magnification in G. A box specifies the area at higher magnification shown in H. (I, J) The dorsomedial and ventrolateral PbN, equivalent to the level shown in C. Boxes in C specify the area at high magnification shown in I and J. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar: 300 nm

Substance P

SP-ir elements were prominent in the PbN at all levels. At the rostral level fibers were present in the KF nucleus, external lateral PbN, central lateral PbN, dorsal lateral PbN, ventral lateral PbN, and the dorsal medial PbN. Fiber density was greatest in the dorsal half of the lateral PbN. Bundles of fibers projected from these dense fiber fields in the medial direction across the peduncle. Cell profiles were difficult to
observe in the lateral PbN but could be examined clearly in the KF nucleus due to a more dispersed fiber field (Figs. 8D & 8E). The cells were triangular with typically three very prominent dendrites. The dorsal medial PbN contained only a few fibers. The fiber network was much less dense than in the lateral PbN.

At the mid level the fiber field along the lateral PbN became increasingly localized to the central lateral PbN (Figs. 8F & 8G). The ventral lateral PbN consisted of a dense fiber network confined to a small area directly adjacent to the border of the peduncle wrapping around to the dorsal medial PbN. The fiber network in the external lateral PbN was less dense and expanded into the KF nucleus. A loose fiber field covered the dorsal medial PbN and medial PbN. The central medial PbN contained a few cell bodies which trailed off from the locus coeruleus. These were the only cell bodies clearly visible due to a lack of SP-ir fibers in this area.

At the caudal level the PbN contained very dense fiber staining. Most notably was the extremely dense, triangular shaped fiber field in the external lateral PbN which extended into KF nucleus (Figs. 8H & 8I). A few cell profiles were visible in this area but were concealed underneath the dense fibers. The entire medial PbN was covered by a moderately dense fiber field and was densest in the external medial PbN. At the ventral portion of the peduncle fibers traversed away from the external medial PbN across the peduncle towards the external lateral PbN and KF nucleus.
Figure 8. (A) SP-ir photoshop drawings of coronal sections through the human brainstem, at the level of the rostral tip of the SCP, as indicated in Fig. 1A. (B) Photoshop drawings of the human brainstem at the mid level. (C) Photoshop drawings of the human brainstem at the caudal level. The drawings correspond approximately to the levels shown by the lines in Fig. 1A. (D, E) The ventrolateral PbN, equivalent to the level shown in A, is illustrated at low magnification in D. A box specifies the area at higher magnification shown in E. (F, G) The dorsolateral PbN, equivalent to the level shown in B, is illustrated at low magnification in F. A box specifies the area at higher magnification shown in G. (H, I) The ventrolateral PbN, equivalent to the level shown in C. A box in H specifies the area at high magnification shown in I. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; dIV: decussation of the trochlear nerve; tV: tract of the trigeminal. Scale bar: 300 nm

*Cebus apella* PbN

*Calbindin*

At the rostral level CAL-ir elements were widely distributed and a loose network of cell bodies and fibers wrapped around the dorsal tip of the peduncle and continued ventrally along the medial PbN. The cells were triangular with well defined, thin dendrites. Numerous fiber projections traversed the rostral top of the peduncle.

At the mid level many uniform triangular cell bodies were found in the area of the dorsal medial PbN at the waist area of the peduncle (Figs. 9D & 9E). The lateral
PbN consisted primarily of fibers which collected into a moderately dense region in the central lateral PbN. The cell bodies in this region were typically ovoid with two dendrites. In the external lateral PbN there was a group of ovoid and triangular cells of moderate size. These cell bodies were enmeshed in an array of fibers that enveloped the ventral portion of the peduncle and continued into the KF nucleus (Figs. 9D & 9F).

At the caudal level there were two cell groups. The cells that were present in the external lateral PbN at more rostral levels were no longer visible and instead a dense band of fibers appeared. Also, a dense cluster of cells emerged in the external medial PbN (Figs. 9G & 9I). It contained an extensive network of triangular cells with two or three long dendrites. The other group was located in the dorsal medial PbN. It was centralized to the waist area of the peduncle and contained cells similar to those in the external medial PbN (Figs. 9G & 9H). Directly adjacent to this cell group was a moderately dense network of fibers in the ventral lateral PbN. Another larger fiber network was seen in the external lateral PbN running parallel to the peduncle (Fig. 9G). This area was essentially devoid of GAL-ir cells.
**Figure 9.** (A) Photoshop drawings of CAL-ir coronal sections through the Cebus monkey brainstem, at the level of the rostral tip of the SCP. Scattered CAL-ir cells and fibers surround the rostral tip of the SCP. (B) Photoshop drawings of the Cebus monkey brainstem at the mid level. (C) Photoshop drawings of the Cebus monkey brainstem at the caudal level. (D,E,F) The PbN at the level shown in B, is illustrated at low magnification in D. Higher magnification views of the same field both dorsally and ventrally are shown in E and in F respectively indicated by boxes. (G,H,I) The parabrachial complex at the level shown in C, is illustrated at low magnification in G. Higher magnification views of the same field both dorsally and ventrally are shown in H and in I respectively indicated by boxes. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; tV: tract of the trigeminal. Scale bar: 300 nm

**Cholecystokinin**

*Cebus* monkey CCK-ir sections were composed of extremely dense fiber fields which made it impossible to identify cell profiles. At rostral level immunoreactive cell bodies were visible probably due to a slightly less dense fiber network around the peduncle. Cell bodies were abundant and consisted of a mix of oval and triangular cells with thick dendrites. The densest collection of cell bodies was found in the central lateral PbN and dorsal lateral PbN. Numerous fiber projections enveloped the most dorsal and rostral portions of the peduncle.
At the mid level fiber density increased drastically. Only a few scattered medium sized triangular cells were visible in the ventral lateral PbN along the perimeter of the peduncle (Fig 5D & 5E). Fibers were distributed around the entire rostral tip of the peduncle in the central lateral PbN, ventral lateral PbN, and dorsal medial PbN.

At the caudal level two networks of cells and fibers in the external medial PbN and external lateral PbN enveloped the ventral portion of the peduncle (Fig 5F & 5G). The two groups were connected via a dense fiber network. The external medial PbN consisted of a web-like matrix of fibers with cell bodies scattered throughout. The external lateral PbN was comprised of a moderately dense assortment of fibers running parallel with the peduncle and cell bodies scattered within. This moderately dense fiber field continued into the central lateral PbN and ventral lateral PbN.
Figure 10. (A) Photoshop drawings of CCK-ir coronal sections through the Cebus monkey brainstem, at the level of the rostral tip of the SCP. (B) Photoshop drawings of the monkey brainstem at the mid level. (C) Photoshop drawings of the monkey brainstem at the caudal level. (D,E) The lateral PbN, equivalent to the level shown in B, is illustrated at low magnification in D. A box specifies the area at higher magnification shown in E. (F,G) The ventral tip of the SCP at the level of the locus coeruleus, equivalent to the level shown in C, is illustrated at low magnification in F. A box specifies the area at higher magnification shown in G. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; tV: tract of the trigeminal. Scale bar (E, G): 100 nm. Scale bar (D, F): 300 nm.

**Dynorphin**

DYN-ir elements were abundant at all levels of the monkey parabrachial region but neither cell bodies nor fibers seemed to distribute within any defined boundaries. At the rostral level, cell bodies were found scattered from the ventral lateral PbN around the dorsal tip of the peduncle and along the entire medial PbN. The dorsal medial PbN contained the highest cell density (Fig. 11D). Both the ventral lateral and dorsal medial PbN consisted of similar cells.

At the mid level immunoreactive cell bodies were found scattered around the peduncle (Figs. 11E & 11F), with the highest density within the ventral lateral PbN and the external medial PbN. Numerous fiber projections traversed the peduncle from
the medial PbN to the lateral PbN. An oval shaped collection of dense fibers and a few cell bodies were located in the dorsal lateral PbN (Fig. 11E).

At the caudal level almost no cell bodies were present in the lateral PbN but a moderately dense collection of fibers remained in the ventral lateral PbN. In the medial PbN cell bodies were present and enmeshed in a network of fibers (Figs. 11G & 11H). Immunoreactive fibers could also be traced from the cell groups in the external medial PbN to the dense fiber field in the external lateral PbN. These two groups surrounded the ventral edge of the peduncle.
Figure 11. (A) Photoshop drawings of DYN-ir coronal sections through the Cebus monkey brainstem, at the level of the rostral tip of the SCP. Box in A corresponds to the area of the photomicrograph in D. (B) Photoshop drawings of the monkey brainstem at the mid level. (C) Photoshop drawings of the monkey brainstem at the level of the caudal tip of the SCP. (E,F) The medial PbN, equivalent to the level shown in B, is illustrated at low magnification in E. A box specifies the area at higher magnification shown in F. (G,H) The ventral tip of the SCP, equivalent to the level shown in C, is illustrated at low magnification in G. A box specifies the area at higher magnification shown in H. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; tV: tract of the trigeminal. Scale bar (D): 100 nm. Scale bar (E-H): 300 nm.

Galanin

Monkey GAL-ir sections contained only fibers. No cell bodies were present at any level, including within the locus coeruleus. The fibers in these sections all had prominent varicosities. At the mid level the fiber distribution was expanded across the central lateral PbN, ventral lateral PbN and medial PbN.

At the caudal level the fiber fields became increasingly contained into two moderately dense areas. The ventral lateral PbN contained a dense cluster of fibers in close association with the border of the peduncle which expanded into the central lateral PbN (Figs. 12C & 12D). The dorsal medial PbN contained the most fiber
density which disappeared at the ventral regions (Figs. 12C & 12E). The ventral bottom of the SCP was completely devoid of GAL-ir elements.
**Figure 12.** (A) GAL-ir photoshop drawings of the monkey brainstem at the mid level. (B) Photoshop drawings of the monkey brainstem at the caudal level. (C,D,E) The medial PbN and lateral PbN, equivalent to the level shown in B, is illustrated at low magnification in C. Boxes specify the area at higher magnification shown in D and E. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; tV: tract of the trigeminal. Scale bar: 300 nm

**Neurotensin**

NT-ir reactive elements surrounded the peduncle at the mid level. A dense fiber group was located in the dorsal lateral PbN and central lateral PbN that projected across the peduncle to medial PbN (Fig. 13C). Cell bodies were obscured by the presence of NT-ir fibers. The ventral tip of the peduncle was enmeshed in a network of fibers and cell bodies between the external medial PbN and external lateral PbN (Fig. 13D).

The caudal level showed the most extensive presence of fibers and cell bodies. Cell bodies were localized to two dense groups in the external lateral PbN and the external medial PbN (Figs 13E-13G). The group in the lateral external PbN was associated in a dense and narrow configuration closely bordering the peduncle and expanding into the central lateral PbN. A dense fiber field in the area made it difficult to observe the cell bodies. The external medial PbN cell group was associated in a circular concentration of cells extensively enmeshed in traversing fiber projections.
across the ventral tip of the peduncle. The cells were small-medium sized and oval shaped. NT-ir elements were not restricted to the groups described. Each portion of the PbN at this level contained cells and fibers although they were mostly contained in the groups described above.
**Figure 13.** (A) NT-ir photoshop drawings of coronal sections through the Cebus monkey brainstem at the mid level. (B) Photoshop drawings of the monkey brainstem at the caudal level. (C,D) The ventral tip of the peduncle, equivalent to the level shown in A, is illustrated at low magnification in C. A box specifies the area at higher magnification shown in D. (E-G) The medial and lateral PbN cell groups, equivalent to the level shown in B, is illustrated at low magnification in D. Boxes specify the area at higher magnification shown in F and G. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; tV: tract of the trigeminal. Scale bar (G): 100 nm. Scale bar (C-F): 300 nm.

**Somatostatin**

SOM-ir elements in the Cebus monkey consisted mostly of dense fibers. At the rostral level two dense fiber fields flanked the peduncle (Fig. 14D). The first region was in the central lateral PbN and the second was in the external medial PbN. In these region it was not possible to identify clearly cell bodies, although some seemed to be present, obscured by the dense fiber network.

At the mid level the SOM-ir fiber group in the central lateral PbN increased in fiber density and began to expand into the ventral lateral PbN and external lateral PbN. Fibers remained along the entirety of the medial PbN but loosely organized, and split into the ventral lateral PbN and external lateral PbN.
At the caudal level the fiber network in the central lateral PbN split into the ventral lateral PbN and external lateral PbN. At this level cell bodies became visible in the external medial PbN and external lateral PbN. These two areas were joined together by thick well-defined traversing fibers at the ventral tip of the peduncle (Figs. 14E & 14F). Cell bodies were most visible in the external medial PbN, with some cell bodies present in the external lateral PbN as well but obscured by the very dense fiber field. A loose mesh of SOM-ir fibers covered the entirety of the medial PbN.
**Figure 14.** (A) SOM-ir photoshop drawings of coronal sections through the Cebus monkey brainstem, at the level of the rostral tip of the SCP. Box in A corresponds to the area of the photomicrograph in D. (B) Photoshop drawings of the monkey brainstem at the mid level. (C) Photoshop drawings of the monkey brainstem at the caudal level. (E,F) The ventral tip of the SCP, equivalent to the level shown in C, is illustrated at low magnification in E. A box specifies the area at higher magnification shown in F. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle; tV: tract of the trigeminal. Scale bar: 300 nm

**Substance P**

The monkey PbN contained a large amount of SP-ir elements at all levels but specifically fibers, which made it difficult to identify cell bodies. At the rostral level the rostral tip of the peduncle was surrounded by fibers. The areas with the highest fiber density were the dorsal lateral PbN, external lateral PbN, and external medial PbN (Fig. 15D). Within these areas some cell profiles could be identified but the dense network of fibers made it impossible to clearly identify any specific characterization of size or shape.

At the mid level the fiber fields in the dorsal lateral PbN began to merge into the central lateral PbN and ventral lateral PbN. The fiber network in the medial PbN expanded across the entire medial edge of the peduncle.
The caudal level contained four areas of interest. The ventral lateral PbN contained an oval shaped, dense network of fibers which continued through the central lateral PbN. Thick fibers traversed across the peduncle to the dorsal medial PbN which contained a larger, but less dense fiber field (Figs. 15E & 15G). Fibers continued along the rest of the medial PbN from this fiber field. Cell profiles were somewhat visible in the ventral lateral PbN but not the dorsal medial PbN. The ventral tip was surrounded by fibers joining the external medial PbN and external lateral PbN (Figs. 15E & 15F). These fibers continued through the KF nucleus.
Figure 15. (A) SP-ir photoshop drawings of coronal sections through the Cebus monkey brainstem, at the level of the rostral tip of the SCP. (B) Photoshop drawings of the monkey brainstem at the mid level. (C) Photoshop drawings of the monkey brainstem at the caudal level. (D) The rostral tip of the SCP, equivalent to the level shown in A. (E) The mid portion of the SCP, equivalent to the level shown in B. Boxes specify the areas at higher magnification shown in F & G. M: medial; V: ventral; D: dorsal; L: lateral; LC: locus coeruleus; LL: lateral lemniscus; SCP: superior cerebellar peduncle; 4V: fourth ventricle. Scale bar: 300 nm

Comparisons

Most of the neuropeptides in this study contained at least partial overlap in distribution. Some of the neuronal groups in the monkey were absent in human brainstem. However, humans exhibited some neuronal groups that were absent in the monkey.

Calbindin

The monkey and human sections shared some similarities in CAL-ir distribution. In both, at the most rostral levels CAL-ir elements were found distributed around the dorsal tip of the peduncle in the ventral lateral PbN and dorsal medial PbN (Figs. 2A & 9A), and these areas contained cell bodies. However, in the monkey CAL-ir elements extended further along the peduncle into the central lateral PbN and central medial PbN which was not seen in the human.
At the mid level the band of dense fibers in the central lateral PbN and the moderately dense field of fibers in the ventral lateral PbN that were present in the monkey were absent in the human. Furthermore, there was a group of cells in the external lateral PbN of the monkey that corresponded with a dense scattered collection of cells and fibers in the external lateral PbN of the human (Figs. 2F & 9F). However, this region contained considerably more CAL-ir cells in the monkey than in the human.

At the caudal level the ventral part of the peduncle was surrounded by the external medial PbN and the external lateral PbN which extended into the KF nucleus. In the human, this cell group occupied a characteristic oval shaped area that we identify as the external lateral PbN in close association with the border of the peduncle. Surprisingly, the external lateral PbN of the monkey only contained fibers which expanded into the central lateral PbN and we did not identify cell bodies (Figs. 2G & 9G). The external medial PbN of the monkey formed a large complex at the caudal edge of the peduncle, including cell bodies that were smaller and more numerous than those in the external medial PbN of the human. The expansion of the peduncle in the human may have split this CAL-ir cell cluster into the external lateral and external medial PbN. Furthermore, in the human this region was more elongated and expanded into the central medial PbN whereas in the monkey it had a circular shape (Figs. 2H & 9I). The monkey contained an additional CAL-ir cell and fiber group in the dorsal medial PbN (Fig. 9H) that was not found in the human.
Cholecystokinin

CCK-ir cells were scattered around the peduncle in both monkey and humans. At the rostral level in both the human and monkey PbN there were abundant immunoreactive cell bodies spanning the KF nucleus, central lateral PbN, ventral lateral PbN, dorsal lateral PbN, dorsal medial PbN, and central medial PbN (Fig. 3D). However, the monkey lacked fibers in the external lateral PbN that were seen in the human, but did contain fibers in the external medial PbN, as were also seen in the human.

At the mid level both the monkey and human contained groups of large, cells and fibers in the ventral lateral PbN and central lateral PbN. Cell bodies in both monkey and human were similar in shape and size and located in close association with the edge of the peduncle in an elongated fashion (Figs. 3F & 10E). In the human, cell bodies and fibers were present and clearly visible in the dorsal medial PbN and the central medial PbN. In the monkey, the high density of fibers made it difficult to observe any cell bodies in the medial PbN. CCK-ir elements also expanded into the external medial PbN and dorsal lateral PbN of the monkey, as in the human.

At the caudal level CCK-ir fibers in the external medial PbN and external lateral PbN surrounded the ventral portion of the peduncle. In the monkey, cell bodies were observed in the external medial nucleus with fiber connections to the external lateral nucleus. The cells of the external lateral PbN of the monkey were arranged in a narrow linear continuum along the peduncle, different from the oval-shaped organization of the cells in this area of the human (3I & 10G). The medial PbN of the
human contained cell bodies and fibers which were scattered through external medial PbN, central medial PbN, and dorsal PbN (Figs. 3G). CCK-ir elements were only present in the dorsal medial PbN and external medial PbN of the monkey.

Additionally, the monkey contained a dense network of parallel fibers in the central lateral PbN (Fig. 10F) that was not found in the human, as the peduncle extends against the border of the brainstem.

**Dynorphin**

DYN-ir cell bodies were more abundant and extensive in the human than the monkey. Cell bodies and fibers were found throughout the central lateral PbN (Fig. 4E), dorsal lateral PbN, ventral lateral PbN, dorsal medial PbN (Fig. 11D), central medial PbN, and external medial PbN. These cells were significantly more organized in the lateral PbN of the human at the rostral level and the mid level. No DYN-ir elements were found in the KF nucleus or external lateral PbN.

At the mid level CCK-ir fibers in the monkey completely surrounded the peduncle and were found in all subnuclei except for the KF nucleus. The most prominent area of staining was the dorsal lateral PbN (Fig. 11E). Distribution of DYN-ir elements in the external lateral PbN and all of the medial PbN subnuclei were similar in the human and the monkey (Fig. 4F). Except for a few scattered cell bodies present in the KF nucleus of the human.

At the caudal level both the monkey and the human contained a moderately dense fiber field and no cell bodies in the lateral PbN (Figs. 4H & 11G). This area was
located at a more extreme lateral position than the CAL-ir and CCK-ir cell groups found in this area. The monkey also contained a moderately dense fiber field in the ventral lateral PbN. The central medial PbN and external medial PbN contained a large amount of cells in the monkey but not in the human (Fig. 11H). Human sections only showed small scattered cell bodies throughout the central medial PbN and dorsal medial PbN (Fig. 4G).

Galanin

There were few common elements in the distribution of GAL in the monkey and human. GAL-ir sections did not contain cell bodies in the PbN of either species. In humans, most GAL-ir fibers were localized to a small fiber field in the central lateral PbN which moved ventrally into the external lateral PbN at caudal levels (Fig. 5E). Staining in the external lateral PbN remained more dorsal than the human sections of CCK-ir, CAL-ir, or DYN-ir. This group of GAL-ir fibers was absent in the monkey, but GAL-ir fibers wrapped around the tip of the peduncle in the monkey through the medial PbN, central lateral PbN, and ventral lateral PbN (12C-12E).

Neurotensin

In contrast to GAL, NT-ir sections showed significant cross species similarities in the distribution of –ir elements in the PbN area. However, at the mid level NT-ir elements were much more clearly defined in human sections than monkey. A dense collection of fibers in the central lateral PbN was found in nearly the same location in
humans and the monkey (Figs. 6D & 13C). This region expanded into the dorsal lateral PbN of the monkey and the external lateral PbN of the human. The dorsal medial PbN was the only other area that contained NT-ir elements in the human. The entire peduncle of the monkey was surrounded by a moderately dense NT-ir fiber field which spread across all subnuclei of the PbN.

At the caudal level the dense fiber field in the central lateral PbN continued into a large network of fibers and cells in the external lateral PbN of the monkey. The external lateral PbN of the human contained a large triangular field of fibers with cell bodies scattered throughout (Figs. 6I & 13G). This group was larger than the same area in human CCK-ir, CAL-ir, DYN-ir, and GAL-ir sections but was very similar to the distribution of SP-ir. A dense cluster of cells was also present in the external medial PbN of the monkey. In the human a less localized cell group appeared in the external medial PbN and expanded dorsally into the central medial PbN and dorsal medial PbN (Figs. 6G, 6H, 13F). These cell bodies were very similar to those found in the external medial PbN and external lateral PbN of the monkey. Additionally, the monkey contained fibers in the ventral lateral PbN and central lateral PbN.

**Somatostatin**

The location of SOM-ir elements was conserved between the human and the monkey. At the rostral level SOM-ir groups appeared in the central lateral PbN and the external medial PbN (Figs. 14D, 7E, 7F). Human sections contained primarily cell
bodies while monkey sections consisted primarily of fibers. The ventral lateral PbN and dorsal medial PbN also contained scattered SOM-ir elements in the human.

At the mid level the central lateral PbN contained a group of cells and fibers in the human and fibers in the monkey which expanded into the dorsal lateral PbN. Additionally, the external medial PbN of the human contained a group of cells which spread along the ventral half of the peduncle. (Fig. 7H). A few loose fibers and cells were also present in the dorsal medial PbN of the human. The entire medial PbN of the monkey was covered by a loose fiber field.

The greatest difference in SOM-ir cytoarchitecture occurred at the caudal level. The human PbN consisted of two areas of SOM-ir cell and fiber concentrations: the first of which was in the dorsal medial PbN (Fig. 7I) and the second was in the external lateral PbN adjacent to a large blood vessel. This area was similar to, although much less dense, than the external lateral PbN of the monkey (Figs. 7J & 14F). The dorsal medial PbN of the monkey also contained a very loose SOM-ir fiber field. The most prominent staining in the monkey occurred in the external lateral PbN, external medial PbN, and ventral lateral PbN (Fig. 14E). The ventral lateral PbN and external medial PbN of the human did not contain any SOM-ir elements.

*Substance P*

There was a large amount of SP-ir elements in the PbN area. At the rostral level prominent staining occurred in the central lateral PbN, external lateral PbN, and external medial PbN of the monkey (Fig. 15D). Human sections showed the greatest
fiber and cell densities in the ventral lateral PbN, central lateral PbN, external lateral PbN, and KF nucleus (Figs. 8D & 8E). Of these areas cells were most visible in the KF nucleus.

At mid level a fiber field extended over the entire area of the lateral PbN. In both the monkey and human most fiber density was localized to the central lateral PbN (Figs. 8F & 8G). Both species also contained a loose fiber field which extended down the entirety of the medial PbN.

The ventral lateral PbN, dorsal medial PbN, external medial PbN, and external lateral PbN contained the densest SP-ir elements in the monkey at the caudal level. The fibers and cells of the external lateral PbN and external medial PbN surrounded the ventral portion of the peduncle in both species (Figs 8H & 15F). SP-ir elements originating in this area extended towards the KF nucleus. Along the medial face of the peduncle, scattered cell bodies and fibers extended along the entire medial PbN with slightly higher density in the dorsal medial PbN. In the monkey, thick fiber projections from this area traversed across the peduncle to the ventral lateral PbN where a thick mesh of fibers and muddied cell bodies were located. The peduncle meets the border of the brainstem in this area of the human so the ventral lateral PbN does not exist.

**Parcellation of the human external lateral PbN**

The external lateral PbN of the human showed well-defined boundaries for each neuropeptide at the caudal level. A characteristic triangular region of NT–ir and SP-ir elements outlined a clear border for the external lateral PbN. Within this region, GAL
fibers appeared in the most dorsal tip of the external lateral PbN, where the border of the brainstem meets the superior cerebellar peduncle. An elongated oval shaped collection of CAL-ir fibers and cells were found directly adjacent to the superior cerebellar peduncle, extending in the ventral and dorsal directions. CCK-ir fibers and cells were found in a circular area in the middle portion of the external lateral PbN. SOM-ir and DYN-ir fibers were found in the lateral corner of the external lateral PbN, typically surrounding a large blood vessel.

Figure 16. A visual representation of the parcellation of the external lateral PbN.

Yellow covers the entire area of the external lateral PbN and represents NT-ir and SP-ir elements. Green represents GAL-ir elements, red CAL-ir, blue CCK-ir, and orange DYN-ir and SOM-ir elements.
Discussion

The PbN serves as a crucial visceral relay center for afferent information from the brainstem to the forebrain in both rats (Ricardo & Koh, 1978) and primates (Beckstead & Morse, 1980). The PbN in the rat has been found to contain at least 12 subnuclei (Fulwiler & Saper, 1984). Homologous CGRP-ir innervation in the PbN of the rat and human has been established in the external medial nucleus and external lateral nucleus (de Lacalle & Saper, 2000). Few other comparative studies have been published focusing on cross species similarities between the rat and primate PbN.

Our observations indicate that the neuropeptides CAL, CCK, DYN, GAL, NT, SOM and SP are present in the primate PbN. The anatomical distribution of these neuropeptides in the rat was strikingly conserved in the primate brainstem. Most notable were the similarities between the primate and the rat in the cytoarchitecture of the external medial nucleus and external lateral nucleus. Clearly, the extreme expansion of the human superior cerebellar peduncle has altered the topographical organization of these cell groups in the human. However, using the peduncle as a topographical marker, we found many similarities in the distribution of cell bodies and fibers. In general, neuronal groups in the primate PbN became increasingly organized in the rostral to caudal direction.

Both the monkey and human expressed CAL-ir elements in the external lateral PbN and external medial PbN. The monkey also contained significant CAL-ir elements in the ventral lateral PbN and dorsal medial PbN. CAL-ir neurons have been identified in the medial PbN of the rat (Sequier et al., 1990) as well as the lateral PbN.
(Zhang & Xiong, 2004). There is a lack of significant literature on CAL projections involving the PbN in any species, so it is possible to determine the role of CAL in these regions of the PbN. However, our work confirms the presence of this neuropeptide in both the lateral PbN and medial PbN in the monkey and human.

The presence of CCK-ir neurons in the external medial PbN and external lateral PbN of the monkey and human was similar. In the rat, CCK-ir fibers and neurons were also described prominently in the external lateral PbN with weaker labeling in the central lateral PbN (Herbert & Saper, 1990). Studies suggest CCK’s role in the lateral PbN of the rat as a satiety promoter (Li & Rowland, 1994) and an inhibitor of NaCl and water intake (Fratucci et al., 2001). The localization of CCK-ir elements to the external lateral PbN in mammals suggest that these functional roles are likely conserved in this subnucleus. The medial PbN of the primate seemed to contain more CCK-ir than what has been described in the rat (Fulwiler & Saper, 1985).

DYN-ir elements were found in the external lateral PbN, central medial PbN, and dorsal medial PbN of the primate. The monkey contained additional DYN-ir elements in the ventral lateral PbN not found in the human. Mu-opioid receptors were expressed in the external lateral PbN and adjacent lateral crescent subnuclei of the rat (Chamberlin et al., 1999). In the rats, DYN neuronal projections from the solitary tract, spinal cord, and trigeminal dorsal horns synapse in these areas and continue to the amygdala (Chamberlin et al., 1999). Our work suggests that DYN-ir cells and fibers in the PbN mediate or modulate some afferent flow of nociceptive information through the brainstem.
By far, GAL-ir was uniquely distributed in the primate, compared with the rat. GAL-ir fibers were present in the central lateral PbN and dorsal medial PbN of the human and monkey. Only human sections contained GAL-ir fibers in the external lateral PbN and only the monkey contained GAL-ir fibers in the ventral lateral PbN. In the rat, GAL-ir fibers have been found in the central lateral PbN, medial PbN, and external lateral PbN. In addition to these subnuclei, GAL-ir neurons were present in the dorsal lateral PbN, superior lateral PbN, and extreme lateral PbN (Herbert & Saper, 1990). However, no GAL-ir neurons were found in the primate. Lesion studies suggest that GAL in the hindbrain contributes to feeding by inhibiting satiety (Koegler et al., 1999) and preferentially increases the consumption of fat and carbohydrates in rats (Tempel et al., 1988), so perhaps these functions could also be located in the PbN in primates.

The primate PbN contained NT-ir elements in the central lateral PbN, medial PbN, external medial PbN, external lateral PbN, and the KF nucleus. The monkey also contained NT-ir fibers in the dorsal lateral PbN. Interactions between CCK and NT at presynaptic receptors modulate excitatory synaptic transmission in the PbN of the rat (Saleh et al., 1997). In our study we found that the distribution of CCK and NT in the primate almost completely overlap. The highest density of CCK and NT fibers in the rat were reported in the ventral lateral PbN (Block & Hoffman, 1986), suggesting that these neuropeptides could have shifted towards the central lateral PbN and the external lateral PbN in the primate. These peptides also seemed more prominently contained in the medial PbN of the primate than in the rat.
SOM-ir immunoreactivity was found in the central lateral PbN, ventral lateral PbN, dorsal lateral PbN, and external lateral PbN of the primate. The external medial PbN also contained SOM-ir neurons in both the human and monkey but were located more rostral in the human than the monkey brainstem. SOM-ir elements in the rat PbN were primarily described in the dorsal lateral PbN (Block & Hoffman, 1987). While we found SOM-ir elements in the dorsal lateral PbN of the primate, the medial PbN and external lateral PbN contained a denser immunoreactivity. SOM neurons of the PbN are considered a component of the descending pathways from the amygdala and are positioned to exert neuromodulatory effects on central taste processing (Magableh & Lundy, 2014).

SP-ir elements were the most prominent out of all the neuropeptides examined in this study. SP-ir distribution was strikingly conserved from rat to primate in the ventral lateral PbN, central lateral PbN, external lateral PbN, external medial PbN, medial PbN, and the KF nucleus. The monkey also contained significant SP-ir elements in the dorsal lateral PbN. SP-ir was most in the ventral lateral PbN of the monkey and the external lateral PbN of the human. The anatomical distribution of SP closely resembled that of NT and CCK in the primate. In the rat, SP projections from the external lateral nucleus to the central amygdala have been established (Yamano & Hillyard, 1988), which our study shows to be conserved in the primate. Dense SP-ir fibers have been described in the dorsal lateral PbN of the rat as well (Block & Hoffman, 1986). The strong presence of SP-ir fibers in the mammalian external lateral PbN and dorsal lateral PbN suggests that these pathways have been conserved. SP’s
role in modulating stress responses throughout the body implicates SP neurons of the PbN in any number of autonomic processes. These responses include vasodilation, inflammation, nociception, anxiety, and vomiting (O'Connor et al., 2004). SP’s specific roles in the PbN have yet to be established. However, SP’s location in the ventral lateral PbN and external lateral PbN coincides with regions that are involved in mechanisms of cardiovascular function (Mraovitch & Kumada, 1982) and gustatory modulation (Norgren & Pfaffmann, 1975) respectively.
Future Directions

Now that the chemical cytoarchitecture of the PbN has been established between the monkey and the human, it is necessary to further examine similarities between the rat and primate. It is still unclear how similar the rat and primate PbN are in their projections to other CNS structures such as the amygdala and hypothalamus. A study of a species of old world monkey found that while most of the projections of the PbN remained consistent with the rat, projections to the hypothalamus were not as widespread or intense (Pritchard et al., 2000). Additional studies investigating species reorganization of afferent and efferent pathways are required to begin to understand what the functional differences between the rat and the primate may be. Our results can be utilized in this regard. Neurochemical identity of the neurons in the PbN of the primate can be compared with the neurons in known areas of projection of the rat. Consistencies will likely confirm conservation of pathways while differences may indicate reorganization of pathways.

Once differences in the afferent and efferent paths between the rat and the primate are established, behavioral and physiological studies should be the next step. At this point in time a fairly developed idea of the function of each subnuclei should exist. That hypothetical function should be the focal point of the study and experiments should be designed to confirm that function. The best course of action would be lesion studies on the PbN of New World monkeys such as the *Cebus* monkey. These experiments would offer the best parallel to the functioning of the human PbN. Lesions to the lateral PbN would be the easiest to accomplish due to its
location directly adjacent to the external border of the brainstem. Specifically, lesions to the external lateral PbN which seems to contain the most neurochemical conservation in the primate. If possible, additional subnuclei should be targeted within the lateral PbN. Lesion studies in the KF nucleus and medial PbN would be the next logical steps.

Particular interest should be placed on the PbN’s role in taste and gustatory modulation. Taste and gustatory regulation in the PbN were its first observed functions. Thus, there is currently more literature defining these modalities in the PbN than any others. The availability of this body of work will make cross species comparisons between the rat and primate easier. Conservation of respiration, thermoregulation, and cardiovascular pathways through the PbN should subsequently be examined.
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References


http://doi.org/10.1073/pnas.77.10.6207


Horst Herbert, Margaret M. Moga, & Clifford B. Saper. (1990). Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary


http://doi.org/10.1002/1096-9861(20001218)428:3<475::AID-CNE6>3.0.CO;2-9


Leibowitz, S., & Akabayashi, A. (1998). Obesity on a high-fat diet: role of hypothalamic galanin in neurons of the anterior paraventricular nucleus projecting to the median eminence. *Obesity on a High-Fat Diet: Role of*
Hypothalamic Galanin in Neurons of the Anterior Paraventricular Nucleus

Projecting to the Median Eminence. Retrieved from
http://www.jneurosci.org/content/18/7/2709.short


Cholecystokinin-and Dexfenfluramine-Induced Anorexia Compared Using Devazepide and c-Fos Expression in the Rat Brain.
http://doi.org/10.1016/0167-0115(94)90003-5


Modulation of Parabrachial Taste Neurons by Electrical and Chemical Stimulation of the Lateral Hypothalamus and Amygdala.
http://doi.org/10.1152/jn.00828.2004


Magableh, A., & Lundy, R. (2014). Somatostatin and corticotrophin releasing hormone cell types are a major source of descending input from the forebrain


http://doi.org/10.1002/cne.902830302


http://doi.org/10.1002/cne.901900205


Homeostasis Revealed by Mice Lacking Both Vitamin D Receptor and Calbindin-D28k. Retrieved from http://www.jbc.org/content/279/50/52406.short