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Functional Organization Of The Paramedian Reticular Nucleus Of The Cat

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FUNCTIONAL ORGANIZATION OF THE PARAMEDIAN RETICULAR NUCLEUS OF THE CAT

by

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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
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London, Canada

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DEDICATED

To my Mother and Father,

My son, Teddy

and to my wife, Candy

ABSTRACT

The functional organization of the paramedian reticular nucleus (PRN) in the medulla oblongata of the cat was investigated neuroanatomically and electrophysiologically in light of its potential role as an integrator of postural and cardiovascular afferent information. Selective injections of lectin-conjugated horseradish peroxidase (HRP) into each of the dorsal (dPRN) and ventral (vPRN) subdivisions of the PRN resulted in retrograde labeling of a large number of afferent sources including the cerebellar deep nuclei, vestibular nuclei, accessory oculomotor nuclei, solitary nuclei, superior colliculus, bulbar reticular formation, sensorimotor cerebral cortex and spinal cord. These afferent projections to the dPRN and vPRN differed somewhat in the origin and relative magnitudes of their inputs with some terminating in only one of the two subdivisions. Collateral axonal projections of PRN neurones were studied with fluorescence histochemistry focusing on their cerebellopetal and spinopetal connections. Approximately 50% of the axonal projections of the PRN to the ipsilateral cortex of the anterior lobe of the cerebellum were shown to have collateral projections to the corresponding contralateral cortex. Fewer axonal projections from the PRN to the ansiform lobule and the lobulus simplex had collateral projections to the corresponding contralateral cerebellar cortex. Approximately 40% of the neurones in the PRN projecting to the region of the intermediolateral nucleus (IML) at and caudal to the T-2 level were found to distribute collateral axons to the region of the IML at and caudal to the T-4 or T-7 levels.

The region of the PRN was then systematically explored for single units antidromically activated by electrical stimulation of

the IML in chloralosed, paralyzed and artificially ventilated cats. These antidromically identified units were tested for their responses to electrical stimulation of the carotid sinus nerve (CSN) and the pressor area of the fastigial nucleus (FN). Sixty-two single units in the PRN were antidromically activated by stimulation of the IML, of which 40% (25/62) responded orthodromically to stimulation of the CSN and/or the FN. All PRN units antidromically activated by electrical stimulation of the IML were confined largely to the caudal half of the vPRN. Under similar anesthetic conditions, the region of the PRN was again systematically explored for single units orthodromically activated by electrical stimulation of the vestibular nuclear complex (VNC) following chronic surgical ablation of the fastigioreticular input to the PRN. These orthodromically identified units were then tested for their responses to electrical stimulation of the IML and the CSN. Forty-seven single units in the PRN were orthodromically activated with a mean latency of 6.3 ± 0.6 ms to stimulation of the VNC, of which 62% (29/47) were antidromically activated with latencies corresponding to a mean conduction velocity of 48.6 ± 3.5 m/s to stimulation of the IML. None of the orthodromically identified PRN units responded to CSN stimulation. Units which responded antidromically to IML stimulation and orthodromically to VNC stimulation were confined largely to the vPRN.

These experiments provide anatomical and electrophysiological evidence which suggests the existence of direct pathways from neurones in the PRN to the region of the IML which mediate cardiovascular afferent information from the CSN and FN as well as vestibular afferent information. In addition to their obvious role in motor regulation, projections of the motor cortex, accessory oculomotor nuclei, superior colliculus and bulbar reticular formation to the PRN may also influence sympathetic activity

through its connections with the IML. These extensive connections and functional autonomic relations of the PRN suggest a central role in mediating orthostatic reflex activity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3N	oculomotor nerve
5ME	mesencephalic trigeminal nucleus
5MN	motor trigeminal nucleus
5PN	sensory trigeminal nucleus (parvocellular division)
5SM	sensory trigeminal nucleus (magnocellular division)
6M	abducens nucleus
7M	facial nucleus
12M	hypoglossal nucleus
AMB	nucleus ambiguus
AP	area postrema
AQ	aqueduct
BC	brachium conjunctivum
BP	brachium pontis
c	cuneate nucleus
CAE	nucleus caeruleus
CC	central canal
CI	inferior central nucleus (raphe)
CM	centromedian nucleus
CNF	cuneiform nucleus
Com	commissural solitary nucleus
CS	superior central nucleus (raphe)
CSN	carotid sinus nerve
CX	external cuneate nucleus
D	nucleus of Darkschewitsch
DMN	dorsal motor nucleus of the vagus
DN	dentate nucleus

DRI	dorsal nucleus (raphe)
EW	Edinger-Westphal nucleus
FN	fastigial nucleus
FTC	central tegmental field
FTG	gigantocellular tegmental field
FTL	lateral tegmental field
FTM	magnocellular tegmental field
FTP	paralemniscal tegmental field
G	gracile nucleus
HRP	horseradish peroxidase
ICA	interstitial nucleus of Cajal
ICC	inferior colliculus (central nucleus)
IFC	infracerebellar nucleus
IML	intermediolateral nucleus
IN	interposed nucleus
INa	interposed nucleus, anterior division
INp	interposed nucleus, posterior division
INC	nucleus incertus
INT	nucleus intercalatus
ION	inferior olivary nucleus
LLV	ventral nucleus (lateral lemniscus)
LP	lateral posterior complex (thalamus)
MGN	medial geniculate nucleus
MLF	medial longitudinal fasciculus
NTS	nucleus of the solitary tract
OTN	nucleus of the optic tract
P	pyramidal tract
PCN	nucleus of the posterior commissure
PF	parafascicular nucleus

PH nucleus praepositus hypoglossi

PPR postpyramidal nucleus (raphe)

PRN paramedian reticular nucleus
- dPRN paramedian reticular nucleus, dorsal division
- vPRN paramedian reticular nucleus, ventral division
- aPRN paramedian reticular nucleus, accessory division

PUL pulvinar

r raphe

RB restiform body

RM red nucleus

RR retrorubral nucleus

SCD superior colliculus (deep layer)

Sin intermediate solitary nucleus

Slt lateral solitary nucleus

Sm medial solitary nucleus

SNC substantia nigra (compact division)

SON superior olivary nucleus

Spc parvocellular solitary nucleus

Svl ventrolateral solitary nucleus

T solitary tract

TB trapezoid body

TDP dorsal tegmental nucleus (pericentral division)

TRC tegmental reticular nucleus (central division)

TV ventral tegmental nucleus

V3 third ventricle

V4 fourth ventricle

VB ventrobasal complex (thalamus)

VIN inferior vestibular nucleus

VLN lateral vestibular nucleus
- VLd lateral vestibular nucleus, dorsal division
- VLv lateral vestibular nucleus, ventral division

VMN	medial vestibular nucleus
VNC	vestibular nuclear complex
VSN	superior vestibular nucleus
WGA	wheat germ agglutinin
ZI	zona incerta

CHAPTER 1 - INTRODUCTION

The mammalian paramedian reticular nucleus (PRN) lies in the caudal medulla oblongata, situated medially within the reticular formation at the level of the middle third of the olivary complex. It has been considered to differ both morphologically and functionally from other parts of the medullary reticular formation (Brodal and Torvik, 1954; Leontovich and Zhukova, 1963). The nucleus consists of large, medium-sized, and small cells and is subdivided into dorsal, ventral, and accessory groups. The subdivisions are not identical in their cellular composition although all three project profusely onto the cerebellum.

The PRN is one of a number of brainstem nuclei which relay impulses from various sources to the cerebellum (Brodal, 1953; Brodal and Torvik, 1954; Brodal and Gogstad, 1957; Sousa-Pinto, 1970; Somana and Walberg, 1978; Kotchabhakdi, Hoddevik, and Walberg, 1980). Using horseradish peroxidase (HRP) as a retrogradely transported protein marker, Somana and Walberg (1978) showed that the cerebellum received a more widespread projection from the PRN than was originally disclosed by retrograde degeneration studies of cerebellopetal fibers from the PRN after selective ablations of parts of the cerebellum (modified Gudden method) (Brodal and Torvik, 1954). The existence of collateral axonal

projections onto different parts of the cerebellum may have accounted for the refractoriness of some cells in the PRN to change after partial ablation of the cerebellum. This refractoriness to change after ablative procedures would explain the discrepancy found between the results of the two methods. Similarly, multiple axonal collaterals from single PRN neurones have been suspected of terminating at a number of levels in the thoracolumbar cord, in particular, the intermediolateral nucleus (Blessing, Goodchild, Dampney, and Chalmers, 1981). Afferents to the PRN arise within the cerebellum, vestibular nuclei, frontoparietal region of the cerebral cortex, and the spinal cord passing upward through its dorsal columns (Brodal, 1953; Brodal and Gogstad, 1957; Sousa-Pinto, 1970). Projections from the pontomesencephalic area to the PRN have also been suggested (Brodal and Gogstad, 1957) however, these may consist entirely or at least in part of fibers within the contralateral descending pathway of the brachium conjunctivum (Faull, 1978) and descending cerebral cortical afferents (Brodal and Gogstad, 1957). Physiological studies have suggested that fibers of the carotid sinus and aortic depressor nerves terminate within the PRN (Crill and Reis, 1968; Miura and Reis, 1969b; Homma, Miura, and Reis, 1970; Miura and Kitamura, 1979). Recent anatomical studies using anterograde transport of HRP have not supported this finding (Ciriello, Hrycyshyn, and Calaresu, 1981).

The function of the PRN as a precerebellar relay nucleus is not well understood. Changes in activity of its neurones secondary to head tilting are similar to responses obtained within the vestibular nuclei under similar experimental conditions (Hiebert and Fernandez, 1965; Fujita, Rosenberg, and Segundo, 1968; Peterson, 1970; Ghelarducci,

Pompeiano, and Spyer, 1974a). Other studies (Ghelarducci, 1973; Ghelarducci, Pompeiano, and Spyer, 1974b) have established a macular input to the rostral portion of the fastigial nucleus of the cerebellum, an area known to project to the PRN (Thomas, Kaufman, Sprague, and Chambers, 1956; Batton, Jayaraman, Ruggiero, and Carpenter, 1977). Direct monosynaptic reciprocal excitatory connections between the interposed nucleus of the cerebellum and the PRN have also been shown (Murakami, Ozawa, Katsumaru, and Tsukahara, 1981). Background activity of cells in the interposed nuclei and the intermediate cortex of the cerebellar anterior lobe have been modified by changes in neck or head position (Boyle and Pompeiano, 1980). This work has suggested a role for the PRN in modifying responses from labyrinthine and cerebellar inputs.

Focal electrical stimulation of the cerebellar cortex (Moruzzi, 1940; Hoffer, Ratcheson, and Snider, 1966; Miura, Kawamura, and Reis, 1969) and the deep cerebellar nuclei (Zanchetti and Zoccolini, 1954; Ramu and Bergmann, 1967) has evoked changes in blood pressure, distribution of blood flow, and modulation of baroreceptor and chemoreceptor reflex activity. In particular, attention has turned to mediation of the fastigial pressor response through the PRN upon focal stimulation of the fastigial nucleus of the cerebellum. Further electrophysiological data has emerged supporting the role of the PRN in cardiovascular regulation with regard to mediation of heart rate and blood pressure (Calaresu and Thomas, 1971; Miura and Kitamura, 1979). Both cerebral and anterior hypothalamic inputs have been thought to influence this role (Löfving, 1961). More recently, using the 2-deoxy-D-(^{14}C) glucose method during hypotension induced by alpha-receptor blocking agents, reflex activity within the PRN and external

cuneate nucleus was abolished in contrast to surrounding areas that showed increases in metabolic activity (Savaki, McCulloch, Kadekaro, and Sokoloff, 1982).

The convergence of baroreceptor, fastigial, and macular influences upon the PRN and the latter's efferent connection with preganglionic sympathetic neurones of the intermediolateral nucleus would suggest a functional role for the PRN as a mediator of cardiac activity and vasomotor tone appropriate to changes in posture.

The purpose of the present investigation is:

(1) to investigate the topographical anatomy of afferent projections to the dorsal and ventral subdivisions of the PRN using the tetramethylbenzidine method of horseradish peroxidase (HRP) histochemistry, and

(2) to study the possibility of axonal collateralization of cerebellopetal and spinal projections from the PRN using fluorochrome double labeling histochemistry.

It has become apparent that the elucidation of the central neural control of cardiovascular regulation cannot be achieved by the sole application of either anatomical or physiological methodology; rather, a combination of the two is needed to complement one another. Knowledge of central autonomic anatomy remains deficient largely because of the polysynaptic nature of pathways which, to a large measure, can only be studied by electrophysiological analysis (Smith, 1974). Therefore, with an understanding of the first order central connections of the PRN from an anatomical study of its afferent and efferent connections, electrophysiological studies of some of these

connections will be done in light of its possible role in cardiovascular and postural control.

CHAPTER 2 - HISTORICAL REVIEW

2.1 Nomenclature

The paramedian reticular nucleus (PRN) has been referred to, in part or in association with other neighbouring cellular groups, by a variety of names. The anterolateral or accessory hypoglossal nucleus of Duval (1876) was situated immediately ventral to the hypoglossal nucleus, surrounding its root fibers and was composed of large cells. Mislawsky (1885) subsequently referred to this area as "Atmungscentrum" in the cat although Koch (1888) still considered it to be part of the hypoglossal nucleus. Later, Goldin (1934) would identify an area ventral to the accessory hypoglossal nucleus of Duval which he named the nucleus comitans hypoglossi dorsalis I. The nucleus funiculi anterioris of Obersteiner (1888) as illustrated by Bechterew (1899) corresponded, in part, to the location of Goldin's nucleus comitans hypoglossi dorsalis which, in turn, occupied the area of the dorsal group of the PRN described by Brodal (1953). The nucleus described by Obersteiner (1888) and later by Marburg (1909) extended to the caudal level of the inferior olive making it unlikely that the nucleus in its entirety corresponded to any subdivision of the PRN as it greatly exceeded the caudal limit of the PRN. Rostrally, Obersteiner's nucleus was situated at the site of the present-day ventral group of the PRN in

the cat. Bechterew (1899) and Barnard (1940), however, indicated the rostral portion of the nucleus of the anterior funiculus at the site of the dorsal group of the PRN.

In man, Jacobsohn (1909) described his "nucleus perpendicularis formationis reticularis albae" corresponding to the nucleus of the anterior funiculus as later defined in man by Obersteiner (1912). The nucleus intermedius formationum reticularum of Ziehen (1954) corresponded to the dorsal group of the PRN. van Hoevell (1911) and Ariëns Kappers, Huber, and Crosby (1936) identified an inferior reticular nucleus which was further subdivided into three parts - a major ventral part, a small-celled area around the hypoglossal nucleus, and a portion surrounding the root fibers of the hypoglossal nerve.

Cell groups corresponding to the PRN of the cat were recognized in reptilian species (van Hoevell, 1911; Schwarz and Schwarz, 1980) as well as in other mammals. The nucleus pyramidalis of Koch (1888) shown in the calf corresponded to the dorsal group of the PRN. The same collection of cells has been observed in the dog by Bechterew (1899) and subsequently again in the cat by Warncke (1904), Cajal (1909), Winkler and Potter (1914), Brodal (1953), Taber (1961), and Berman (1968). Barnard (1940) compared the region in both the dog and monkey labeling it the nucleus of the anterior funiculus. Faull (1977) compared the PRN in the rat, cat, and monkey as a site of origin of large numbers of cerebellopetal fibers. The medial reticular nucleus of Kimmel (1940) was described as an irregular column of small reticular neurons medial to the rostral part of the hypoglossal nucleus in a fourteen-day rabbit embryo. This irregular column of small cells was

not evident in the adult rabbit (Kimmel, 1940) although the PRN appeared well defined more ventrally (Meessen and Olszewski, 1949). Pitts (1940) identified a cell group which he labeled the dorsal part of the inferior reticular nucleus corresponding to the dorsal group of the PRN. His ventral group was situated further rostrally to what is considered the ventral group of the PRN. In man, Olszewski and Baxter (1954) did not delineate a distinct nuclear group corresponding to the PRN seen in the cat (Brodal, 1953). They considered the cells in this area to be scattered elements derived from adjacent nuclei and intermingled among the fibers of the medial longitudinal fasciculus and tectospinal tract. Using the modified Gudden method to display retrograde cellular changes (axonal reaction) in cerebellectomized cats, Brodal (1953) described his "nucleus reticularis paramedianus medullae oblongatae" or paramedian reticular nucleus deciding on a new collective name for the site of origin of cerebellopetal fibers rather than relying on the variety of names ascribed to the various parts of cell groups in the region. The inferior reticular nucleus of van Hoevell (1911) and Ariens Kappers, Huber, and Crosby (1936) was felt to include not only the PRN but the perihypoglossal cells and more rostrally situated cells of the medullary reticular formation and was therefore thought to be inappropriate.

2-2 Location

The PRN, in transverse section, is an irregular column of cells arranged within a triangular field on each side of the midline at the level of the middle third of the olivary complex in the medulla oblongata (Figure 1). In the cat medulla, the caudal pole is a small

FIGURE 1

The structure and location of the PRN.

a. A dorsal view of the medulla oblongata of the cat. The location of the PRN is marked in black on the floor of the fourth ventricle. The level of the transverse hemisection through the PRN below is marked "A".

b. A transverse hemisection of the medulla oblongata (labeled "A" above) that has been stained with cresyl violet. The PRN is bordered by the raphe medially, the hypoglossal fibers laterally, the hypoglossal nucleus dorsally, and the inferior olivary nucleus ventrally (x 11.8).

r - raphe

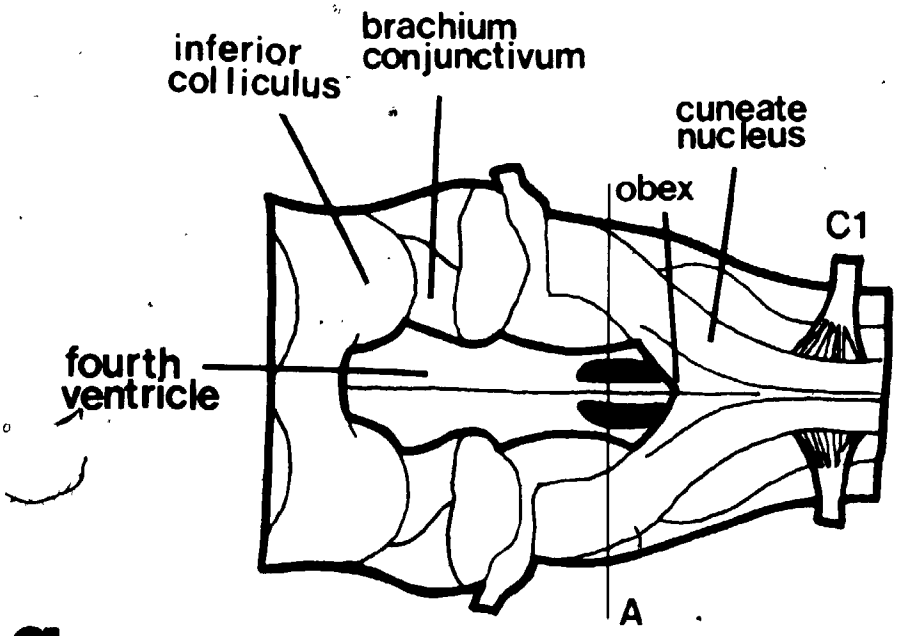
v - fourth ventricle

XII n. - hypoglossal nucleus

XII root - hypoglossal rootlets

ION - inferior olivary nucleus

PRN - paramedian reticular nucleus


inferior colliculus
brachium conjunctivum
cuneate nucleus
obex
C1
fourth ventricle
A

a.


v
r
ION

b.

aggregate of cells lying slightly caudal to the transition between the lower and middle third of the olivary complex and is situated dorsally with respect to the center of the nucleus. The rostral pole is found slightly rostral and ventromedial to the rostral tip of the hypoglossal nucleus with occasional scattered cells marking a further rostral prolongation.

In transverse section, the triangle is bordered laterally by the root fibers of the hypoglossal nerve, ventrally by the olivary complex and medially by the cell groups of the raphe. The latter cell groups are separated from the PRN by a zone of longitudinally running fibers belonging to the tectospinal tract and medial lemniscus. Large cells scattered amongst the root fibers of the hypoglossal nerve along the lateral border of the PRN represent Jacobsohn's "nuclei interfasciculares hypoglossi" and are not considered part of the PRN although they give rise to cerebellopetal fibers (Brodal, 1953). The dorsal acute angle of the triangular space of the PRN is found ventral to the rostral two-thirds of the hypoglossal nucleus.

2.3 Cytoarchitecture

In spite of certain individual variations, a rather uniform pattern of cell groups collectively called the paramedian reticular nucleus is found in most mammalian species. The three reticular cell groups of the PRN were characterized by Brodal (1953) although Winkler and Potter (1914) had earlier distinguished three groups in the region of the PRN which went unlabeled in their atlas of the cat's brain.

The dorsal group (dPRN) is the most caudally situated and increases in size on transverse section further rostrally. Ventrally, a progressively more intimate relationship forms between the dPRN and the ventral group (vPRN) until at their rostral poles the two appear to fuse. Continuity also is apparent laterally with reticular cells surrounding the hypoglossal nerve. A progressively more intimate relationship forms at rostral levels where also the nucleus of Roller is closely related. The rostral end of the dPRN is situated slightly above the rostral tip of the hypoglossal nucleus. The caudal pole of the vPRN lies slightly further rostrally than that of the dPRN. It is a distinct grouping of cells in its caudal two-thirds with cellular processes connecting it with reticular cells surrounding the hypoglossal root fibers, the dPRN and the cell groups of the raphe (Brodal, 1953). In its caudal half also, the vPRN approaches the dorsalmost aspect of the magnocellular portion of the lateral reticular nucleus and is separated from it by the hypoglossal root fibers and associated reticular cells. Its borders become less distinct rostrally as connections with the reticular formation surrounding the hypoglossal root fibers as well as those with the dPRN and the cells of the raphe become more intimate. Fiber bundles running in rostrocaudal, dorsoventral, and mediolateral directions often separate cells within the vPRN and the dPRN into smaller units. An intricate matrix of fine and coarse fibers is noted within each, the former encircling individual cells in a dense fashion (Brodal and Gogstad, 1957). The smallest of the three groups is the accessory (aPRN). Its caudal pole is situated much further rostrally than the caudal ends of the dPRN and the vPRN. It lies as a relatively distinct group mediodorsally within

the triangular area immediately lateral to the medial longitudinal fasciculus. In transverse section, it appears as a dorsoventral cellular column which may be subdivided into dorsal and ventral units. Its rostral end is found at the level of the rostral dPRN although occasional scattered cells may prolong its termination.

2.4 Cytology

The PRN in the cat possesses cells of large, medium, and small size. The large cells are multipolar and contain a central nucleus and large, angulated, intensely stained Nissl granules (Taber, 1961). The polygonal-shaped, medium-sized cells contain an eccentric nucleus and fine to coarse moderately stained Nissl granules. The small cells are usually stellate-shaped bearing a large round nucleus and minimal cytoplasm.

The dPRN and the caudal two-thirds of the vPRN consist chiefly of large cells mixed with a smaller number of medium-sized and even fewer small cells (Brodal, 1953). Small, loosely structured, cell-poor areas are found within the dPRN which are variable in location from one animal to the next. At levels of the rostral half of the vPRN where progressive fusion is occurring with the more diffuse ventral reticular formation of the upper medulla, the proportion of large cells increases. The latter cells begin to approximate in size those of the gigantocellular tegmental field (Brodal, 1953; Berman, 1968). The aPRN consists mostly of compactly arranged small cells with an intermingling of a small number of medium-sized cells.

2.5 Golgi and Silver Impregnation Studies

Valverde (1961) in his Golgi study of the medullary reticular formation demonstrated that the axons of cells of the PRN divided into long branches one of which was frequently found to cross the raphe. Axons were generally directed caudally before turning toward the inferior cerebellar peduncle on their way to the cerebellum.

The normal PRN has been studied with silver impregnation techniques also (Brodal, 1953; Brodal and Gogstad, 1957) and found to have variable numbers of characteristic terminal boutons, most being small. These were frequently seen on cell surfaces. Those found in the aPRN were of the smallest type and were arranged compactly. The largest boutons were found only on large and medium-sized neurones within the dPRN and the vPRN although boutons of different sizes were also found on these same cells.

2.6 Neuroanatomical Studies of Connectivity

A. Afferent Connections

Earlier anatomical studies have shown afferent projections to the PRN from one or more of the deep nuclei of the cerebellum, the dorsal columns of the cervical cord, vestibular nuclei, and the frontoparietal cerebral cortex. Suspicion of a primary source of afferent projections from the pontomesencephalic region of the brainstem requires further elucidation. Along with several other brainstem nuclei the PRN has been designated a precerebellar relay nucleus because of its prominent cerebellopetal projections.

i. Cerebellar Afferents

Cerebelloreticular connections with regard to the PRN were first described using the silver impregnation method of Glees and given to be moderate in number (Brodal and Gogstad, 1957). Hemicerebellectomy with some involvement of the contralateral vermis including the fastigial nucleus (FN) resulted in a few degenerating terminal fibers within the vPRN and the dPRN bilaterally with an ipsilateral predominance. No degeneration was noted in the aPRN. A lesion involving mainly the vermis and intermediate part of one anterior lobe and both fastigial nuclei produced a less marked pattern of degeneration within the PRN. This suggested that the fastigial nucleus (FN) was not the sole contributor of afferents to the PRN but rather that the cortex itself projected onto the PRN. Marchi degeneration studies following unilateral lesions of the FN showed approximately equal numbers of degenerating fibers projecting to the dorsomedial medullary reticular formation on either side of the midline (Carpenter, Brittin, and Pines, 1958). Bilateral fastigiobulbar projections to the PRN in the cat were confirmed in similar studies with the Nauta technique (Thomas, Kaufman, Sprague, and Chambers, 1956; Carpenter, 1959). Terminal degeneration in the medullary reticular formation was chiefly medial and contralateral occurring in both the dPRN and the vPRN. It exceeded that found in both the lateral reticular nucleus and perihypoglossal nuclei. Complete destruction of the FN resulted in abundant degeneration within the contralateral dPRN, vPRN, and aPRN in order of declining intensity (Walberg, Pompeiano, Westrum, and Hauglie-Hanssen, 1962). Slightly less intense degeneration was found ipsilaterally indicating coincident damage of the neighbouring

uncinate fasciculus carrying fibers of the contralateral FN on their way to the medullary reticular formation. After discrete lesions of the FN in the rat, Achenbach and Goodman (1968), using the Laidlaw modification of the Nauta-Gygax technique, determined that the majority of contralateral degenerating fastigiobulbar fibers originated in the caudal half of the FN. Autoradiographic studies performed in the monkey later showed the fastigioreticular fibers to be almost entirely crossed, passing via the uncinate fasciculus, and arising from all rostrocaudal levels of the FN except for its extreme rostral pole (Batton, Jayaraman, Ruggiero, and Carpenter, 1977). Rostral parts of the FN appeared to contribute all of the fibers destined to the PRN. No label was seen in the PRN following injections of the caudal FN. Labeled fibers within the ipsilateral juxtarestiform body were found not to terminate in the PRN. Injections of tritiated amino acids into dorsolateral regions of the rostral half of the FN produced discretely labeled terminations about cells of the dPRN. Earlier work in the opossum using the Fink-Heimer method following lesions confined to the posteromedial FN revealed terminal degeneration in the PRN (Martin, King, and Dom, 1974). This contrasted with the results obtained after lesions of the anterolateral FN in which no terminal degeneration was found in the reticular formation. Evidence for projections from the dentate (DN) and interposed (NIP) nuclei of the cerebellum to the PRN has been conflicting. Using the Laidlaw modification of the Nauta-Gygax technique following lesions of either the DN or the NIP in the cat, terminal degeneration in the PRN has been found (Carpenter and Nova, 1960) and not found (Cohen, Chambers, and Sprague, 1958). When present, it appeared about cells of the vPRN after lesions of the DN. In the

monkey, similar studies also revealed a few degenerating fibers in the PRN (Mehler, Vernier, and Nauta, 1958). Application of HRP into the PRN in the opossum failed to retrogradely label cells in the DN and the NIP (Martin, Henkel, and King, 1976). However, injections of tritiated leucine confined to the DN and NIP resulted in some labeling in the region of the PRN (Graybiel, Nauta, Lasek, and Nauta, 1973). The Fink-Heimer technique has been used following lesions of the DN and the NIP in the opossum (Martin, Henkel, and King, 1976) and following destruction of the brachium conjunctivum in the rat (Faull, 1978). A few degenerating fibers could be traced caudally from the brachium conjunctivum to enter the PRN contralaterally. Sites of terminal degeneration in the PRN were noted, particularly in the rat. A common mammalian pattern has been shown in the overall distribution of cerebellofugal fibers forming the contralateral descending pathway of the brachium conjunctivum as evidenced in the rat (Chan-Palay, 1977), opossum (Martin, King, and Dom, 1974; Martin, Henkel, and King, 1976; Yuen, Dom, and Martin, 1974), cat (Brodal and Szikla, 1972; Graybiel, Nauta, Lasek, and Nauta, 1973), and monkey (Chan-Palay, 1977).

ii. Spinal Afferents

Only very moderate ascending connections to the PRN have been demonstrated in the cat (Brodal, 1953; Brodal and Gogstad, 1957). Degenerating terminal fibers and boutons within all three groups of the PRN were seen only with lesions of the dorsal funiculi at the C2 - C3 level. Studies following lesions of ventral and/or lateral funiculi in the cat (Brodal and Gogstad, 1957) and monkey (Mehler, Feferman, and Nauta, 1960) revealed no signs of terminal degeneration within the

PRN. Lesions performed within the dorsal column nuclei and immediate surroundings produced degeneration in the PRN which was not more marked than that following lesions restricted to the dorsal columns. The degeneration could have resulted from damage to dorsal column fibers bypassing the cuneate and gracile nuclei. Degenerating fascicles were found in the PRN bilaterally following unilateral cervical cord lesions in the opossum although no terminal degeneration was noted in the nucleus (Hazlett, Dom, and Martin, 1972). Similarly, lesions of the dorsal column nuclei have not resulted in any notable terminal degeneration in the PRN (Ebbesson, 1968; Hazlett, Dom, and Martin, 1972).

iii. Vestibular Afferents

Golgi studies of cells of all four main vestibular nuclei have demonstrated axonal and collateral branches distributed mainly to the medial two-thirds of the pontomedullary reticular formation (Cajal, 1909, Lorente de Nó, 1933, Scheibel and Scheibel, 1958). Fibers of the vestibulospinal tract were noted to emit collaterals to the reticular formation.

Lesions of various portions of the vestibular nuclear complex produced terminal degeneration mainly within the vPRN and less markedly in the dPRN (Brodal and Gogstad, 1957). In only one of the four cases studied was there convincing degeneration in the aPRN. Carpenter (1960) produced lesions in the inferior and lateral vestibular nuclei of the cat and found bilateral degeneration with ipsilateral predominance in the reticular formation of the pons and medulla. Fine degenerated fibers intimately surrounding the cell bodies of neurones in the ventral

part of the reticular formation were again noted. Following an extensive lesion of the inferior vestibular nucleus, a few degenerating fibers were found in the ventral portion of the ipsilateral PRN (Ladpli and Brodal, 1968). The latter result, however, may have been due to interruption of bypassing fastigioreticular fibers and therefore cannot be taken as firm evidence for a vestibuloreticular projection. In the monkey, lesions of the medial vestibular nucleus produced a modest amount of preterminal degeneration in the PRN bilaterally (McMasters, Weiss, and Carpenter, 1960). The ipsilateral contribution was stronger. More profuse degeneration was seen in the medial medullary reticular formation following lesions of both the inferior and lateral vestibular nuclei although fastigioreticular fibers may have been interrupted also. The projections again appeared stronger ipsilaterally. Destruction of either the inferior or superior vestibular nucleus produced no fiber degeneration in the PRN in agreement with previous work (Weiss, McMasters, and Carpenter, 1964).

iv. Cerebral Afferents

Ablations of the frontoparietal cortex of a single cerebral hemisphere in the cat resulted in a preponderance of terminal degeneration within the ipsilateral vPRN (Brodal and Gogstad, 1957). In particular, destruction of the anterior and posterior sigmoid gyri, anterior aspect of the lateral gyrus and middle suprasylvian gyrus caused degenerating boutons to appear on small and large cells most intensely within the vPRN and less markedly in the ventral part of the dPRN with only a few degenerating fibers found in the aPRN. With further ablations restricted to the motor area, convincing degeneration was not demonstrable in the aPRN but a similar pattern otherwise arose.

Conspicuous degeneration in the vPRN and less intense reaction in the ventral portion of the dPRN remained. No clear somatotopical pattern of projection could be demonstrated. Cortical lesions in the occipital, temporal, medial, and basal regions of the cerebral hemispheres produced no convincing terminal degeneration within the nucleus.

Sousa-Pinto (1970), using the Nauta-Laidlaw technique, later confirmed and further defined these corticoreticular projections. The cortical projection consisted mainly of fibers originating within the first somatomotor area (anterior sigmoid and ventral coronal gyri) and terminating within the vPRN. A less conspicuous projection from the first somatosensory area (posterior sigmoid and dorsal coronal gyri) and area 6 of Hassler and Muhs-Clement (Hassler and Muhs-Clement, 1964) in the medial wall of the anterior sigmoid gyrus was found also to project upon the vPRN as well as the dPRN. Both the perihypoglossal nuclei and the dPRN received fibers primarily from the ventral part of the coronal gyrus, the designated 'face' region of the first somatomotor area. In a similar study, projections from the parietal cortex of the cat, namely the anterior portions of the middle suprasylvian and lateral gyri, were found not to terminate within the PRN nor the lateral reticular or perihypoglossal nuclei (Mizuno, Mochizuki, Akimoto, Matsushima, and Sasaki, 1973).

v. Other Afferents

Extensive lesions of the pontomesencephalic area have produced considerable terminal degeneration within the PRN (Brodal, 1953; Brodal and Gogstad, 1957). Near complete transection of the brainstem at the intercollicular level produced degenerating fibers alongside the medullary raphe as well as some passing lateral to the PRN.

All three subdivisions of the PRN contained significant numbers of degenerating fine terminal fibers and degenerating terminal boutons. Although lesions in these studies were not all purely unilateral some in which a close approximation was obtained indicated a preponderance of degeneration ipsilaterally. A diffuse system of descending hypothalamo-bulbar fibers was found concentrating lateral and adjacent to the motor nuclei of the vagus after lesions were placed in the rostral part of the posterior hypothalamic nuclei (Cheatham and Matzke, 1966). No projection to the medial portion of the medullary reticular formation was evident. The severity of degeneration in these cases exceeded that produced by frontoparietal cortical lesions suggesting to the earlier authors that other sources of supranuclear afferents existed. However, stereotaxic electrolytic lesions of the caudate, putamen, globus pallidus, thalamus, hypothalamus, and parvocellular red nucleus have failed to show terminal degeneration within the PRN. Papez and Stotler (1940) had shown earlier that rubroreticuloolivary fibers came into close relation with paramedial reticular structures caudal to the decussation of the brachium conjunctivum although later studies would not confirm any termination upon cells within the PRN (Courville, 1966). In the rat, however, lesions in the anterior midbrain involving the dorsal reticular formation produced contralateral degeneration throughout the dorsoventral extent of the PRN (Smith, 1965). Carpenter, Harbison, and Peter (1970) and Kawamura, Brodal, and Hoddevik (1974) have shown afferent projections to the PRN from the ipsilateral interstitial nucleus of Cajal and contralateral superior colliculus, respectively, using selective orthograde degeneration techniques in the cat.

Since heavier degeneration within the PRN of the cat

occurred following an almost unilateral section of the pons than after a near complete mesencephalic transection, an origin of afferent fibers from the pontomesencephalic junction was considered likely (Brodal and Gogstad, 1957). The modest contralateral descending cerebellar projection from the brachium conjunctivum (Martin, Henkel, and King, 1976; Faull, 1978) coupled with a corticonuclear projection from the cerebral hemispheres, and projections from the interstitial nucleus of Cajal and superior colliculus may account for the observations of Brodal and Gogstad (1957).

Direct projections of afferent cranial nerve fibers onto the cells of the PRN have not been identified despite electrophysiological studies having shown them to be present in the case of the carotid sinus nerve (Crill and Reis, 1968; Miura and Reis, 1969b; Homma, Miura, and Reis, 1970; Miura and Kitamura, 1979). Histological studies of the terminations of primary afferent glossopharyngeal and vagal fibers in the opossum (Dubois, 1929), rabbit (Kimmel, 1941), rat (Torvik, 1956), cat (Foley and Dubois, 1934; Ingram and Dawkins, 1945; Cottle, 1964), and man (Schwartz, Roulhac, Lam, and O'Leary, 1951) have shown no involvement of the PRN. After complete intracranial sectioning of the rootlets of both glossopharyngeal and vagus nerves, Cottle (1964), using the Nauta technique, found terminal degeneration predominantly within the nucleus of the solitary tract (NTS) and none within the dorsal motor nucleus of the vagus or the PRN.

Horseradish peroxidase (HRP) studies of glossopharyngeal and vagal afferent projections onto the brainstem of the cat (Ciriello, Hrycyshyn, and Calaresu, 1981a) as well as selective applications of HRP into cardiac buffer nerves in the cat (Ciriello, Hrycyshyn, and Calaresu,

1981b) did not result in uptake of the tracer within the PRN. The major site of termination in each of these cases has been the NTS. Other sites of termination of afferent fibers in the case of the carotid sinus nerve included the dorsal motor nucleus of the vagus, the area immediately ventral to the solitary nuclear complex, and the external cuneate nucleus. The projections of the NTS have been studied in the cat using the Nauta and Fink-Heimer techniques (Cottle and Calaresu, 1973). Fibers were observed to descend medioventrally from the NTS into the PRN and a very limited amount of preterminal fiber degeneration was evident in the PRN. Lack of evidence for a more definitive projection to the PRN was thought to be due possibly to insufficient damage to the NTS. However, autoradiographic tracing methods following injection of tritiated amino acids into the NTS of the rat failed to confirm a projection to the PRN (Norgren, 1978).

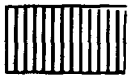
B. Efferent Connections

Reticulocerebellar projections, crossed and uncrossed, were first described using the Marchi method in the rabbit (van Gehuchten, 1904) and cat (Papez, 1926). Brodal (1953), using the modified Gudden method for identifying retrograde changes in PRN neurones after cerebellar lesions, was the first to recognize the exact origin of some of these cerebellopetal fibers as the PRN and to establish this nucleus as a site of integration of impulses destined for the cerebellum. Previous authors (Duval, 1876; Koch, 1888; Obersteiner, 1888; Goldin, 1934; Barnard, 1940; Pitts, 1940) had referred to the component groups of the PRN under varying names and had not considered them to be parts of a functional precerebellar unit. Most of the cells were observed to project to the cerebellum via the inferior cerebellar peduncle with

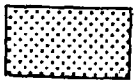
ipsilateral connections being the more abundant (Brodal, 1953; Brodal and Torvik, 1954). A very limited number of cells within both the dPRN and the vPRN were found not to project to the cerebellum. These cells, however, were thought to belong to various parts of the reticular formation diffusely bordering the PRN. With selective lesions applied to the cortex and deep nuclei of the cerebellum, retrograde changes were consistently produced within all subgroups and among all cell types of the PRN. Efferent projections of the PRN were found to terminate, predominantly ipsilaterally, upon the anterior lobe, pyramis, uvula, and fastigial nuclei but not upon the ansoparamedian lobule, flocculus, paraflocculus, and dentate nuclei. Projections from the PRN to the anterior lobe and paramedian lobule of the cerebellum were later confirmed using HRP methods (Cheek, Rustioni, and Trevino, 1975; Batini, Corvisier, Hardy, and Jassik-Gerschenfeld, 1977). Somana and Walberg (1978), using the HRP technique, established a more widespread distribution of PRN projections onto the cerebellum than was originally disclosed using retrograde degenerative techniques (Figure 2). Collateral axonal projections onto the cerebellum may have allowed certain cells within the PRN to remain refractory to axonal injury from partial cerebellar ablations. The chromatolytic changes usually encountered may have been slow to develop under such circumstances and would have gone unnoticed in the earlier studies. Bilateral projections with a stronger ipsilateral component were demonstrated in the more recent HRP study. The majority of fibers terminated within the anterior lobe and the vermis of the posterior lobe while a less marked projection was found to the ansiform lobule, lobulus simplex, and the flocculus. Weak connections were noted with the cerebellar nuclei, paramedian lobule, and paraflocculus. No topographical organization was apparent

FIGURE 2

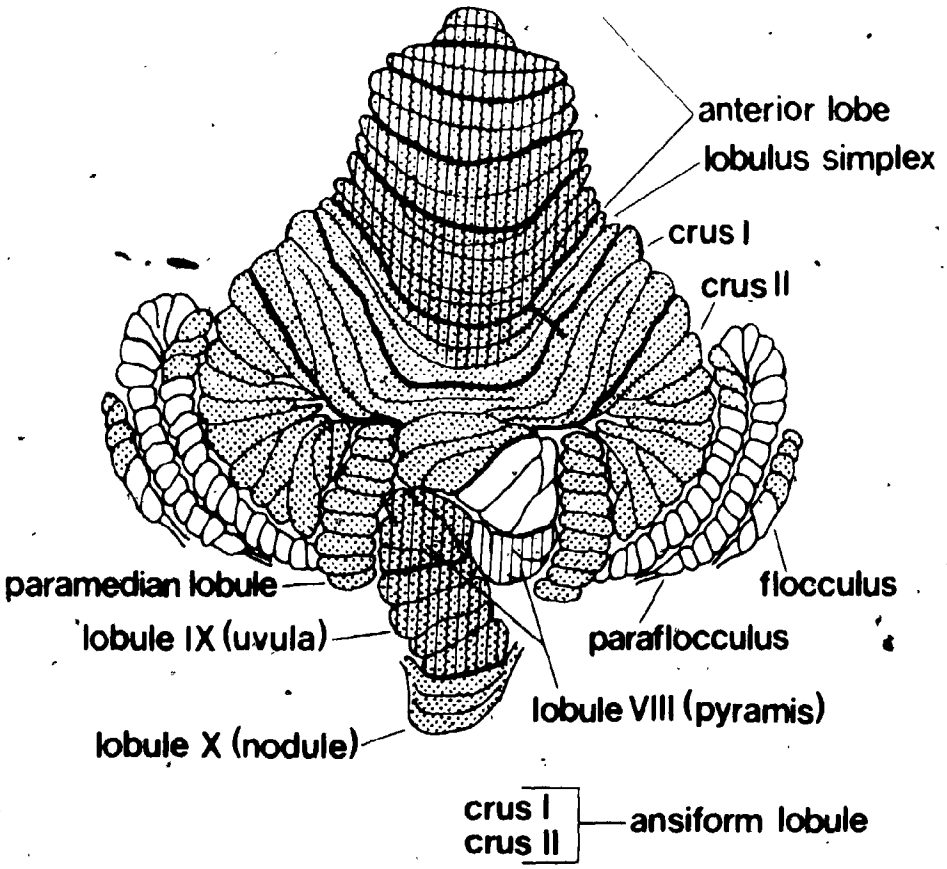
A schematic demonstration of the projection of cerebellopetal fibers from the PRN. These results are obtained from studies using the techniques of retrograde degeneration (Brodal and Torvik, 1954) and HRP neurohistochemistry (Somana and Walberg, 1978).



Brodal and Torvik (1954)



Somana and Walberg (1978)



BRODAL and TORVIK, 1954

anterior lobe
pyramis
uvula
fastigial nucleus

SOMANA and WALBERG, 1978

anterior lobe
lobulus simplex
ansiform lobule
paramedian lobule
posterior vermis
(lobules VII,VIII,IX,X)
paraflocculus
flocculus
cerebellar nuclei (3)

although each of the three PRN groups did not share identical projections. All three cerebellar nuclei and most of the cerebellar cortex, excluding large portions of the flocculus and paraflocculus, received projections from the dPRN whereas the vPRN lacked connections with lobule IX, the flocculus, and paraflocculus. The largest contributions of the aPRN were to lobule I, flocculus, vermal lobules VII to X, and the fastigial nucleus. Selective injection of HRP into the lateral nucleus of the cerebellum in the rat showed no retrograde labeling of cells within the PRN (Eller and Chan-Palay, 1976). Similar studies in the cat involving the anterior interposed nucleus and dentate nucleus of the cerebellum identified no retrograde filling of cells within the PRN (Bishop, McCrea, and Kitai, 1976).

Injections of HRP into the vestibular nuclear complex failed to show retrogradely labeled cells in the PRN (Pompeiano, Mergner, and Corvaja, 1978). Similarly, diencephalic injections of HRP resulted in no uptake in cells of the PRN (Cheek, Rustioni, and Trevino, 1975).

Reticulospinal projections from cells in the PRN have been demonstrated, although, not specifically referred to using the HRP technique (Blessing, Goodchild, Dampney, and Chalmers, 1981). Following injections of HRP centered upon the intermediolateral nucleus at the T4 or the L2 spinal levels, a similar pattern of retrogradely labeled cells appeared bilaterally within the medial medullary reticular formation. An ipsilateral predominance was noted. Both the dPRN and the vPRN were involved and cells of all sizes were labeled. An earlier study of medullary reticulospinal projections using silver impregnation

methods had shown no terminations within the intermediolateral nucleus but rather within laminae VII and IX of the spinal gray matter (Nyberg-Hansen, 1965). Recently, Miura, Onai, and Takayama (1983) have demonstrated retrogradely labeled cells within the PRN after discrete injection of HRP into the region of the intermediolateral nucleus.

C. Summary

In addition to rather extensive afferent connections with the cortex and deep nuclei of the cerebellum, the PRN has been shown to receive first order afferent connections from the cerebral cortex, vestibular nuclei, and spinal cord (Figure 3). Evidence for the existence of cerebellar cortical projections to the PRN has been based upon gross cerebellar ablations involving the deep nuclei coincidentally and studied by silver impregnation techniques (Brodal and Gogstad, 1957). The more recent neuroanatomical methods of autoradiography and HRP and fluorochrome histochemistry have not been used to confirm these results. Similarly, projections from the cerebral cortex and cervical cord have been based solely upon degeneration studies (Brodal and Gogstad, 1957; Sousa-Pinto, 1970).

An efferent projection to the thoracolumbar cord from the PRN has been demonstrated (Blessing, Goodchild, Dampney, and Chalmers, 1981; Miura et al., 1983). The remaining efferent connections of the PRN have involved projections onto both the cerebellar cortex and the fastigial nuclei establishing reciprocal relationships with these two areas. Collateral axonal projections both in the case of cerebellopetal fibers and reticulospinal fibers have been proposed.

FIGURE 3

A schematic diagram summarizing connections of the PRN that have been established using the techniques of retrograde degeneration (modified Gudden method), silver impregnation of degenerating nerve fiber terminals (Glees', Nauta-Gygax, Nauta-Laidlaw, and Fink-Heimer methods), HRP neurohistochemistry, and autoradiography.

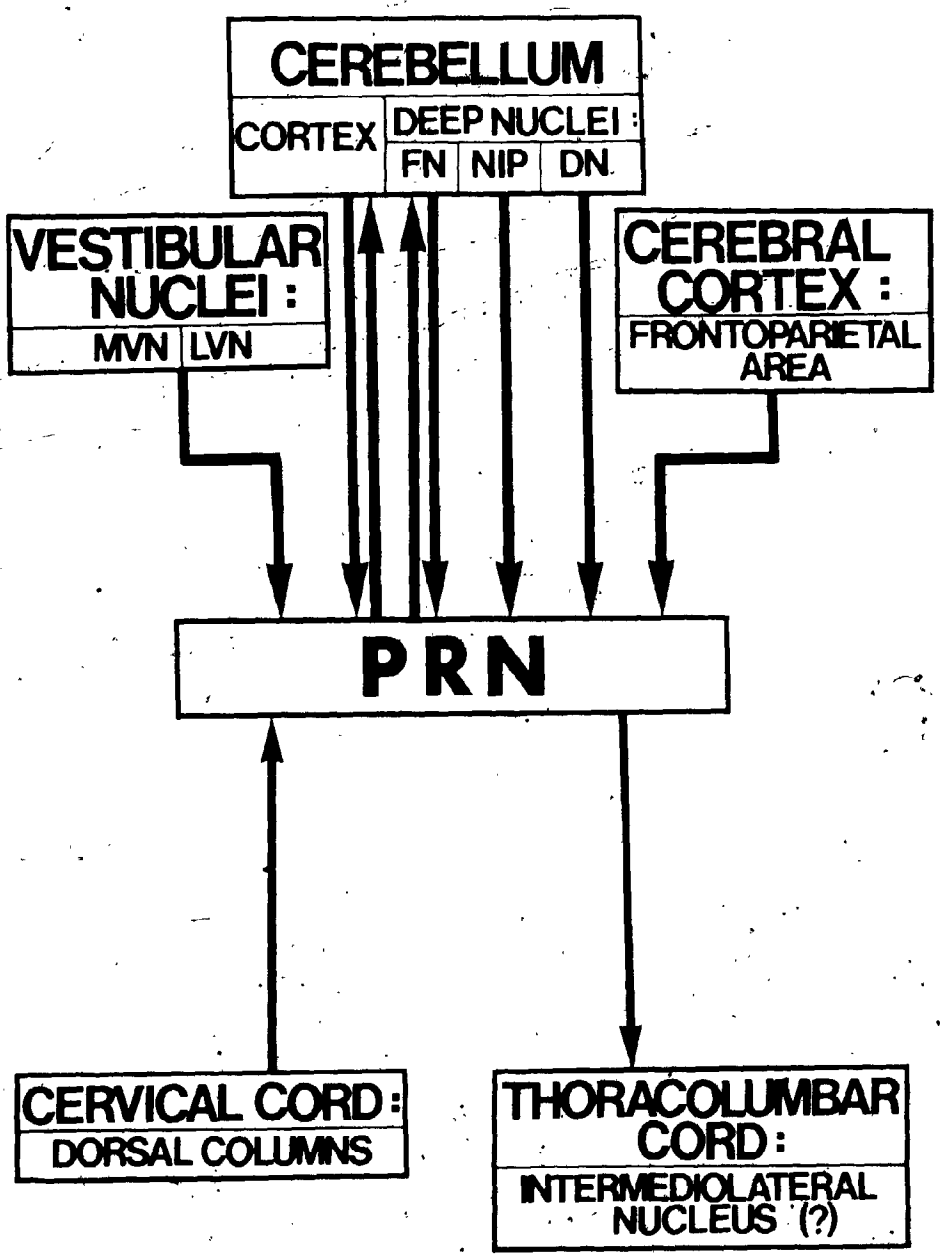
FN - fastigial nucleus

NIP - interposed nucleus

DN - dentate nucleus

MVN - medial vestibular nucleus

LVN - lateral vestibular nucleus


CEREBELLUM
CORTEX
DEEP NUCLEI :
FN
NIP
DN
VESTIBULAR NUCLEI :
MVN
LVN
CEREBRAL CORTEX :
FRONTOPARIETAL AREA
PRN
CERVICAL CORD :
DORSAL COLUMNS
THORACOLUMBAR CORD :
INTERMEDIOLATERAL NUCLEUS (?)

2.7 Neurophysiological Studies of Connectivity and Function

Despite its anatomical subgrouping, the PRN has been described as a pharmacologically homogeneous group of neurones in the cat (Avanzino, Bradley, and Wolstencroft, 1975) and rat (Duggan, Headley, and Lodge, 1974). In contrast to the surrounding reticular structures, the cells of the PRN were found to be uniformly responsive to three transmitter substances: acetylcholine, noradrenaline, and 5-hydroxytryptamine. Almost all cells within the PRN that were excited by acetylcholine could be antidromically activated from stimulation within the cerebellum or the inferior cerebellar peduncle (Avanzino, Bradley, and Wolstencroft, 1966). Synaptic excitation by acetylcholine, however, was thought to be unlikely (Duggan, Headley, and Lodge, 1974) and, in contrast to the neighbouring nucleus of the solitary tract, no significant adrenergic innervation has been demonstrated (Dahlström and Fuxe, 1965).

Attention in early physiologic studies of the medial bulbar reticular formation which included the PRN focused on its involvement in electroencephalographic arousal upon electrical stimulation (Moruzzi and Magoun, 1949) and in motor inhibition whether initiated reflexly in decerebrate rigidity or from the motor cortex (Magoun and Rhines, 1946). Although the approximate region of the PRN had been outlined schematically in these studies (McCulloch, Graf, and Magoun, 1946; Magoun and Rhines, 1946; Niemer and Magoun, 1947; Moruzzi and Magoun, 1949), no correlation with any cell groups was made; in fact, the rostrocaudal extents of the regions of stimulation yielding positive results far exceeded the limits of the PRN. Both the dPRN and the vPRN were situated within the region of the inhibitory center of Magoun and

Rhines (1946) however their function as distinct entities in this regard was not studied.

Functional aspects of the PRN have been considered primarily from two viewpoints in the present day:

A. as one of a number of central neural sites of cardiovascular regulation;

B. as a site of integration of static and dynamic postural information destined for the cerebellum.

A. Cardiovascular Regulation

In their investigation of cardioregulatory sites in the cat brainstem, Ranson and Billingsley (1916) identified a medial medullary area on the floor of the fourth ventricle near the obex which when stimulated would cause a fall in arterial pressure. A close anatomical juxtaposition of sites within the caudal medulla mediating circulatory reflexes was suspected (Bard, 1929) however not proven conclusively until Wang and Ranson (1939a) reexplored the depths of the medulla within the same region and found that decreases in blood pressure were produced most often by stimulating the medial reticular formation. The region of the PRN is included in one of their planes of section illustrated schematically at the level of the middle of the inferior olivary nucleus. A tonically active depressor area producing a hypotensive effect and cardiac slowing upon stimulation was confirmed in the same area (Alexander, 1946) although Bach (1952) demonstrated both pressor and depressor responses to stimulation here. The PRN was histologically verified as the site of stimulation producing a decrease

in arterial pressure in a much later study (Pórszász, Barnankay, Szolfsány, Pórszász-Gibiszer, and Madarász, 1962).

Studies of supramedullary influences upon cardiovascular action concentrated largely upon the hypothalamus and its descending pathway. Initial reports of a distinct pathway within the medial medullary reticular formation appeared contradictory (Beattie, Brow, and Long, 1930; Magoun, Ranson, and Hetherington, 1938; Wang and Ranson, 1939b; Thompson and Bach, 1950), although, according to Thompson and Bach (1950), discrete lesions within the medial medulla in the area of the PRN produced a considerably enhanced rise in arterial pressure upon stimulation of lateral areas of the posterior hypothalamus. Simultaneous stimulation of the same region opposed hypothalamically induced vasopressor responses. Stimulation of a depressor area localized to the preoptic region and anterior hypothalamus was found to produce a response pattern similar to that elicited by baroreceptor stimulation (Hilton and Spyer, 1971). Bilateral ablations of this hypothalamic zone reduced the reflex response to baroreceptor afferent stimulation as did similar lesions within the medullary depressor area. The reflex could only be abolished, however, when both hypothalamic and medullary depressor sites were ablated suggesting therefore that the entire brainstem depressor area functioned as a unit integrating baroreceptor information. Baroreceptor reflexes evoked by natural or direct nerve stimulation have been altered by simultaneous stimulation of the hypothalamus (Hilton, 1963; Ninomiya, Wilson, Judy, and Caldwell, 1969). Primary afferent depolarization in the carotid sinus nerve (CSN) has been produced following electrical stimulation of the posterior hypothalamus (Weiss and Crill, 1969). This suggested a presynaptic

inhibitory influence by the hypothalamus upon fibers of the CSN as well as a possible mechanism of interaction between supramedullary regions and baroreceptor input without direct synapsing upon medullary neurones. Similar inhibition of baroreceptor reflex activity was demonstrated by stimulation in the region of the inferior olive (Smith and Nathan, 1966).

Decerebration and cerebellectomy in anesthetized and vagotomized cats each resulted in sustained changes in baroreceptor reflex responsiveness (Reis and Cuenod, 1965). This suggested that a tonic influence was exerted by telencephalic and cerebellar structures upon pressor and depressor responses mediated by the caudal medulla. Initial decerebration resulted in a significant reduction of the normal pressor response to carotid artery occlusion, however, a subsequent cerebellectomy restored the pressor response to control levels. The normal reflex drop in systemic pressure seen after stretching of the carotid sinus was augmented by decerebration. A subsequent cerebellectomy further potentiated this reflex drop in pressure. With the vagi intact, baseline systemic arterial pressure remained unchanged after decerebration and cerebellectomy. These results suggested that both the forebrain and cerebellum tonically influenced only those medullary centers involved in reflex changes in arterial pressure rather than centers involved in the maintenance of the resting systemic pressure.

Sympathoinhibitory effects elicited by stimulation of the rostral portion of the cingulate gyrus were markedly diminished or abolished following electrolytic lesions of the medullary depressor area including the PRN (Löfving, 1961). A general sympathoinhibitory pathway

originating in the rostral cingulate gyrus and relaying through both the anterior hypothalamus and medullary vasodepressor region was suggested. Somatotopically organized sympathoinhibitory responses have been evoked through stimulation of the motor cortex producing an increase in muscle blood flow (Clarke, Smith, and Shearn, 1968). This has suggested a role for the cortical projections onto the PRN (Reis, 1972). Depressor responses evoked from muscle nerves were abolished by lesions in the ventral medulla including the PRN (Johansson, 1962). Electrical stimulation of sites in the midbrain and diencephalon which, in turn, were shown to project upon the PRN and surrounding area, elicited a pressor response similar to that occurring during exercise (Smith, Rushmer, and Lasher, 1960; Smith, 1965).

Stimulation of the region of the PRN and raphe nuclei produced evoked activity in the inferior cardiac nerve (Coote and Downman, 1966). Complete inhibition of splanchnic nerve activity accompanying a fall in arterial pressure was produced by stimulation in the same area (Gootman and Cohen, 1970, 1971). The latency of this depressor response was found to be consistently shorter than a pressor response obtained by stimulation of the nucleus reticularis parvocellularis. This suggested a direct descending pathway to spinal preganglionic neurones in the intermediolateral nucleus of the thoracolumbar spinal cord from the region of the PRN rather than an action on neurones within medullary pressor regions. Direct cardioinhibitory fibers from the PRN have not been demonstrated (Hopkins and Armour, 1982).

Humphrey (1967) recorded long latency evoked potentials from the region of the PRN during stimulation of the carotid sinus nerve (CSN) and attributed the delay of afferent input to synaptic transmission

through the nucleus of the solitary tract (NTS). Single unit activities and evoked potentials were recorded exclusively in the NTS on stimulation of the CSN in another study (Seller and Illert, 1969). However, myelinated CSN fibers were found to be antidromically excited by microelectrode stimulation in the region of the PRN (Crill and Reis, 1968) and an evoked response of short latency having the characteristics of a monosynaptic reflex response could be recorded extracellularly in the same area upon orthodromic electrical stimulation of the CSN (Miura and Reis, 1969b). Homma, Miura, and Reis (1970) subsequently found short latency responses in a small number of neurones within the PRN using intracellular recordings of evoked responses from electrical stimulation of the CSN. Similar work using extracellular recordings of neuronal activity in the caudal medulla identified the NTS and an area ventrolateral to the hypoglossal nucleus as the major sites of projection of CSN afferents while no projections to the PRN were evident (McAllen and Spyer, 1972).

Stimulation of the glossopharyngeal, carotid sinus, superior laryngeal, and aortic depressor nerves produced extracellular positive field potentials and intracellularly recorded hyperpolarizations in the region of the PRN with mean latencies that implied polysynaptic connections with most cells (Biscoe and Sampson, 1970a). A number of spontaneously active cells were inhibited by nerve stimulation and a few were thought to have been monosynaptically activated (Biscoe and Sampson, 1970b). More recently, recordings by intracellular methods of polysynaptic potentials from neurones within the PRN have been made upon electrical stimulation of the CSN (Miura and Kitamura, 1979). Most of the synaptic activation occurred in the dorsolateral quadrant

of the nucleus with monosynaptic latencies.

After earlier experiments had established that cerebellar stimulation influenced cardiovascular function (Moruzzi, 1940; Zanchetti and Zoccolini, 1954; Hoffer, Ratcheson, and Snider, 1966; Ramu and Bergmann, 1967) attention focused upon the fastigial nucleus in particular as it was shown to evoke a marked pressor response upon stimulation (Miura and Reis, 1969a, 1970; Miura, Kawamura, and Reis, 1969; Achari and Downman, 1970). Stimulation of either fastigial nucleus produced excitatory postsynaptic potentials with monosynaptic latencies in cells within the area of the PRN (Ito, Udo, Mano, and Kawai, 1970). Bilateral lesions of the PRN completely abolished the response whereas unilateral ablation reduced the amplitude of the response by one-half (Miura and Reis, 1969a, 1970). Acute lesions of the fastigial nucleus (FN) in the cat impaired orthostatic reflexes although the baseline systemic arterial pressure remained unchanged (Reis and Doba, 1972; Doba and Reis, 1972). Reflex pressor responses to tilt in both monkeys and cats after acute lesions of the FN were significantly impaired although improvement was noted with time (Huang, Carpenter, and Wang, 1977). A markedly increased carotid sinus sensitivity in animals with chronic lesions of the FN may have accounted for the recovery of the reflex. Mutually inhibitory responses in arterial pressure were produced by electrical stimulation of the fastigial nucleus and the CSN, both of which excited neurones within the PRN polysynaptically (Miura and Reis, 1971). Cells excited by both the CSN and fastigial nucleus were very rare.

Rhythmic synchronous firing of neurones within the PRN has been demonstrated in association with changes in cardiac

rhythm and phasic alterations in the cardiac cycle (Pórszász and Pórszász-Gibiszer, 1968; Biscoe and Sampson, 1970; Miura and Reis, 1972; Middleton, Woolsey, Burton, and Rose, 1973). Miura and Reis (1972) showed the same cells to cease firing with occlusion of the ipsilateral common carotid artery (carotid sinus baroreceptor neurones) and to be excited by chemical stimulation of carotid body chemoreceptors. The suggestion of a dual cell population within the PRN (Miura and Reis, 1971), one responding to CSN stimulation and the other to stimulation of the FN, was supported by work involving direct PRN stimulation in decerebrate anesthetized and decerebrate vagotomized cats (Calaresu and Thomas, 1971). One population of cells appeared to be inhibitory to cardioinhibitory neurones, producing an increase in heart rate, while the other appeared to be inhibitory to cardioacceleratory neurones. Cardioacceleration was observed during PRN stimulation when both sympathetic and parasympathetic pathways were active in the decerebrate animal suggesting that during simultaneous inhibition of both neural inputs to the heart, inhibition of the vagal input had the greater influence. In the vagotomized animal, PRN stimulation produced cardiac slowing. Inhibition of reflex vagal bradycardia through stimulation of the fastigial nucleus has resulted in cardioacceleration (Achari and Downman, 1970) suggesting an activation of one of the proposed inhibitory cell populations of the PRN projecting upon the cardioinhibitory center of the brainstem. Controversy has existed regarding a baro- or chemoreceptor input to the PRN. Antidromically identified neurones in the PRN which projected to the cerebellum were not observed to alter their activity in response to stimulation of the CSN and vagus nerves, hypothalamic depressor area or

to peripheral somatic stimulation (Spyer and Wolstencroft, 1971; Lipski, McAllen, and Spyer, 1973; Duggan and Game, 1975a,b). However, a number of PRN neurones which were excited synaptically from the cerebellar peduncles responded to vagal stimulation while others were excited on stimulation of the hypothalamic depressor area (Spyer and Wolstencroft, 1971).

The second thoracic (T2) segment of the intermediolateral nucleus contains the largest number of cardioacceleratory neurones (Henry and Calaresu, 1972a,b, 1973) and has been shown by means of antidromically evoked field and single unit potentials to receive contra- and ipsilateral afferent fibers from the PRN (Henry and Calaresu, 1974a,b,c). Single unit responses within the intermediolateral nucleus of the T2 segment from stimulation of the PRN were of short and long latency (Henry and Calaresu, 1974d). Long latency responses were felt to be recorded from preganglionic neurones whereas short latency responses came from interneurones between medullospinal and preganglionic neurones.

The metabolic activity in a variety of nuclei involved in the central control of blood pressure was studied using the 2-deoxy-D-(^{14}C) glucose method during hypotension induced by alpha-receptor blocking agents (Savaki, McCulloch, Kadekaro, and Sokoloff, 1982). Reflex activity within the PRN and external cuneate nucleus was abolished under hypotension in contrast to increased activity located within the nucleus of the solitary tract, dorsal motor nucleus of the vagus, nucleus ambiguus, periventricular and supraoptic nuclei.

B. Postural Control

Both the vestibular nuclear complex and rostral fastigial nucleus receive discrete macular inputs and respond in a specified patterned fashion according to the degree and direction of head and trunk tilting experienced (Fujita, Rosenberg, and Segundo, 1968; Peterson, 1970; Shimazu and Smith, 1971; Arduini and Pompeiano, 1957; Ghelarducci, 1973; Ghelarducci, Pompeiano, and Spyer, 1974b; Stanojevic, 1981). Similar response patterns to tilt have been recorded in a number of precerebellar relay nuclei including the PRN (Spyer, Ghelarducci, and Pompeiano, 1973; Ghelarducci, Pompeiano, and Spyer, 1974a). Neurones in the dPRN and the vPRN were identified by antidromic activation through electrical stimulation of juxtafastigial areas in the cerebellum. Some were activated synaptically on stimulating the ipsilateral anterior lobe. Three response types were elicited during lateral tilt of the head and body. Some units responded by either increasing or decreasing their rates of discharge to both contralateral or ipsilateral tilting while still others increased their firing frequency on ipsilateral tilt but decreased it on contralateral tilt (Ghelarducci, Pompeiano, and Spyer, 1974a). The notable absence of units demonstrating a response pattern opposite to the latter situation (i.e., a decrease in firing frequency on ipsilateral tilt and an increase of contralateral tilt) contrasted with the prevalence of this response in the lateral reticular nucleus, vestibular nuclei (Peterson, 1970) and the main reticular formation (Spyer, Ghelarducci, and Pompeiano, 1973) during tilt. The absence of this response in the PRN persisted after cerebellectomy despite pattern changes in the lateral reticular nucleus which no longer showed a predominance of this same response.

The rostral fastigial nucleus has been implicated as the final output station of a cerebellar loop for the macular influence on postural tone (Ghelarducci, 1973). However, the interposed nucleus (NIP) of the cerebellum has also been shown to be responsive to sinusoidal stimulation of neck and labyrinth receptors by rotatory motion of the head and trunk along the longitudinal axis of the animal (Boyle and Pompeiano, 1980). Neurones of the PRN have been excited both antidromically and orthodromically by stimulation of the NIP while recording intra- and extracellularly (Eccles, Nicoll, Schwarz, Taborikova, and Willey, 1976; Murakami, Ozawa, Katsumaru, and Tsukahara, 1981). Moreover, monosynaptic reciprocal connections between the two nuclei were shown.

Cardiovascular responses to tilting as studied through electrical stimulation of the rostral fastigial nuclei (fastigial pressor area) closely resembled reflex cardiovascular responses evoked by assumption of an upright posture (Reis and Doba, 1972; Doba and Reis, 1972). Bilateral ablation of the rostral fastigial nucleus impaired reflex cardiovascular responses to tilting. Lesions in the NIP failed to alter these reflex responses to tilting. As both macular and baroreceptor influences have been demonstrated within the PRN, a similar functional coupling between postural changes and cardiovascular adjustments has been proposed (Reis, 1972; Calaresu, Faiers, and Mogenson, 1975) - a coupling of somatic and autonomic motor systems.

C. Summary

A widespread distribution of monosynaptic and paucisynaptic connections of the PRN has been disclosed

electrophysiologically (Figure 4). In agreement with neuroanatomical studies, monosynaptic afferent projections to the PRN from the FN and the NIP of the cerebellum have been shown. Reciprocal innervation with the PRN was also apparent.

The similarity in neuronal response patterns to macular inputs within both the vestibular nuclei and the PRN has, in part, confirmed a connection established neuroanatomically between the two. Similarly, little direct evidence has been presented for monosynaptic projections from the cerebral cortex and hypothalamus to the PRN although studies involving excitation of these areas as well as surgical interruption of descending fibers have suggested an influence exerted through paucisynaptic relays.

Baroreceptor afferents have been shown to project monosynaptically onto cells of the PRN although a number of studies have denied this. Relaying of baroreceptor input through the NTS has also been suggested.

An efferent projection from the PRN to the intermediolateral nucleus has been shown by antidromic excitation confirming neuroanatomical studies showing similar results with HRP histochemistry.

The FN and the CSN were shown to project upon separate cell populations within the PRN (Figure 5a). Rarely, a single cell responded to stimulation of both the FN and the CSN. The fastigial pressor response and barorecepter (CSN) input have both been shown to influence neuronal activity in the PRN. Mediation of pressor responses from these two sources could then be accomplished by an interaction of

two respective groups of cells in the PRN receiving input from them.

The convergence of extrinsic cardiovascular (baroreceptor) and macular inputs on the PRN suggests a combined functional role for the nucleus in integrating postural and cardiovascular inputs (Figure 5b). The FN has been shown to mediate both pressor and postural information and to be intimately related with the PRN. Similarly, cells within the NIP have been shown to respond to vestibular stimulation and to influence PRN neurones. Regulation, by the PRN, of systemic vasomotor tone and cardiac activity appropriate to specific postural changes could then be accomplished through its connection with sympathetic preganglionic neurones in the intermediolateral nucleus.

FIGURE 4

A schematic diagram summarizing monosynaptic and paucisynaptic connections of the PRN that have been proposed using a variety of physiological techniques (see text). Both direct and indirect projections of baroreceptor afferent fibers (CSN) onto the PRN have been suggested. Presynaptic depolarization of baroreceptor afferents by hypothalamic centers provides a means of modulating baroreceptor input to the PRN and other medullary centers. Reciprocal connections between the PRN and both the fastigial (FN) and interposed (NIP) nuclei of the cerebellum provide a continuous framework of activity upon which inhibitory influences from the cerebellar cortex may act.

NTS - nucleus of the solitary tract

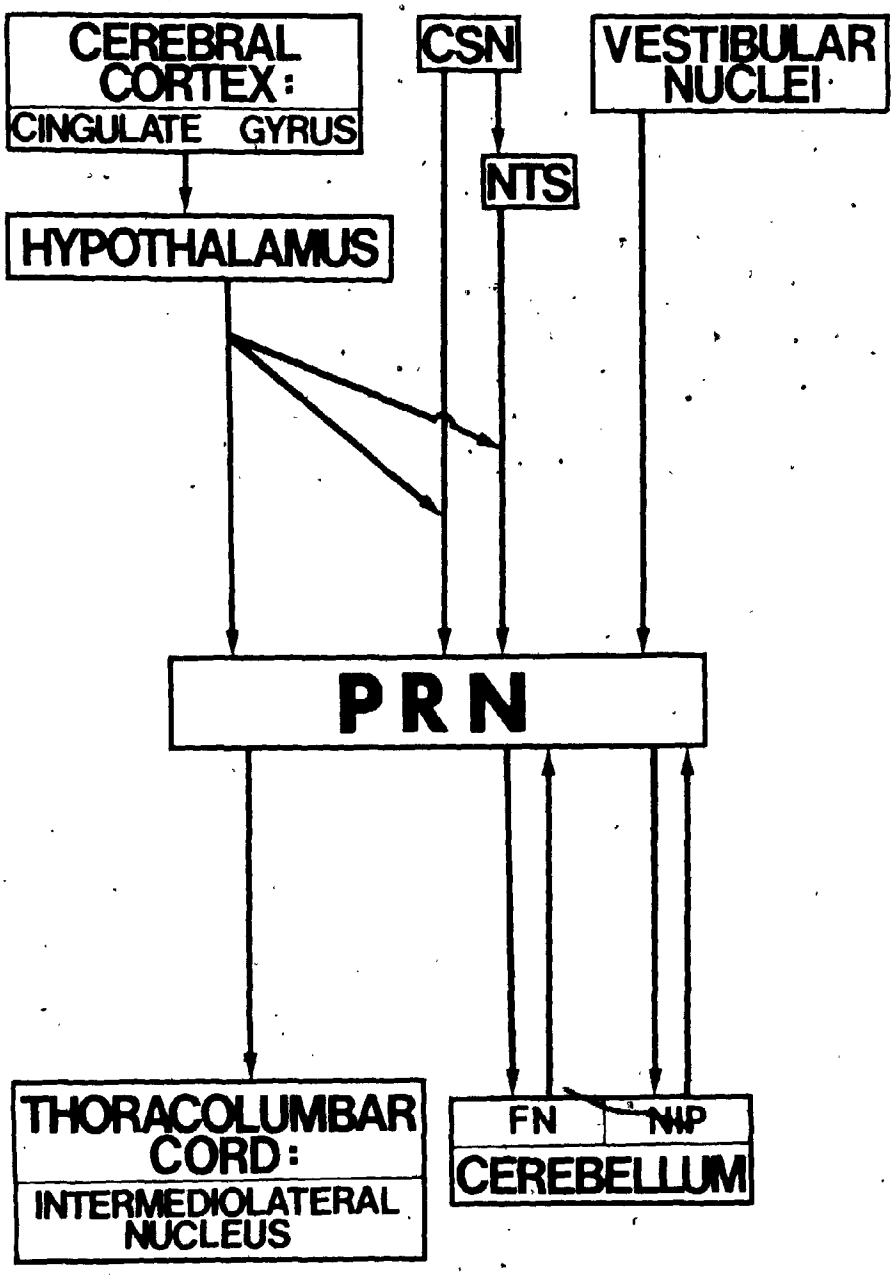

CEREBRAL CORTEX:
CINGULATE GYRUS
CSN
VESTIBULAR NUCLEI
NTS
HYPOTHALAMUS
PRN
THORACOLUMBAR CORD:
INTERMEDIOLATERAL NUCLEUS
FN
NIP
CEREBELLUM

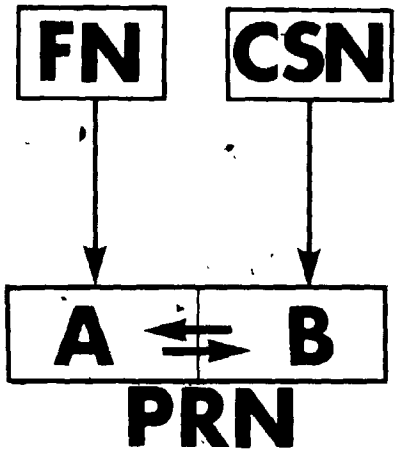
FIGURE 5

The functional organization of the PRN.

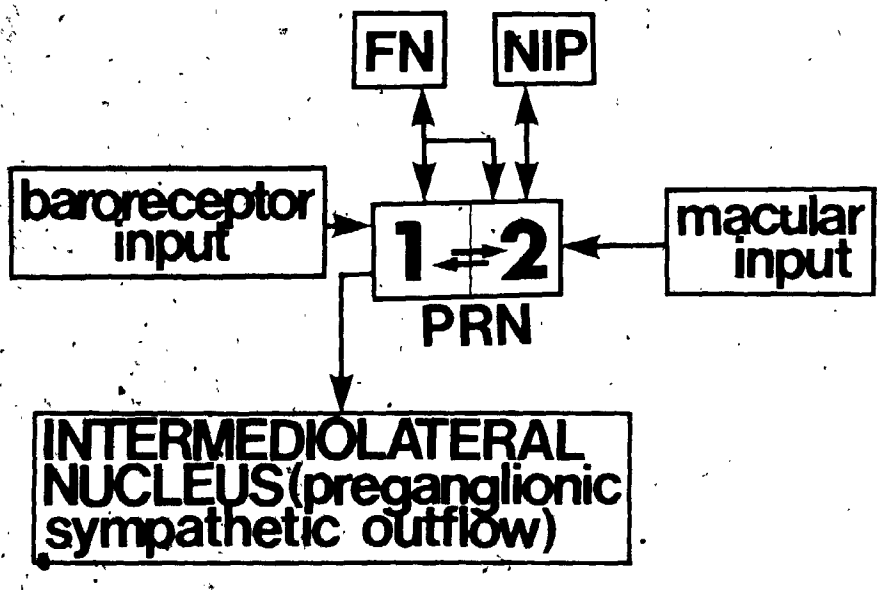
a. The fastigial nucleus (FN) and carotid sinus nerve (CSN) appear to project onto two separate cell populations, A and B, within the PRN which communicate with one another to mediate pressor responses and heart rate. . Both the fastigial pressor response and baroreceptor inputs have been shown to influence the activity of PRN neurones individually.

b. Two functional compartments, 1 and 2, within the PRN are suggested by inputs relaying baroreceptor and macular inputs respectively. Both the fastigial (FN) and interposed (NIP) nuclei reciprocally innervate the PRN. The fastigial nucleus mediates both pressor and postural information whereas the interposed nucleus seems to be solely involved with postural control in its connection with the PRN. The PRN may therefore have a combined functional role as a regulator of cardiovascular activity appropriate to specific postural changes. An efferent limb to the sympathetic vasoactive neurones of the intermediolateral nucleus in the thoracolumbar cord may be the means by which the PRN exerts control over this response.

a.


FN
CSN
A
B
PRN

b.


FN
NIP
baroreceptor input
1
2
PRN
macular input
INTERMEDIOLATERAL NUCLEUS(preganglionic sympathetic outflow)

CHAPTER 3 - MATERIALS AND METHODS

3.1 Topographical Anatomy of the Origins of Afferent Projections to the PRN

A. Surgical Procedure

Results were obtained in 20 adult cats of either sex weighing 2.0 - 4.7 kg. Anesthesia was induced by a mixture of ketamine hydrochloride (KETASET, Rogar/STB, London, Ontario) (10 mg/kg, i.m.) and xylazine (ROMPUN, Cutter Laboratories, Mississauga, Ontario) (2.2 mg/kg, i.m.) and maintained with sodium pentobarbital (SOMNOTOL, M.T.C. Pharmaceuticals, Hamilton, Ontario) (6.5 mg/30 - 60 min, i.v.). A pre- and postoperative dose of benzathine penicillin G (50,000 units, i.m.), procaine penicillin G (50,000 units, i.m.) and dihydrostreptomycin (125 mg, i.m.) (PENLONG S, Rogar/STB, London, Ontario) was given. The head of the animal was fixed in a Kopf stereotaxic apparatus (David Kopf Industries, Tujunga, CA). Fine glass pipettes with a tip diameter of 30 - 50 μm O.D. were prepared from Kimax glass tubing. The pipettes were filled by capillarity with a concentrated 1:1 solution of HRP conjugated with wheat germ agglutinin and unconjugated HRP (Sigma, Type VI, St. Louis, MO). The solution was allowed to dry at the tip for 1 - 2 hours. The pipette was fixed to a dissecting needle and attached to a carrier which was then mounted on the Kopf stereotaxic apparatus. An occipital craniectomy was performed exposing the posterior cerebellum which was then gently pushed forward to expose the floor of the caudal fourth ventricle.

The pipette was introduced at a 40° angle relative to the plane of the floor of the ventricle 1 - 2 mm lateral to the midline to avoid penetrating the medial longitudinal fasciculus or interrupting tectospinal fibers which pass in the dorsal part of the medulla immediately next to the midline. The pipette was positioned transependymally at variable depths ranging from 1.0 to 2.0 mm for injection of the dorsal (d)PRN or from 2.3 to 3.2 mm for injection of the ventral (v)PRN. The obex and midline were used as external landmarks for placement of the pipette within the region of the PRN. Placement time ranged from 2 to 5 minutes. The size of the deposit was readily controlled by an appropriate combination of pipette diameter and placement time (Contestabile and Flumerfelt, 1981).

3. Tetramethylbenzidine Method for HRP Histochemistry

Following a postoperative survival period of 3 - 5 days, the animals were reanesthetized with ketamine hydrochloride (20 mg/kg, i.p.) and sacrificed using sodium pentobarbital (40 mg/kg, i.v.). A thoracoabdominal incision and a wide open thoracotomy were performed. All animals were perfused transcardially using: (1) 500 - 800 ml NaCl (0.9%) at 20°C for 8 min; (2) 2000 ml of 0.1 M phosphate buffer (pH 7.4) containing 1% paraformaldehyde and 1.25% glutaraldehyde at 20°C for 30 min; and (3) 2000 ml of 0.1 M phosphate buffer (pH 7.4) containing 10% sucrose at 4°C for 30 minutes. The brain was removed and stored in perfusate (3) at 4°C for 12 - 36 hours. Frozen serial sections through the entire brainstem, diencephalon, cerebellum, cerebral hemispheres and spinal cord segmental areas C1-3, C5, C8, T2, T4, T7, T10, L1, L3, L7 and S2 were cut with a sledge microtome at a thickness of 40 µm. The cerebellum was sectioned sagittally and the remaining tissue transversely. One from every 2 serial sections through the injection site and 1 from every 5

serial sections through the remaining tissue was placed in 0.1 M phosphate buffer (pH 7.4) at 4°C, rinsed 3 times with deionized water and processed according to the method of Mesulam (1978) and Mesulam et al. (1980) using tetramethylbenzidine (TMB, Sigma, St. Louis, MO) as the chromogen. Sections were placed in a freshly prepared pre-reaction soak for 20 min at 19 - 23°C. The soak was prepared by mixing a solution containing 100 mg of sodium nitroferricyanide in 92.5 ml of distilled water and 5 ml of 0.2 M sodium acetate buffer (pH 3.3) with a solution containing 5 mg of TMB in 2.5 ml of absolute ethanol prepared by heating to 35 - 40°C. Addition of 1.0 - 2.5 ml of 0.3% H_2O_2 per 100 ml of pre-reaction soak initiated the required enzymatic reaction which continued for a further 20 min at 19 - 23°C. The tissue was rinsed in 6 changes of sodium acetate buffer (pH 3.3) at 4°C following the reaction. Sections were then mounted on chrome-alum gelatinized slides counterstained with 1% aqueous neutral red solution (pH 4.8) for 3 - 5 min, coverslipped and viewed under bright- and dark-field illumination.

Frozen transverse sections of cervical, thoracic, lumbar and sacral segments of the spinal cord were examined for anterograde unconjugated HRP label within the terminals of expected fibers of passage through the site of the HRP injection. In particular, labeled terminals of interstitiospinal fibers were sought in the dorsal part of Rexed's lamina VIII and adjacent parts of lamina VII bilaterally throughout the spinal cord (Nyberg-Hansen, 1966). Similarly, labeled terminals of tectospinal fibers were sought in Rexed's laminae VI and VII in the upper cervical segments (C1-3, C5) (Nyberg-Hansen, 1964). Absence of anterograde label in the respective laminae of these fibers of passage supported the premise of primary uptake by terminals in the region of the PRN.

Retrogradely labeled cells were identified by a granular blue reaction within their perikarya following conversion with TMB.

3.2 Collateral Efferent Projections of PRN Neurones to the Cerebellum and Spinal Cord

A. Surgical Procedure

Experiments were done in 9 adult cats of either sex weighing 2.1 - 4.6 kg. Each experiment required two operative exposures as the fluorochrome injections were temporally spaced to allow for differences in transport time. Anesthesia was induced and maintained in each phase by a mixture of ketamine hydrochloride (KETASET; 20 mg/kg, i.m.) and xylazine (ROMPUN; 1.0 mg/kg, i.m.). A pre- and postoperative dose of benzathine penicillin G (50,000 units, i.m.), procaine penicillin G (50,000 units, i.m.) and dihydrostreptomycin (125 mg, i.m.) (PENLONG S) was given. In addition, a preoperative dose of dexamethasone phosphate (DEXAGEN-S, Rogar/STB, London, Ontario) (0.5 mg, i.m.) was administered to reduce cord swelling from local toxicity.

In 5 animals, cerebellar injections of the fluorochromes 'Fast Blue' (FB) and 'Nuclear Yellow' (NY) were performed. The head of the animal was fixed in a Kopf stereotaxic apparatus (David Kopf Industries). A wide craniectomy of the occipital and parietal bones and the central tentorium facilitated selective placements of the fluorochromes within the cerebellum. Pressure injections of approximately 2.0 μl of 2% FB were made into a predetermined region of the right side of the cerebellum. The animals were allowed to awaken and after an interoperative period of 72 hours, similar injections of 1% NY in a corresponding 'mirror-image' fashion were made in the contralateral cerebellum. Three to five

penetrations using a glass micropipette (tip diameter 50 - 150 μm) mounted on a 5.0 μl Hamilton syringe were made for each injection.

In the remaining 4 cats, spinal cord injections of FB and NY were performed. The animals were positioned in a modified Kopf stereotaxic apparatus with the head fixed rostrally and tail clamped caudally. The spinous processes of the vertebral bodies were exposed through dorsal midline incisions to allow placement of spinal clamps on either side of the segmental level of the cord at which injection of the tracer agent was to be made. The T2 segmental level was chosen for injection of FB as it has been shown to contain the largest segmental number of sympathetic preganglionic neurones within the intermediolateral nucleus (IML) (Henry and Calaresu, 1972) and hence, the largest number of nerve terminals. After a laminectomy of the T2 vertebra was performed, a total volume of approximately 2.0 μl of 2% FB was pressure injected into the region of the right IML. Positioning of the injection within the region of the IML was done by penetrating the dorsal cord for a distance of 1.1 - 1.5 mm using the dorsolateral sulcus as a surface landmark. The animals were allowed to awaken and after an interoperative period of 2 - 4 weeks, a similar injection of 1% NY was made into the region of the IML at the T4 level in 2 cats and at the T7 level in the remaining 2 cats. Each cat received 5 injections (0.4 μl) of FB and NY equally spaced in the respective cord segment. Glass micropipettes (tip diameter 30 - 50 μm) mounted on a 5.0 μl Hamilton syringe were used.

B. Preparation of Tissues for Fluorochrome Histochemistry

After survival periods of 18 - 24 hours in the case of the cerebellar injections and periods of 30 - 48 hours in the case of the

spinal cord injections, the animals were reanesthetized with a combination of ketamine hydrochloride (20 mg/kg, i.p.) and xylazine (2.2 mg/kg, i.m.) and sacrificed using sodium pentobarbital (SOMNOTOL; 40 mg/kg, i.v.). Following a wide open thoracotomy, all animals were perfused transcardially according to the protocol of Huisman et al. (1982): 2 liters NaCl (2.7%) followed by 3 liters cacodylate-buffered (pH 7.2) formalin (30%) followed by 2 liters cacodylate-buffered (pH 7.2) sucrose (8%). Frozen serial transverse sections through the injection sites and the region of the PRN in the caudal medulla oblongata were cut at 40 μm with a sledge microtome. The cerebellum was sectioned sagittally and the spinal cord segments were sectioned transversely. All sections were immediately mounted on chrome-alum gelatinized slides and air dried. Alternate sections were counterstained with 1% aqueous neutral red solution (pH 4.8) for 3 - 5 min and coverslipped for comparative viewing under bright-field illumination. Fluorochrome labeling was identified under a Leitz Ploemopack fluorescence microscope equipped with a filter mirror system (360 nm excitation wavelength). The location of single- and double-labeled neurones in the PRN was mapped on a representative series of transverse hemimedullary projection drawings made from the neutral red-stained material.

3.3 Electrophysiological Characteristics of PRN Neurones Relaying Cardiovascular Afferent Input Directly to Spinal Sympathetic Centers of the Thoracic Cord

A. General Procedures

Results were obtained in 9 adult cats of either sex (2.3 - 3.4 kg) anesthetized with alpha-chloralose (60 mg/kg, i.v. followed by 30 mg/kg q8 - 10 h) after ethyl chloride and ether induction. After tracheal cannulation the animals were paralyzed with decamethonium bromide (Sigma;

0.5 mg/kg, i.v. followed by 0.25 mg/kg q2 - 3 h) and artificially ventilated. The femoral artery and vein were cannulated with polyethylene catheters for recording of arterial pressure and drug administration, respectively. Arterial pressure was recorded through a Statham P23Dd transducer. A 7P44A Grass tachograph triggered by the arterial pressure pulse monitored the heart rate. Both parameters were continuously recorded on a Grass model 7 polygraph. Rectal temperature was maintained at $37 \pm 0.2^{\circ}C$ by a heating pad controlled by a Yellow Springs 73 temperature regulator.

B. Surgical Procedures

i. Exposure of the Carotid Sinus Nerve

The right carotid sinus nerve (CSN) was identified as described previously by Ciriello and Calaresu (1979). A lateral curvilinear incision was made at the level of the tympanic bulla. The sternocleidomastoid and digastric muscles overlying the sinus region were reflected; the large jugulodigastric lymph node was removed hemostatically. The underlying CSN was identified and isolated from surrounding tissue. A 1 - 2 cm segment of the hypoglossal nerve underlying the CSN was resected. The distal portion of the CSN was crushed and the central end of the isolated nerve was placed on a bipolar stainless steel electrode and surrounded with cotton pellets saturated in warm 360 medical fluid (Dow Corning, Midland, MI).

ii. Exposure of the Spinal Cord

The animal was secured in a modified Kopf stereotaxic frame (David Kopf Industries) with the head fixed rostrally and the tail clamped caudally. The spinal cord at the level of the T2 segment was approached through a dorsal midline incision. The paraspinal muscles were

retracted laterally and the cord held rigidly by clamps fastened to the T1 and T3 spinous processes. A laminectomy of the T2 vertebra was performed. Hemostasis was secured with electrocautery and application of bone wax and absorbable gelatin sponge (Gelfoam, Upjohn, Don Mills, Ontario) to bleeding bone edges and soft tissues, respectively.

iii. Exposure of Central Structures

The floor of the caudal fourth ventricle was exposed through an occipital craniectomy by removal of a portion of the posterior vermis by suction. Exposure of the dorsal surface of the cerebellum to facilitate placement of electrodes into the fastigial nucleus (FN) necessitated a rostral extension of the occipital craniectomy to include part of the parietal bones and tentorium centrally. Bone wax and absorbable gelatin sponge (Gelfoam) were again employed for hemostasis. All nerve tissue was covered with cotton pellets soaked in warm 360 medical fluid (Dow Corning).

C. Electrical Stimulation of the CSN and Central Structures (IML, FN) to Elicit Responses in PRN Neurones

i. Stimulation of the CSN

The stimulus applied through the bipolar stainless steel stimulating electrodes was a rectangular pulse of 0.2 ms duration at 0.5 Hz and at current intensities of 0.8 - 1.3 mA. Current intensities used were up to five times the current required to elicit a threshold response in arterial pressure when using a 15 s stimulus train at 25 Hz and a pulse duration of 0.3 ms.

ii. Stimulation of the IML

Stainless steel microelectrodes (tip diameters 80 - 100 μm;

resistance in saline 150 - 400 kΩ), etched from 00 insect pins (Green, 1958) and insulated with Insl-X (Insl-X Products, Yonkers, NY) were placed in the region of the right (ipsilateral) IML. Penetrations were made through the dorsolateral sulcus for distances of 1.4 - 1.7 mm beneath the dorsal surface. The stimulus applied to antidromically excite single units in the PRN was a rectangular pulse of 0.2 ms duration at 0.5 Hz and current intensities of 0.6 - 1.1 mA. Current intensities used were up to five times that needed to elicit a threshold increase in arterial pressure. The threshold intensity for the IML was defined as the current required to elicit an increase in arterial pressure of 25 mm Hg when a 15 s train of pulses (0.3 ms; 25 Hz) was applied. The indifferent electrode was an alligator clip attached to paraspinal muscle.

iii. Stimulation of the FN

Concentric bipolar stainless steel electrodes (SNEX-100, David Kopf Industries, Tujunga, CA; 0.25 mm tip diameters, 50 - 90 kΩ initial DC resistance in saline) were placed stereotaxically in the left (contralateral) FN (Berman, 1968). Rectangular pulses of 0.2 ms duration at 0.5 Hz were delivered through the central electrode using current intensities of 0.50 - 1.0 mA. Current intensities were up to 5 times the current required to elicit a threshold response in arterial pressure when using a 15 s stimulus train at 60 Hz and a pulse duration of 0.3 ms. The indifferent electrode was an alligator clip attached to exposed scalp muscle. Units in the PRN antidromically excited by stimulation of the IML were subsequently tested for orthodromic responses to stimulation of the FN and CSN.

3.4 Electrophysiological Characteristics of PRN Neurones Relaying Vestibular Afferent Input Directly to Spinal Sympathetic Centers of the Thoracic Cord

A. General Procedures

Results were obtained in 5 adult cats of either sex (2.1 - 3.2 kg). To avoid electrically stimulating fastigioreticular fibers destined for the PRN and which pass through the vestibular nuclear complex (VNC), the experimental model necessitated a two stage surgical procedure, the first stage of which would eliminate the fastigioreticular fibers. In the first stage, an ablation of both fastigial nuclei was performed followed by an interoperative period of at least two weeks allowing the fastigioreticular fibers to degenerate. Anesthesia in the first stage was induced and maintained in the manner described previously (section 3.1 i). Antibiotic and steroid coverage was also provided as described previously (section 3.2 i). In the second stage, the animals were reanesthetized and prepared for electrical stimulation and recording as before (see section 3.3 i).

B. Surgical Procedures

i. Ablation of the Fastigial Nuclei

The dorsal surface of the cerebellum was exposed through an occipital craniectomy. The midline vermis was electrocauterized and a subpial suction ablation of the vermis performed to the roof of the fourth ventricle ventrally and to the anterior lobe cortex rostrally. The ablated volume of tissue was widened ventrally in a conical fashion in order that both fastigial nuclei located in the paramedial area would be eliminated. In addition, the majority of the fastigioreticular fibers crossing the cerebellar midline from the contralateral FN were destroyed. Hemostasis

was secured with absorbable gelatin sponge (Gelfoam). The animals were allowed to awaken at the end of the procedure and to recuperate.

ii. Exposure of the CSN and Central Structures

After an interoperative period of 15 - 52 days, the animals were reoperated to prepare them for electrical stimulation and recording. Procedures for exposure of the CSN and spinal cord have been described previously (section 3.3 iia,b). Exposure of the cerebellum and medulla required only the extension of the craniectomy performed in the first stage to include the parietal bones sagittally and the central tentorium.

C. Electrical Stimulation of the CSN and Central Structures (IML, VNC) to Elicit Responses in PRN Neurones

Electrical stimulation of the CSN and IML was done using the same materials and parameters described previously (section 3.3 iiia,b). Concentric bipolar stainless steel electrodes (SNEX-100, David Kopf Industries; 0.25 mm tip diameters, 50 - 100 kΩ initial DC resistance in saline) were placed stereotaxically in the right (ipsilateral) VNC (Berman, 1968). Stimulation parameters were the same as those described for the FN (see section 3.3 iii). Units in the PRN orthodromically activated by VNC stimulation were tested for antidromic responses to stimulation of the IML.

Stimuli were delivered to the CSN and central sites in all animals from a Grass S88 stimulator through a Grass PSIU6D stimulus isolation and constant current unit.

3.5 Recording of Single Unit Activity in the PRN

Stainless steel microelectrodes, etched from 00 insect

pins (Green, 1958) insulated with Insl-X with tip diameters of 1 - 3 μm and a resistance in saline of 1.2 - 2.3 MΩ were used for extracellular recording. The indifferent electrode was a hypodermic needle inserted into the brain tissue through a small right parietal craniectomy. The region of the right PRN was systematically explored from 0.2 mm to 1.5 mm rostral to the obex, from 0.5 mm to 1.3 mm lateral to the midline and from 1.0 mm to 3.0 mm ventral to the surface of the fourth ventricle. The reference points for positioning the recording electrode were the obex and dorsal median sulcus. Unit activity was amplified through a Grass P15 differential preamplifier with a band pass of 0.3 - 10 kHz and displayed on a Tektronix R5103N oscilloscope for observation and photography.

3.6 Histological Localization of Recording and Stimulation Sites

Recording and stimulation sites were identified by iron deposition from the electrode tip (20 μA for 20 s, tip positive). The locations of most of the antidromically and orthodromically activated units within the PRN were then derived by interpolation using the site(s) of iron deposition as a reference. Animals were perfused transcardially with 1 liter of NaCl (0.9%) followed by 1% potassium ferrocyanide in 10% formalin-saline solution (1 liter) to reveal the iron deposits by the Prussian blue reaction. The brains were fixed in 10% formalin for at least 10 days and 40 μm frozen sections were cut transversely through the medulla and T2 segmental region of the cord and sagittally through the left paramedial cerebellum. All sections were stained with 1% neutral red. Stimulation and recording sites were mapped on transverse sections of the spinal cord and medulla reproduced from projection drawings of the cord and medulla and on sagittal sections of the cerebellum modified from a stereotaxic atlas (Berman, 1968).

3.7 Data Analysis

Conduction velocities of evoked antidromic action potentials were calculated using the total conduction distance measured for each animal (range 81 - 106 mm) from the stimulation site in the IML to the recording site in the RRN. Means $\pm$ standard errors of latencies, conduction velocities and parameters of single unit responses were calculated. The latency of antidromic responses was the time interval between the stimulus artifact and start of the rising phase of the extracellular action potential. The latency of orthodromic responses to electrical stimulation of the FN, VNC and CSN was taken as the time interval from the stimulus artifact to the first spike in 5 consecutive traces. Statistical analysis of conduction was done using the Student's t-test ($p < 0.01$ was considered to be statistically significant).

CHAPTER 4 - RESULTS

4.1 Topographical Anatomy of the Origins of Afferent Projections to the PRN

A. Description of Injection Site

To study the topographical organization of afferent input to the PRN, the dPRN and vPRN were separately injected with HRP. The dPRN was injected in 12 of the 20 cats and the vPRN was injected in the remaining eight (Figure 6). In each case, the effective zone of uptake within the dense inner zone of the HRP deposit was confined to either division of the PRN. The zone of secondary diffusion from the initial deposit produced a lightly shaded halo which in some cases extended medially towards the medial longitudinal fasciculus, dorsally and rostrally towards the perihypoglossal nuclei or ventrally to the inferior olive. These sites of secondary diffusion do not contribute significantly to retrograde neuronal labeling (Jones and Leavitt, 1974; Vanègas et al, 1977). Furthermore, LaVail and LaVail (1974) have shown that HRP uptake by damaged fibers of passage does not occur within the diffuse periphery of injection sites. In the present study, retrograde neuronal labeling was therefore considered to result from uptake within the dense core of the HRP injection site.

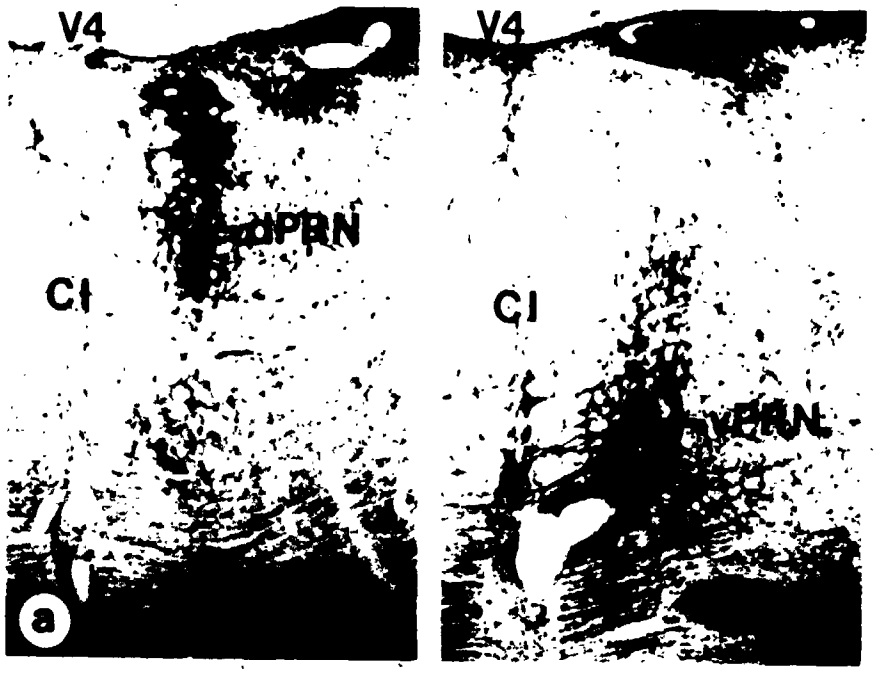
Although it has been shown that lectin conjugated HRP is not taken up by intact fibers of passage and is transported anterogradely for only short distances by damaged fibers of passage (Brodal et al, 1983), free HRP is known to be taken up and transported anterogradely to the nerve

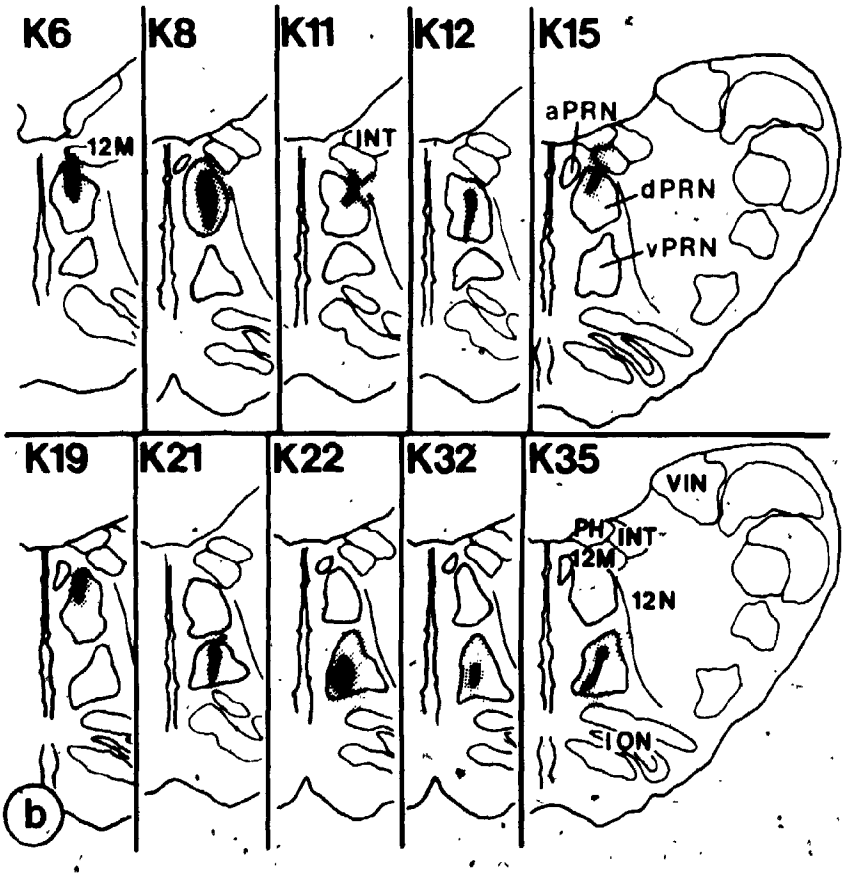
FIGURE 6

Sites of HRP deposition in the right PRN.

a. Photomicrographs of neutral red counterstained sections showing injection sites confined to the dPRN (case K8) and vPRN (case K22) (x 22).

b. Schematic representations of injection sites in ten studied cases. The dense core of the injection site marks the zone of effective uptake; the shaded periphery shows the extent of secondary diffusion from the initial deposit. Note that most zones of secondary diffusion are also confined to the region of the PRN.


V4
dPRN
CI
a
V4
CI
vPRN


K6
K8
K11
K12
K15
12M
INT
aPRN
dPRN
vPRN
K19
K21
K22
K32
K35
VIN
PH
INT
12M
12N
ION
b

fiber terminal by both intact and damaged fibers of passage. The latter property was used to determine the amount of uptake of injected HRP by fibers of passage at the site of injection.

Henceforth, all references to horseradish peroxidase, both conjugated and unconjugated, will be noted simply by 'HRP' unless otherwise stated.

B. Deep Cerebellar Nuclei

Neurones within all three contralateral deep cerebellar nuclei and the ipsilateral fastigial nucleus (FN) were labeled following separate injections of HRP into the dPRN and vPRN. No cerebellar cortical labeling was noted.

i. Fastigial Nucleus

In the case of dPRN injections, most labeled neurones in the contralateral FN appeared within its dorsomedial portion (Figure 7a). A large number of these cells was concentrated posteriorly with relatively few cells found scattered throughout the entire anterior aspect of the FN. Labeling in the ipsilateral FN was relatively sparse and concentrated within the ventromedial aspect of the anterior half of the nucleus. The labeled perikarya were mostly small or medium-sized, round and multipolar neurones with a few scattered larger labeled cells found throughout (Figure 8a). Injection of the vPRN resulted in relatively more cell-labeling along the anterior aspect of the medial half of the FN in addition to labeling of cells in its dorsal half (Figure 9a). Labeled neurones in the ipsilateral FN were again less abundant and largely confined to its ventromedial portion.

FIGURE 7

Distribution of labeled neurones in the contralateral deep cerebellar nuclei and ipsilateral fastigial nucleus (inset) after injection of the right dPRN (photomicrograph) (case K8). Numbers indicate distance from the midline in mm. Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

a. Fastigial nucleus

b. Interposed (posterior division) and dentate nuclei

c. Dentate nucleus

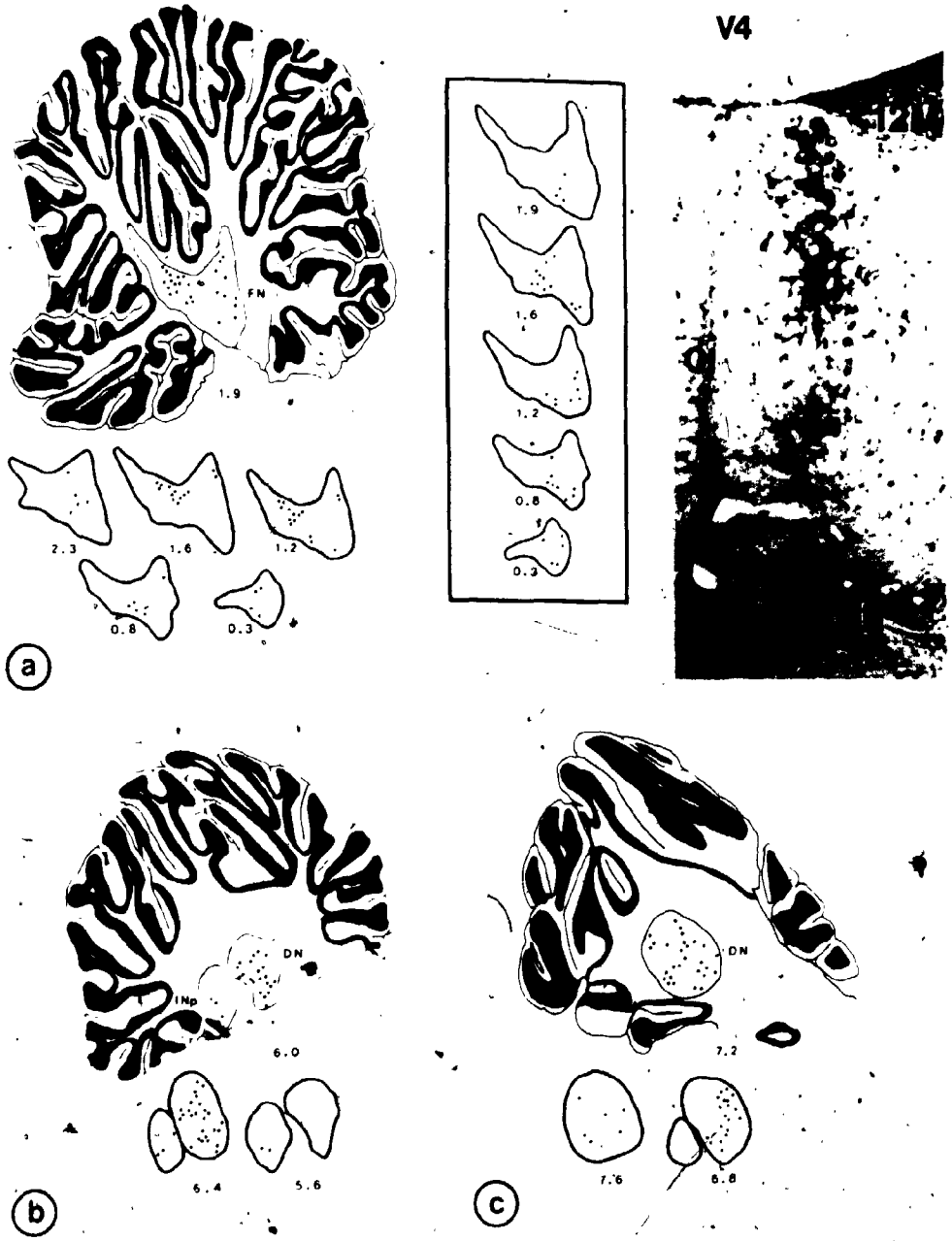

V4
FN
1.9
2.3
1.6
1.2
0.8
0.3
a
1.9
1.6
1.2
0.8
0.3
DN
INp
6.0
6.4
5.6
b
DN
7.2
7.6
6.8
c

FIGURE 8

Photomicrographs (bright-field microscopy) of labeled neurones in the deep cerebellar nuclei (x 470).

a. Fastigial nucleus

b. Interposed nucleus (posterior division)

c. Dentate nucleus

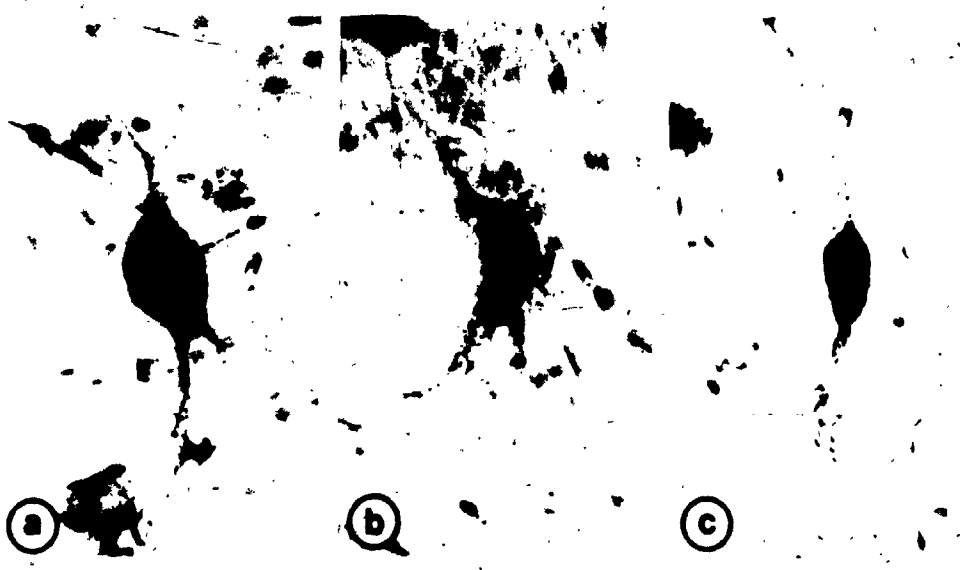

a
b
c

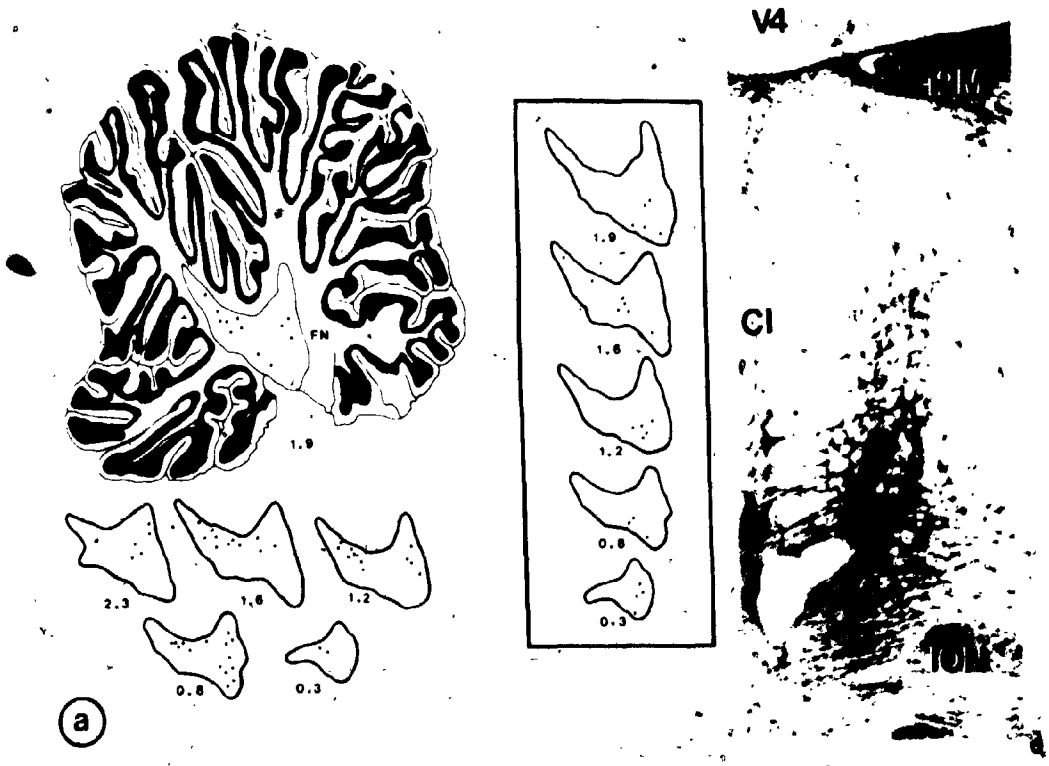
FIGURE 9

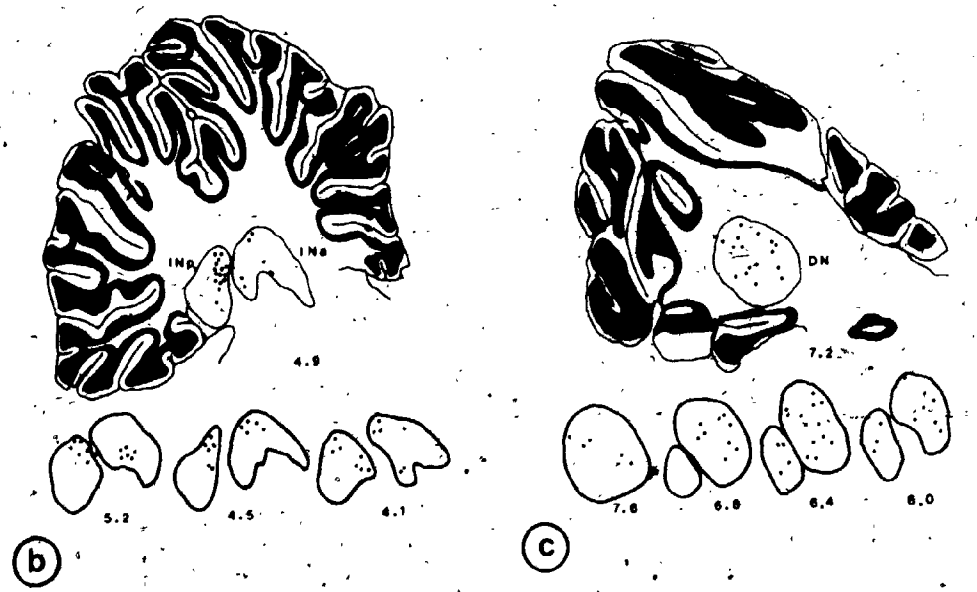
Distribution of labeled neurones in the contralateral deep cerebellar nuclei and ipsilateral fastigial nucleus (inset) after injection of the right vPRN (photomicrograph) (case K22). Numbers indicate distance from the midline in mm. Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

a. Fastigial nucleus

b. Interposed nucleus (anterior and posterior divisions)

c. Dentate nucleus


V4
CI
FN
1.9
2.3
1.6
1.2
0.8
0.3
1.9
1.6
1.2
0.8
0.3
a


INB
INA
4.9
5.2
4.5
4.1
b
DN
7.2
7.6
6.8
6.4
6.0
c

ii. Interposed Nucleus

A paucity of labeled cells within the interposed nucleus (IN) was noted after dPRN injections and these were confined to its posterior subnucleus (INp) (Figure 7b). Injection of the vPRN resulted in more abundant neuronal labeling in both the anterior (INa) and posterior (INp) subnuclei (Figure 9b). Most of the labeled cells were concentrated in the anterodorsal portion of the INp with fewer scattered labeled cells found posteriorly in the INa. Most labeled cells were medium-sized and multipolar (Figure 8b).

iii. Dentate Nucleus

Labeled cells were found throughout the anteroposterior extent of the dentate nucleus largely central with respect to its medial and lateral borders (Figures 7c, 8c, 9c). This was the case with both dPRN and vPRN injections although in the latter case, relatively fewer labeled cells were encountered in the dentate nucleus. Many cells were lightly labeled in comparison to those in the fastigial and interposed nuclei.

c. Vestibular Nuclear Complex

The majority of labeled vestibular neurones were found in both lateral vestibular nuclei after separate injections of either the dPRN or the vPRN (Figures 10, 11; Table I). A larger number of neurones was labeled within the dorsal division (VLd) than within the ventral division (VLv) of both nuclei and a heavier concentration of labeled cells was found after injection of the dPRN than of the vPRN. The giant cells of the VLd and the large and medium-sized multipolar cells of the VLv were labeled. Relatively fewer neurones within the inferior and medial vestibular nuclei of both sides were lightly labeled (Figures 10, 11, 12c,d,e). Of the latter two nuclei, labeling was more frequent in the ipsilateral inferior vestibular nucleus particularly after injections of

FIGURE 10

Distribution of labeled neurones in the medulla oblongata after injection of the right dPRN (case K12). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

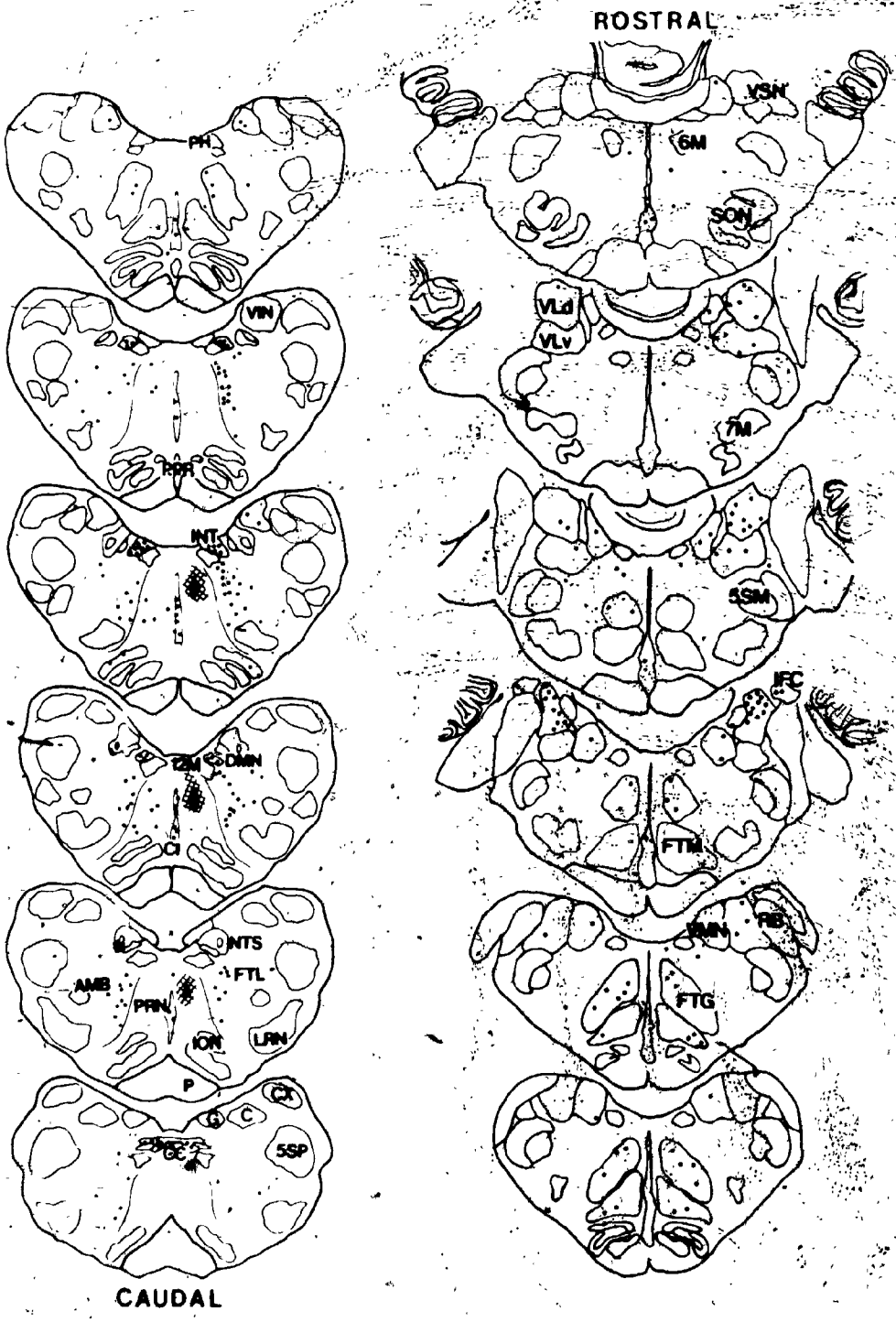

ROSTRAL
PH
VIN
RPR
INT
12M
DMN
CI
NTS
FTL
AMB
PRN
ION
LRN
P
CX
G
C
GC
5SP
CAUDAL
VSN
6M
SON
VLd
VLv
7M
5SM
IFC
FTM
VMN
RB
FTG

FIGURE 11

Distribution of labeled neurones in the medulla oblongata after injection of the right vPRN (case K21). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

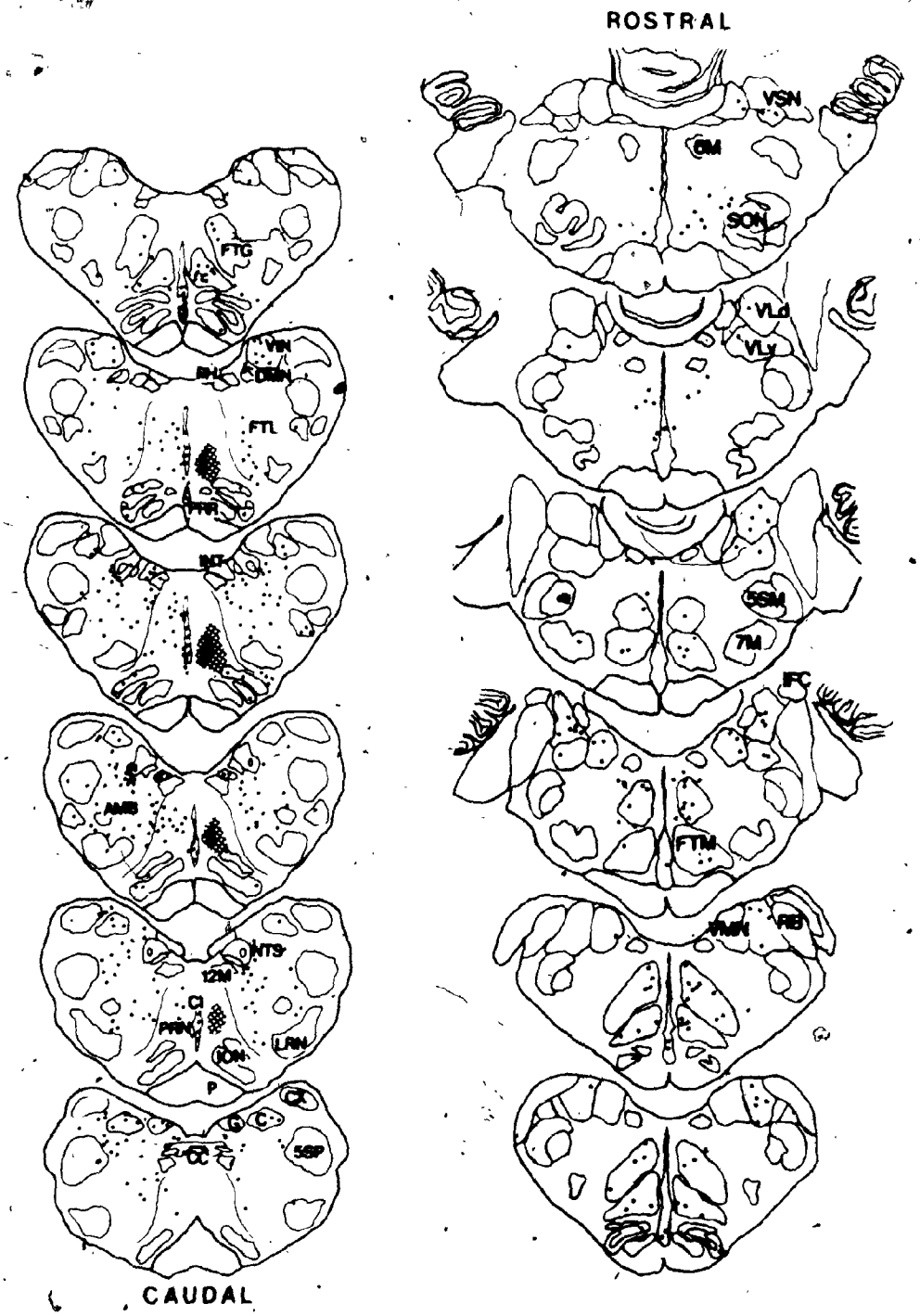

ROSTRAL
VSN
GM
SON
VLd
VLv
5SM
7M
IFC
FTM
VMN
RB
FTG
Vc
VIN
DRN
FTL
MTN
NCT
AMB
NTS
12M
CI
PRN
IOM
LRN
P
CC
5SP
CAUDAL

FIGURE 12

Photomicrographs (bright-field microscopy) of neutral red counterstained sections showing labeled neurones in the medulla oblongata and cervical cord (x 470).

a Left solitary nucleus

b Right nucleus intercalatus (of Staderini)

c Right medial vestibular nucleus

d. Right inferior vestibular nucleus

e Left lateral vestibular nucleus (dorsal division)

f Right lamina VI

g. Right lamina VII

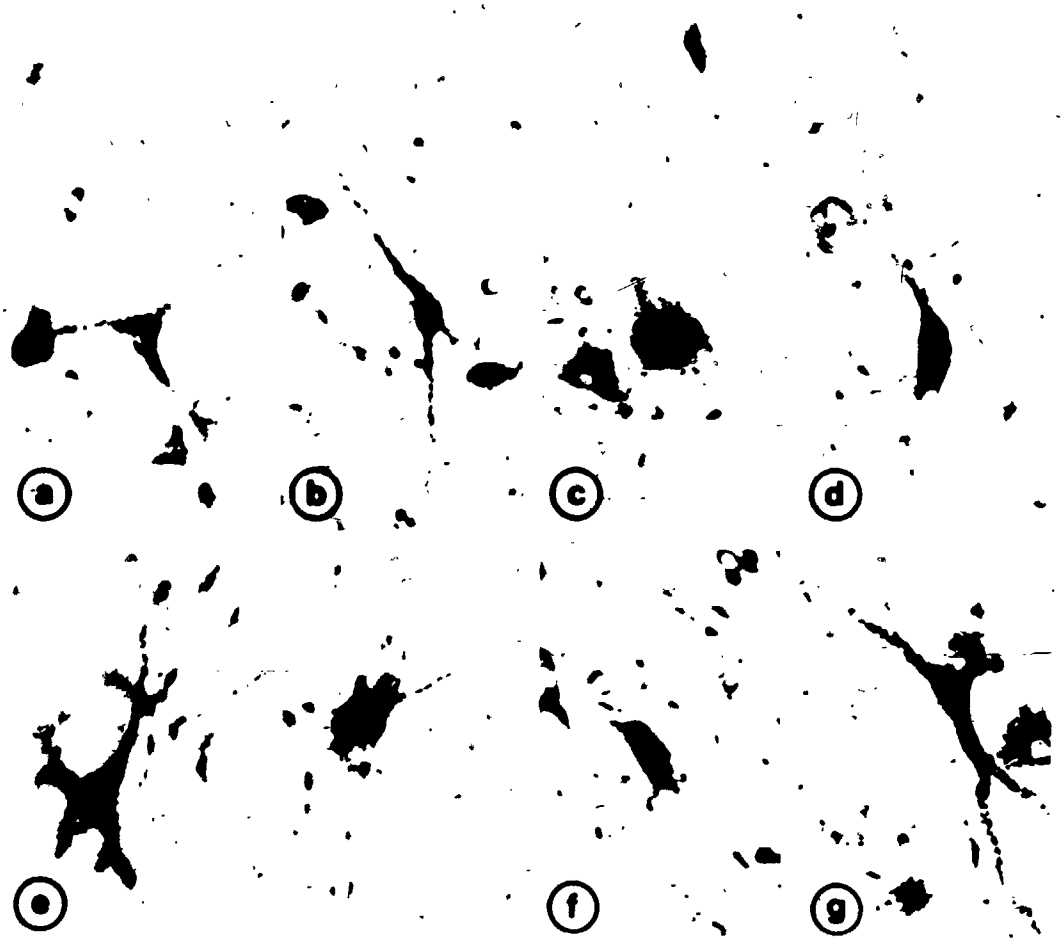

a
b
c
d
e
f
g

TABLE I

Number of labeled neurones within medullary nuclei after injection of the dPRN (K8) and vPRN (K22)

NUCLEUS	dPRN (K8)				vPRN (K22)			
	LEFT	RIGHT	TOTAL		LEFT	RIGHT	TOTAL	
LNT	13	12	25		10	9	19	
VLd	13	24	37	41	6	9	15	19
VLv	0	4	4		1	3	4	
VMN	5	9	14		5	6	11	
VIN	2	6	8		7	20	27	
FTM	4	6	10		11	23	34	
FTG	13	13	26		14	21	35	
Svl	19	1	20		8	2	10	
Sin	7	0	7	40	0	0	0	12
Com	8	5	13		2	0	2	

the vPRN (Table IX). No labeled cells were found in the superior vestibular nuclei. Sparse neuronal labeling of the infracerebellar nucleus (cell group y) on both sides appeared after injection of the dPRN.

D. Accessory Oculomotor Nuclei.

Many neurones within the interstitial nucleus of Cajal and the nucleus of Darkschewitz were labeled following injection of the PRN (Figure 13). Neurones in areas of the reticular formation ventral and lateral to the accessory oculomotor group in addition to the intervening area between the interstitial nucleus of Cajal and the nucleus of Darkschewitz were also labeled. The latter area included cells within the periaqueductal gray region of the rostral midbrain. A sparse bilateral projection to the dPRN from the nucleus of the posterior commissure was also noted, although it could not be demonstrated in all cases. Examination of frozen sections from segmental levels in all regions of the spinal cord failed to identify consistently a bilateral distribution of terminal anterograde label in Rexed's laminae VII and VIII (dorsal aspect), the sites of termination of interstitiospinal fibers.

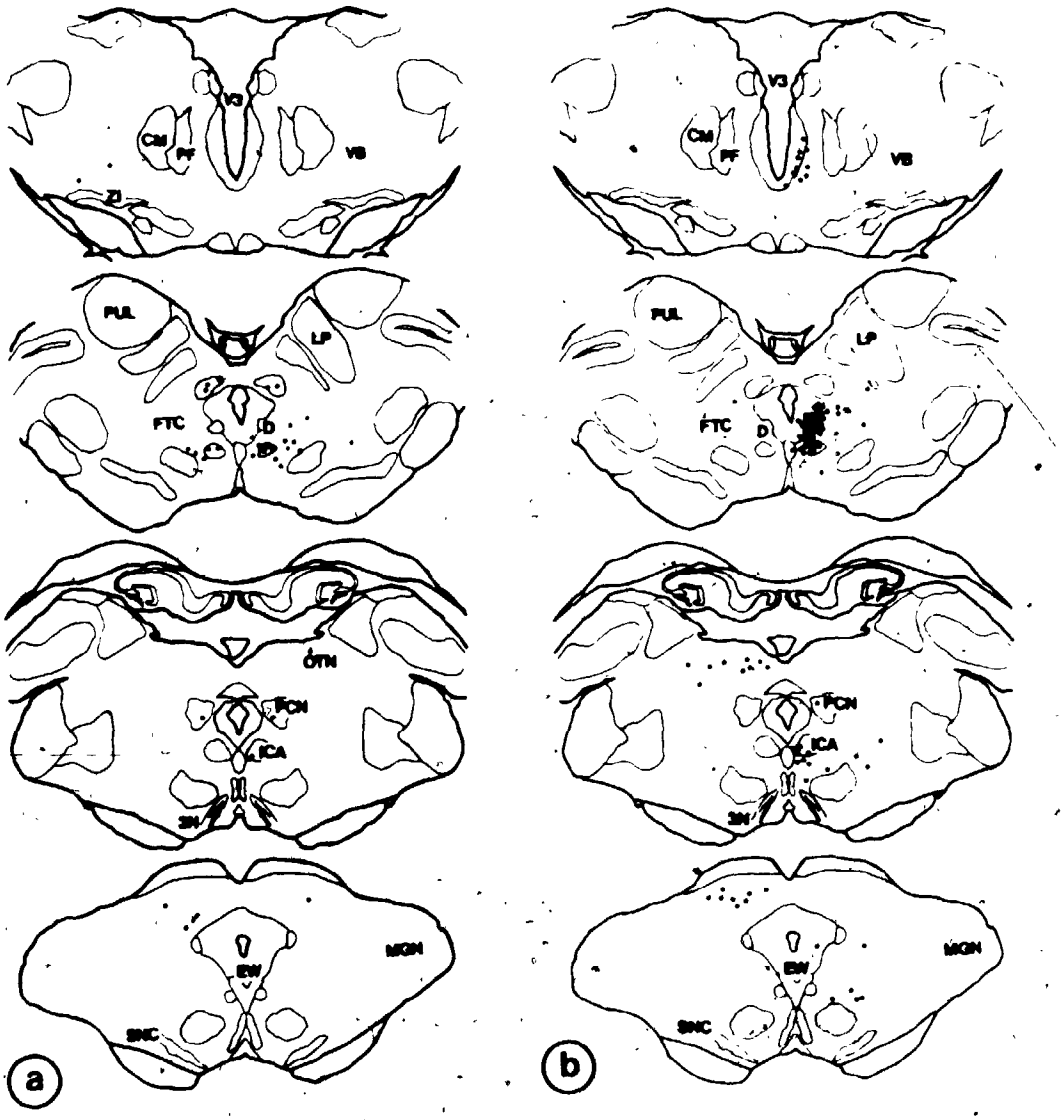
i. Interstitial Nucleus of Cajal

The interstitial nucleus of Cajal which lies ventral to the periaqueductal gray of the rostral midbrain is an ill-defined nucleus traversed by fibers of the medial longitudinal fasciculus (MLF). Injection of the dPRN resulted in labeling of neurones in the interstitial nucleus bilaterally with an ipsilateral predominance (Figure 13a). The labeled cells were confined to the rostral half of the nucleus. The majority were medium-sized, multipolar and densely-labeled (Figure 14a). The neighbouring medial reticular formation (central tegmental field) also contained labeled neurones bilaterally. Injections of the vPRN resulted in labeling

FIGURE 13

Distribution of labeled neurones in the rostral midbrain after injection of the right dPRN (case K19) (a) and right vPRN (case K35) (b). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

ROSTRAL



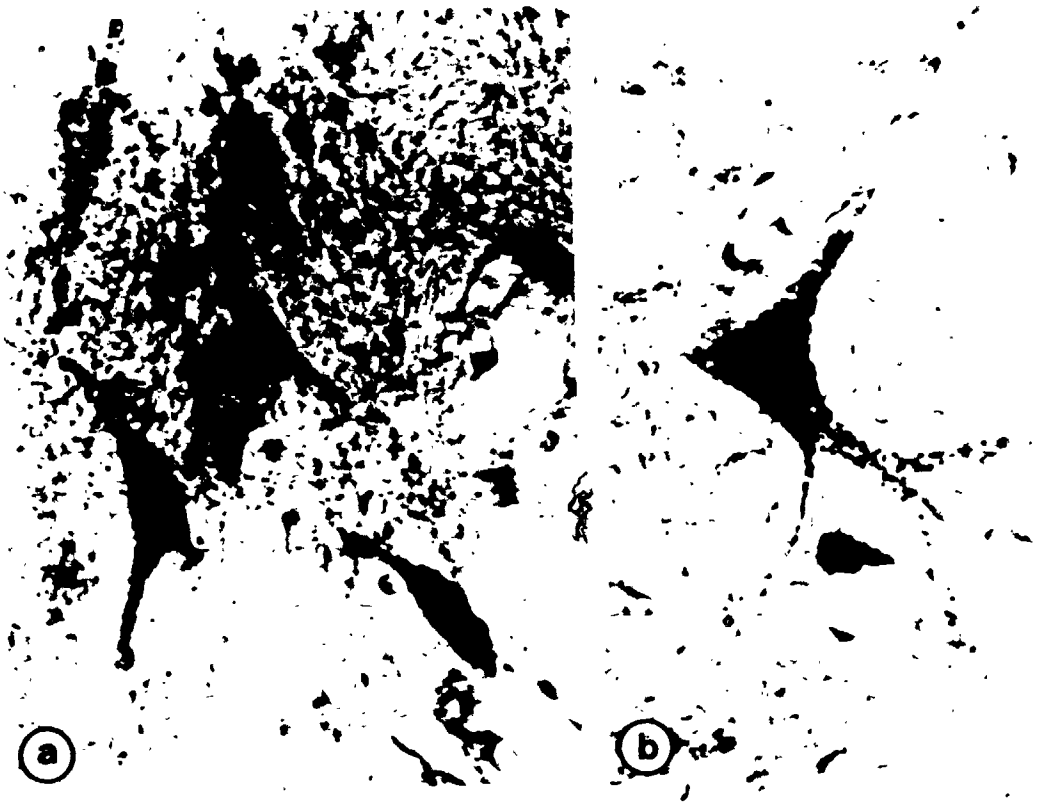
CAUDAL

FIGURE 14

Photomicrographs (bright-field microscopy) of neutral red counterstained sections showing labeled neurones in the accessory oculomotor nuclei (x 510).

a. Interstitial nucleus of Cajal

b. Nucleus of Darkschewitsch


a
b

of neurones only in the ipsilateral interstitial nucleus and the surrounding reticular formation. Slightly more labeled cells were found medially in the interstitial nucleus throughout its rostrocaudal extent.

ii. Nucleus of Darkschewitsch

The nucleus of Darkschewitsch is a small cluster of cells in the ventrolateral portion of the periaqueductal gray slightly rostral to the caudal pole of the interstitial nucleus of Cajal. No cellular labeling in the nucleus of Darkschewitsch appeared after injections of the dPRN In contrast, particularly dense labeling of numerous cells within the nucleus ipsilaterally was noted after injections of the vPRN (Figure 13b). Cellular labeling was also prominent in the neighbouring periaqueductal gray and the region intervening between the nucleus of Darkschewitsch and the interstitial nucleus of Cajal. A rostral prolongation of the area of cellular labeling in the periaqueductal gray extended into the caudal diencephalon. Labeled cells in the nucleus of Darkschewitsch were medium-sized and multipolar or fusiform in shape (Figure 14b).

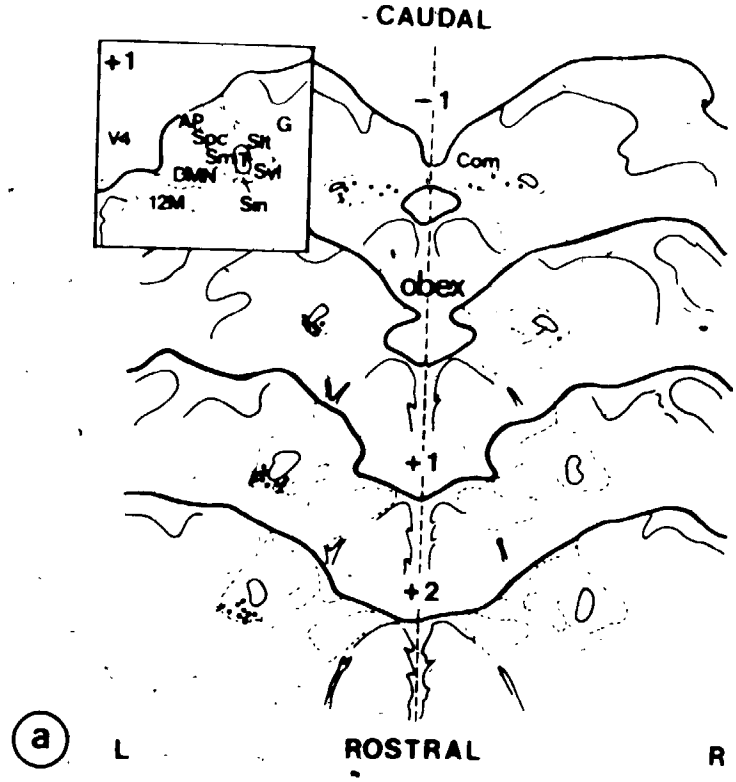
E. Remaining Brainstem Nuclei

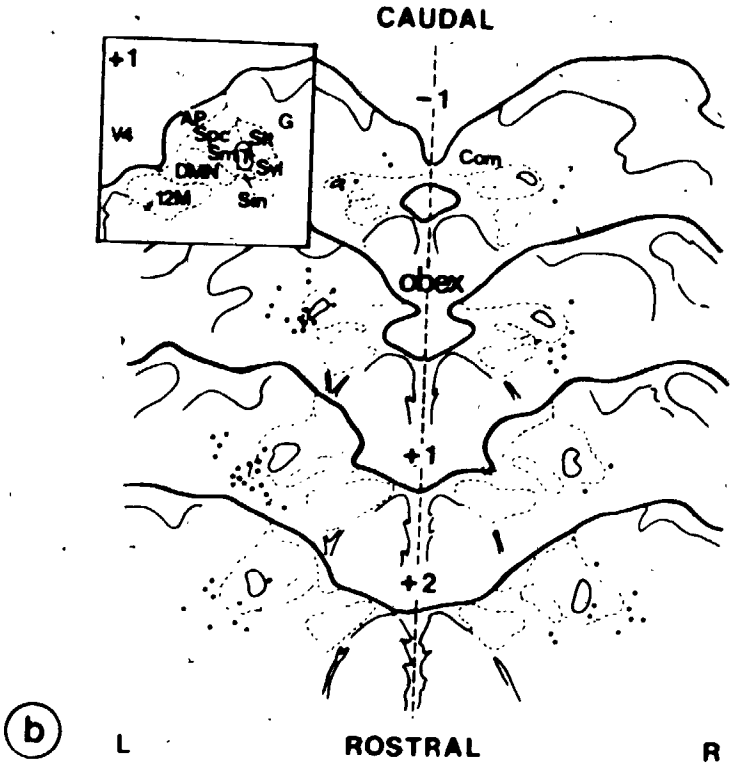
i. Solitary Nuclear Complex

Injection of the dPRN resulted in labeling of cells mainly within the opposite ventrolateral solitary nucleus (Svl) and a few scattered cells of the commissural and intermediate solitary nuclei (Figure 15a). Injection of the vPRN resulted in less marked labeling of cells in both the contralateral and ipsilateral Svl although more labeled cells appeared in the area immediately ventral to the solitary complex (Figure 15b). The afferent projection to the PRN from the solitary complex was heavier from its caudal half. Labeled neurones were mainly small and medium-sized and appeared spindle-shaped or multipolar (Figure 12a).

FIGURE 15

Distribution of labeled neurones in the solitary tract nucleus and immediate area following injection of the right dPPN (case K8) (a) and the right vPPN (case K12) (b). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.


CAUDAL
+1
V4
AP
Spc
Sm
G
Sm
Sm
DMN
12M
Sm
-1
Com
obex
+1
+2
a
L
ROSTRAL
R


CAUDAL
+1
V4
AP
Spc
Sm
G
Sm
Sm
DMN
12M
Sm
-1
Com
obex
+1
+2
b
L
ROSTRAL
R

ii. Superior Colliculus

Labeling of cells was found predominantly in the intermediate layer of the contralateral superior colliculus after injections of HRP into the vPRN (Figures 13b, 16). A few scattered labeled neurones appeared in the deep layer of the superior colliculus. Injections of the dPRN resulted in labeling of comparably much fewer scattered neurones in the contralateral superior colliculus (Figure 17). This may have resulted from spurious labeling of passing fibers destined for the vPRN. Individual neurones were moderately- to densely-labeled and usually medium-sized.

iii. Nucleus Intercalatus (of Staderini)

Labeled neurones appeared in approximately equal numbers in the nucleus intercalatus of both sides when either the dPRN or vPRN was injected (Figures 10, 11). These appeared generally throughout most of the rostrocaudal extent of the nucleus. Injection of the dPRN produced a distribution of labeled cells within the nucleus intercalatus which extended further rostrally than did that of the vPRN. The labeled cells were usually small while only a few were medium-sized (Figure 12b). Most had oval cellular outlines or were fusiform in shape.

iv. Raphe and Reticular Formation

Labeling of large, medium-sized and small fusiform cells occurred throughout most of the inferior central nucleus (nucleus raphe obscurus) of the medulla with injection of both the dPRN and vPRN, the latter resulting in a larger number of labeled cells in the raphe. In addition, the postpyramidal nucleus of the raphe (nucleus raphe pallidus) contained labeled cells resulting from injection of the vPRN. Labeling of cells appeared throughout the rostrocaudal extent of the medullary raphe

FIGURE 16

Distribution of labeled neurones in the pons and caudal midbrain after injection of the right vPRN (case K21). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

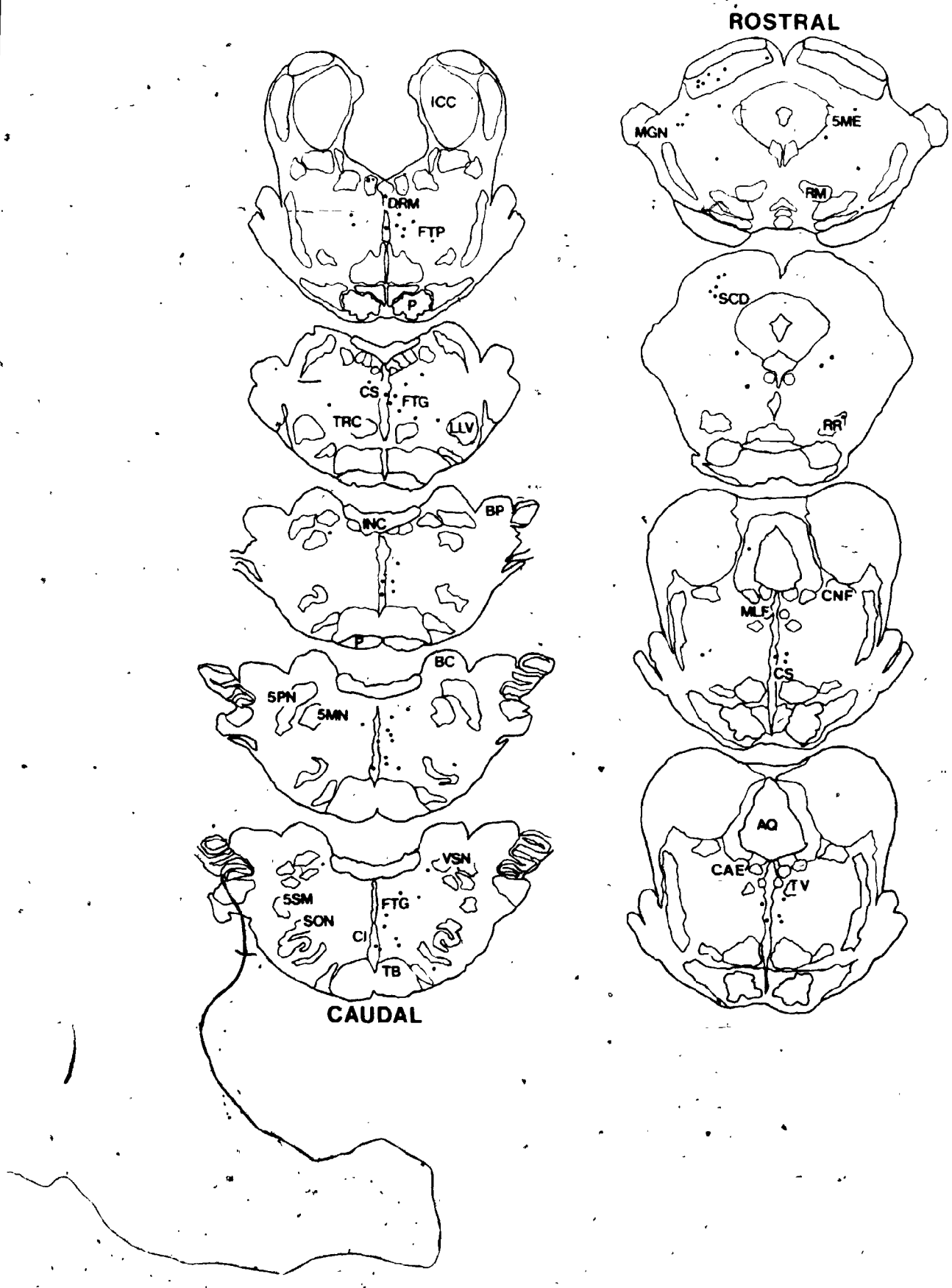
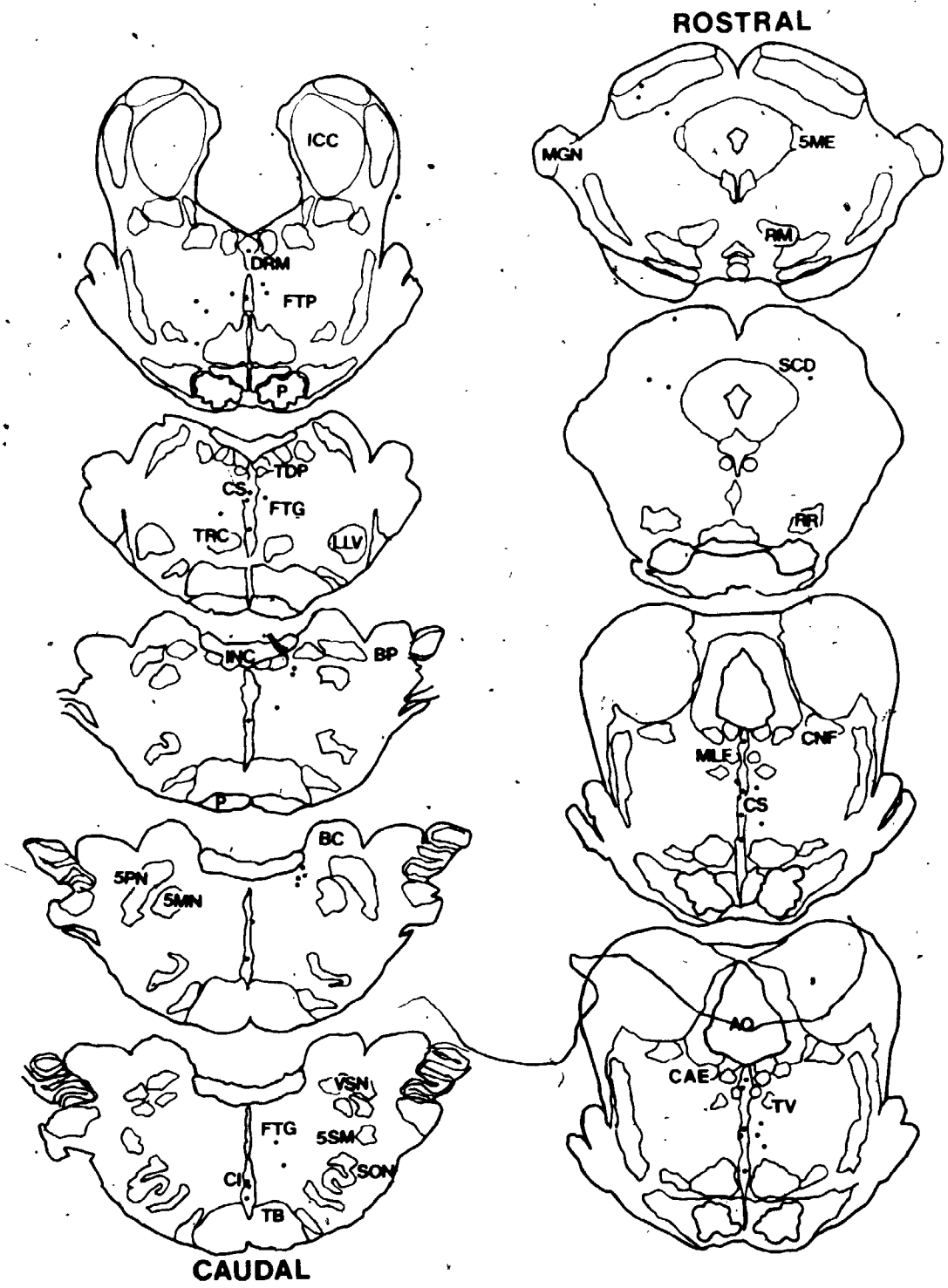

ROSTRAL
ICC
DRM
FTP
P
CS
FTG
TRC
LLV
INC
BP
P
BC
5PN
5MN
VSN
5SM
SON
CI
FTG
TB
CAUDAL
MGN
5ME
RM
SCD
RR
MLF
CNF
CS
AQ
CAE
LV

FIGURE 17

Distribution of labeled neurones in the pons and caudal midbrain after injection of the right dPRN (case K19). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.


ROSTRAL
ICC
MGN
5ME
DRM
RM
FTP
SCD
P
TDP
CS
FTG
RR
TRC
LLV
INC
BP
CNF
MLF
CS
P
BC
5PN
5MN
AQ
CAE
TV
VSN
FTG
5SM
SON
CI
TB
CAUDAL

but was concentrated more in its caudal portion.

A sparse and variable pattern of cell labeling occurred throughout the raphe region in the pons and midbrain after injections of HRP into either the dPRN or vPRN (Figures 16, 17). The most consistently labeled cells appeared within the inferior and superior central nuclei of the raphe. Other nuclei containing labeled cells were the nuclei raphe magnus, pontis and dorsalis. Individual cells often showed moderate to light granular labeling. Most labeled cells were medium-sized although a few large cells in the inferior central nucleus of the pons were also labeled.

The lateral (FTL), gigantocellular (FTG) and magnocellular (FTM) tegmental fields ipsilateral and contralateral to the site of injection also contained labeled neurones (Table I). These were mainly large or medium-sized and multipolar in appearance. An HRP injection of the lateral tegmental field at the level of the PRN resulted in anterograde as well as retrograde labeling of some cells within the ipsilateral and contralateral PRN, contralateral FTL and the raphe indicating that at least some of the labeled cells seen within the PRN, FTL and raphe on previous injections of the PRN could have resulted from interruption of fibers traversing the area of injection. Similarly labeling of cells within the cuneate and gracile nuclei predominantly on the contralateral side after injection of the vPRN resulted largely from interruption of fibers of the medial lemniscus and olivopetal fibers.

Scattered labeled cells appeared in the medial reticular formation of the pons and midbrain. The ipsilateral gigantocellular, paralemniscal and central tegmental fields contained the majority of

labeled cells (Figures 16, 17). Injections of the dPRN resulted in labeling of a small number of cells both ipsilaterally and contralaterally. Injections of the vPRN produced a pattern of retrogradely labeled cells which was largely ipsilateral particularly within the gigantocellular and paralemniscal tegmental fields closely applied to the inferior and superior central nuclei of the raphe.

v. Contralateral PRN

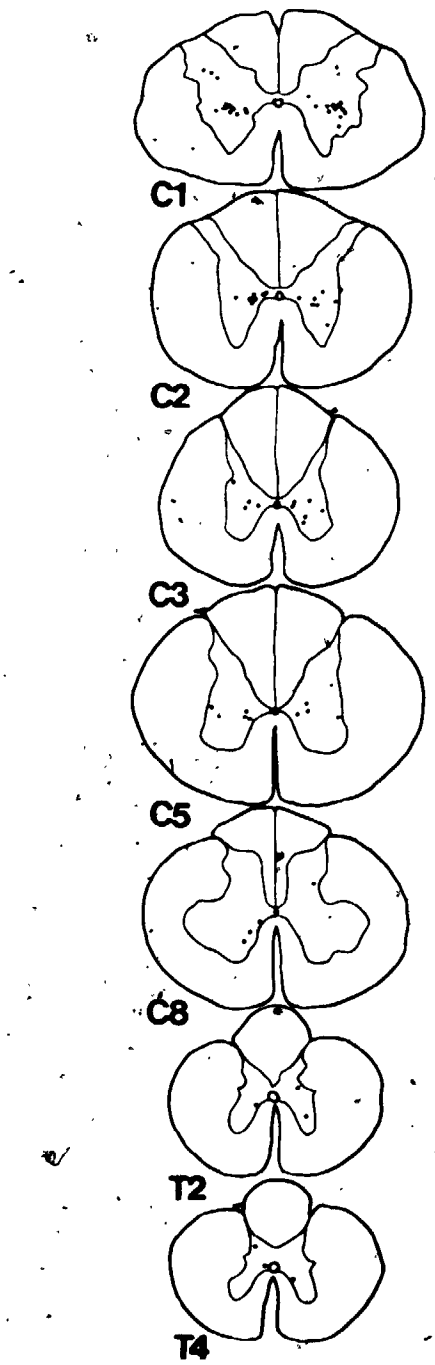
Labeled neurones appeared within the contralateral dPRN and vPRN after injections of either the dPRN or vPRN. Medium-sized multipolar and polygonal cells were most often labeled.

F. Spinal Cord

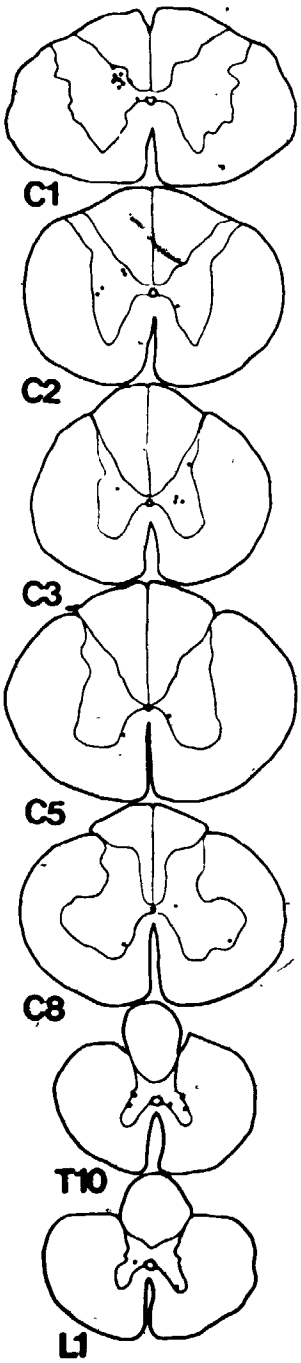
The majority of retrogradely labeled spinal neurones were found in the cervical cord with injection of either the dPRN or vPRN. In the case of the dPRN, labeled cells were distributed bilaterally in approximately equal proportions in laminae VII and VIII (Rexed, '54) (Figure 18a). A few additional scattered labeled cells were found in laminae V and VI of the cervical cord as well as laminae VI, VII and VIII of the upper thoracic cord. Injection of the vPRN produced labeling of cells which was concentrated largely in the medial zone of lamina VI in the contralateral C1 region of the cervical cord (Figure 18b). A few additional labeled neurones appeared bilaterally in laminae VII and VIII of the remaining cervical and upper thoracic cord as well as the lower thoracic and upper lumbar cord. Small labeled cells appeared largely within the medial zone of lamina VI and were oval or triangular in shape (Figure 12f).

FIGURE 18

Distribution of labeled neurones in the spinal cord following injection of the right dPRN (case K8) (a) and the right vPRN (case K22) (b). Each dot represents a single cell and each level indicates the cumulative labeled cell population of five tissue sections.



(a)



(b)

G. Cerebral Cortex

Examination of serial sections of the cerebral hemispheres after injection of HRP into the PRN showed retrograde cellular label confined to the sensorimotor cortex with no label appearing posterior to the coronal gyrus. Some segregation of populations of labeled cells was possible by separate injection of HRP into either the dPRN or vPRN. Similar densities of cells were labeled bilaterally. In the cases involving injection of the dPRN, most labeled cells appeared in the ventral coronal gyrus (Figure 19a). Other sites of cell labeling included the medial portion of the anterior sigmoid gyrus in the pericruciate cortex and a few scattered cells in the posterior sigmoid gyrus. Injection of the vPRN produced retrograde cell labeling chiefly in both the lateral and medial portions of the anterior sigmoid and ventral coronal gyri (Figure 19b). Additional labeled cells were found scattered in the posterior sigmoid gyrus. Labeled neurones were medium-sized with pyramidal or oval-shaped cell bodies (Figure 20). All appeared within the internal pyramidal layer (layer V) of the cortex. As the micropipette penetrations did not encroach upon the pyramidal tract, spurious retrograde labeling of corticospinal fibers was not likely to have occurred.

4.2 Collateral Efferent Projections of PRN Neurones

A. Projections to the Cerebellum

The sites of injection of the fluorochromes, FB and NY, are shown in Figure 21. The percentage of FB-labeled reticulocerebellar neurones in the PRN which were NY double-labeled was calculated according to the method of Huisman et al. (1982). FB single labeled and FB-NY double-labeled neurones were counted in every fifth section through the PRN and the numbers multiplied by 5 with a percentage calculated according

FIGURE 19

Distribution of labeled neurones in the cerebral cortex after injection of the right dPRN (case K8) (a) and the right vPRN (case K22) (b). Each dot represents a single cell and each level indicates the cumulative labeled cell population of three tissue sections.

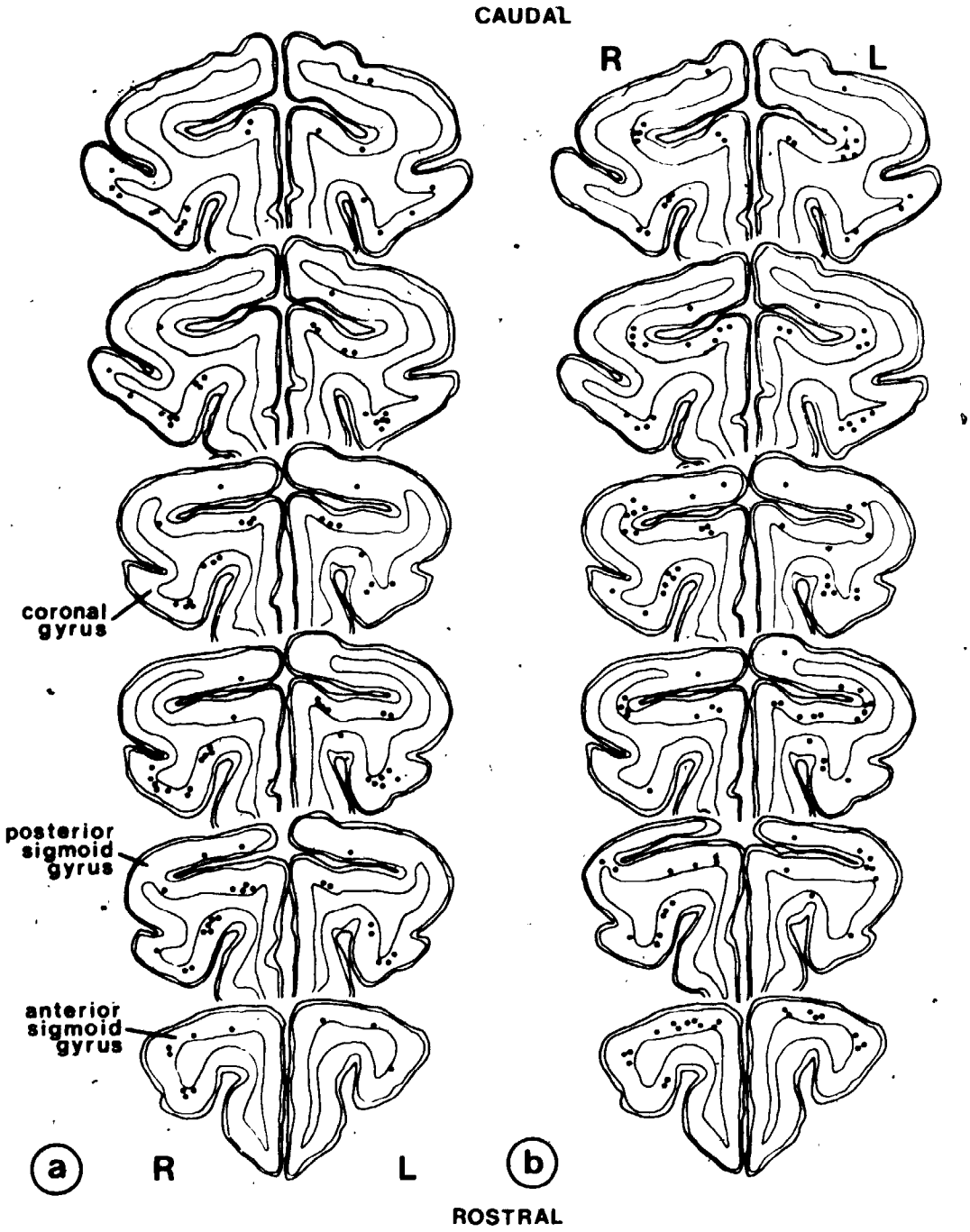

CAUDAL
R
L
coronal gyrus
posterior sigmoid gyrus
anterior sigmoid gyrus
a
R
L
b
ROSTRAL

FIGURE 20

Photomicrographs (bright-field microscopy) of neutral red counterstained sections showing labeled neurones in the cerebral cortex (x 480).

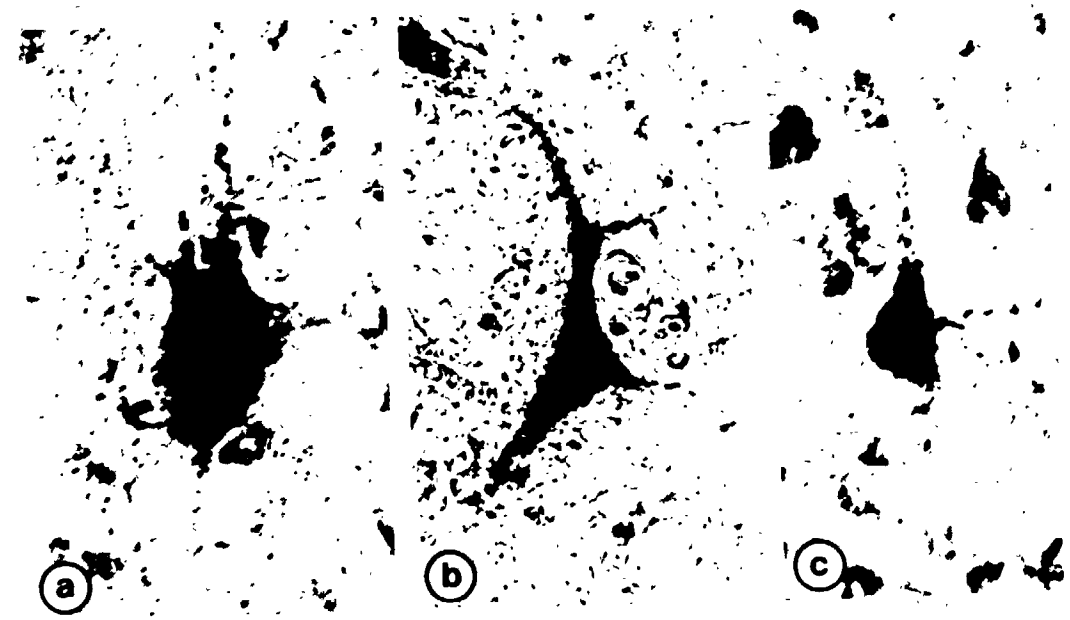
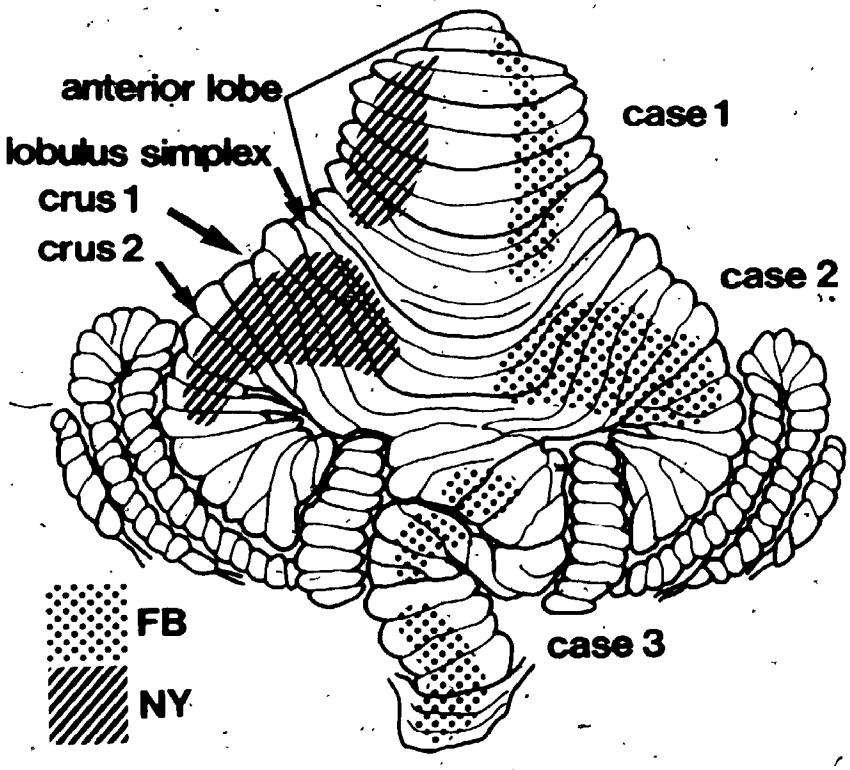

a
b
c

FIGURE 21

Diagram of the cerebellar folia showing the extent of FB and NY injections in the anterior lobe (case 1), the lobulus simplex, crus I and crus II (case 2) and of the FB injection in the posterior vermis (case 3).


anterior lobe
lobulus simplex
crus 1
crus 2
case 1
case 2
case 3
FB
NY

to the formula:

$$\frac{\text{FB-NY double-labeled neurones}}{\text{single FB + FB-NY double-labeled neurones}} \times 100\%$$

Injections of the anterior lobe (case 1) resulted in an equal distribution of FB and NY label in both the dorsal and ventral divisions of the right PRN. Double-labeled neurones appeared in both divisions although predominantly in the dPRN (Figure 22a). The percentage of FB-labeled neurones in the dPRN and vPRN which were double-labeled with NY were 35% and 15%, respectively (Table II). Injections of the lobulus simplex, crus I and crus II (case 2) resulted in smaller numbers of single-labeled neurones and considerably fewer double-labeled neurones in both the dPRN and vPRN (Figure 22b). Again, most single- and double-labeled cells appeared in the dPRN. The percentages of FB-labeled neurones in the dPRN and vPRN which were double-labeled with NY were 27% and 17%, respectively (Table II). In case 3, a single FB injection field of the posterior vermis was accompanied later by bilateral injections of NY into the lobulus simplex and crus I. Some double-labeling of neurones was again noted in both the dPRN and vPRN. The accessory division of the PRN (aPRN) contained no double-labeled cells despite having considerably more FB single-labeled neurones than in the previous two cases (Figure 22c). A greater number of double-labeled cells appeared in the dPRN. Labeled cells were clearly distinguishable on the basis of their fluorescent characteristics and cytological features (Figure 23).

B. Projections to the Spinal Cord

The distribution of PRN cells sending fibers to the region of the IML in the T2 segment of the thoracic cord was examined in the case material of Caverson et al. (1983) in which discrete placements of WGA-HRP

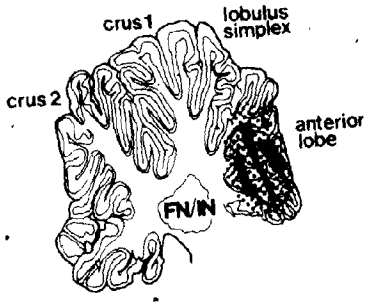
FIGURE 22

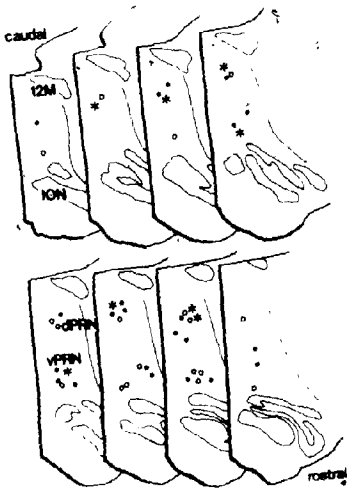
Retrograde fluorochrome labeling of PRN neurones after injection of the cerebellum. Semidiagrammatic sagittal sections of representative FB injection sites in the cerebellum. Sites of tissue necrosis are marked in black and are surrounded by a gradient of fluorescence most intense centrally and least intense at the outer border indicated. Corresponding transverse sections of the caudal medulla at the level of the PRN show the distribution of single- and double-labeled neurones. ● , FB single-labeled neurone; ○ , NY single-labeled neurone; ✱ , FB-NY double-labeled neurone.

a. Injection of the anterior lobe (case 1).

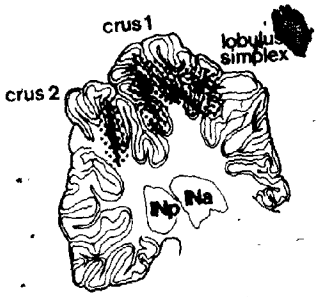
b. Injection of the lobulus simplex, crus I and crus II (case 2).

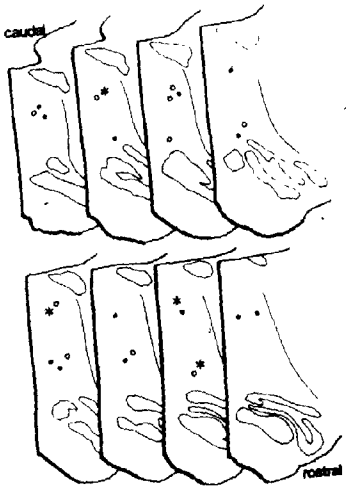
c. Injection of the posterior vermis (bilateral NY injections of the lobulus simplex and crus I) (case 3).


crus 1
lobulus simplex
crus 2
anterior lobe
FN/IN

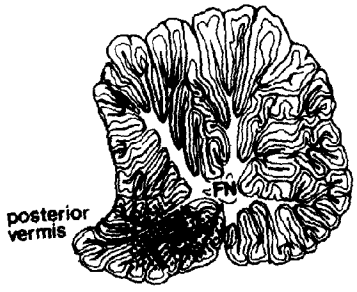

caudal
l2M
lON
dPPN
vPPN
rostral

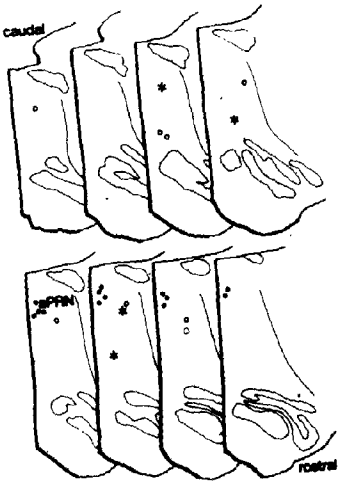
a


crus 1
lobulus simplex
crus 2
Np
iNa


caudal
rostral

b


FN
posterior vermis


caudal
dPPN
rostral

c

TABLE II

Distribution of double-labeled neurones in the dPRN and vPRN after cerebellar injections of FB and NY

	FB-labeled and FB-NY double-labeled neurones	FB-NY double-labeled neurones	percentage of FB-labeled neurones which were double-labeled with NY
case 1			
dPRN	85	30	35%
vPRN	65	10	15%
case 2			
dPRN	55	15	27%
vPRN	30	5	17%

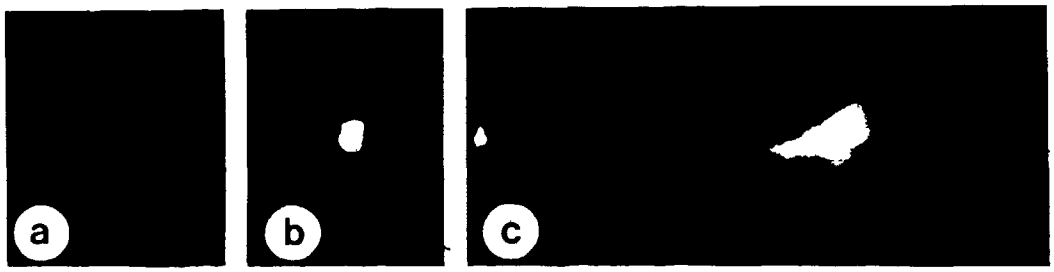
FIGURE 23

Photomicrographs (ultra-violet illumination) of fluorochrome-labeled neurones in the PRN after injection of the cerebellum (x 400).

a. FB single-labeled neurone showing cytoplasmic labeling and a nuclear shadow indicating the absence of nuclear labeling.

b. NY single-labeled neurone showing nuclear labeling.

c. FB-NY double-labeled neurone showing both cytoplasmic and nuclear labeling.


a
b
c

were made in the IML region. Figure 24 summarizes the results obtained in one representative animal which had an HRP diffusion site localized primarily to the region of the IML (Figure 24a). The majority of retrogradely labeled neurones were found throughout the rostrocaudal extent of the vPRN (Figure 24b). A smaller number was also observed in the ventral aspect of the dPRN, particularly in the middle to rostral portions of the nucleus. Labeled neurones were usually medium- and large-sized, and multipolar (Figures 24d-f).

After injection of FB and NY into the IML region, the patterns of distribution of single- and double-labeled neurones in the PRN appeared similar and showed no topological variation among cases injected at either the T2, T4 or T7 levels (Figure 25). The percentages of FB-labeled neurones which were double-labeled with NY after injection of NY into the region of the IML at the T4 and T7 segmental levels were 42% and 35%, respectively; the corresponding percentages of double-labeled cells taken from the total number of neurones found labeled within the PRN were 30% and 24%, respectively. Single-labeled (FB- or NY-) and double-labeled neurones were clearly distinguishable from one another on the basis of their fluorescent characteristics and cytological features (Figure 26). Slightly more FB single-labeled neurones were identified than NY single-labeled neurones. This may have occurred from the labeling of a greater number of fibers likely terminating in the IML region at the T2 segmental level which has been shown to contain the largest segmental number of sympathetic preganglionic neurones (Henry and Calaresu, 1972).

FIGURE 24

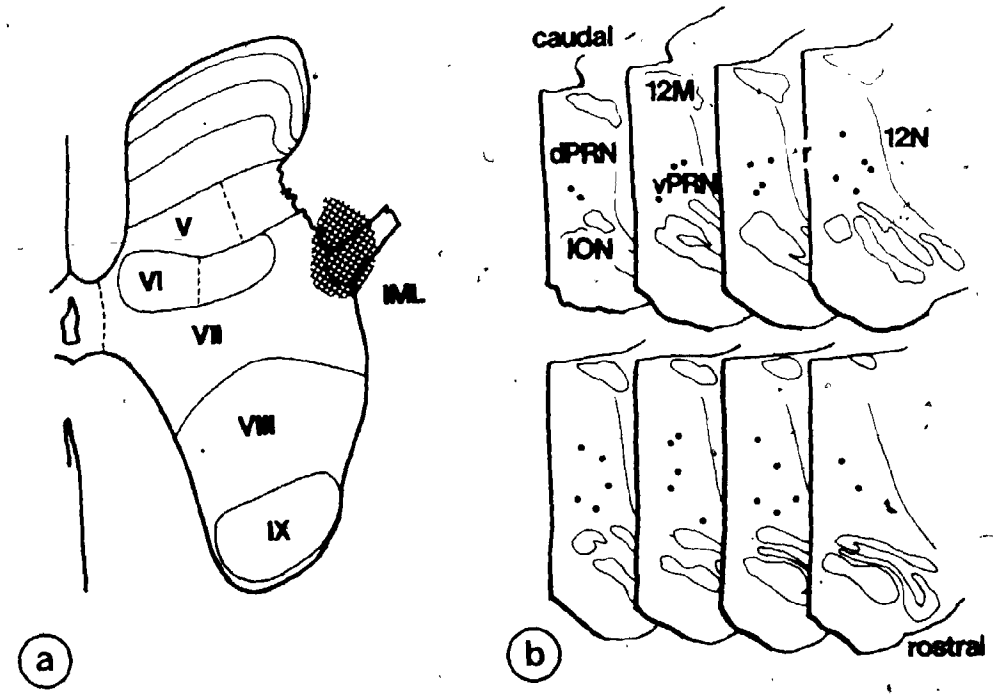
Retrograde labeling of neurones in the ipsilateral PRN after diffusion of HRP into the region of the IML.

a. Cross-section of the spinal cord (modified after Rexed, 1954) at the T2 level showing the maximum extent of diffusion of the HRP (crosshatch).

b. Transverse hemimedullary sections through the region of the PRN (0.2 to 1.6 mm rostral to the obex). Each dot represents a single labeled neurone.

c. Bright-field photomicrograph of a neutral red counterstained section showing HRP retrogradely labeled neurones in the PRN (x 24).

d-f. Bright-field photomicrographs of neutral red counterstained sections showing examples of HRP labeled neurones in the PRN (x 420).


V
VI
VII
VIII
IX
IML
a
caudal
12M
dPRN
vPRN
ION
12N
rostral
b

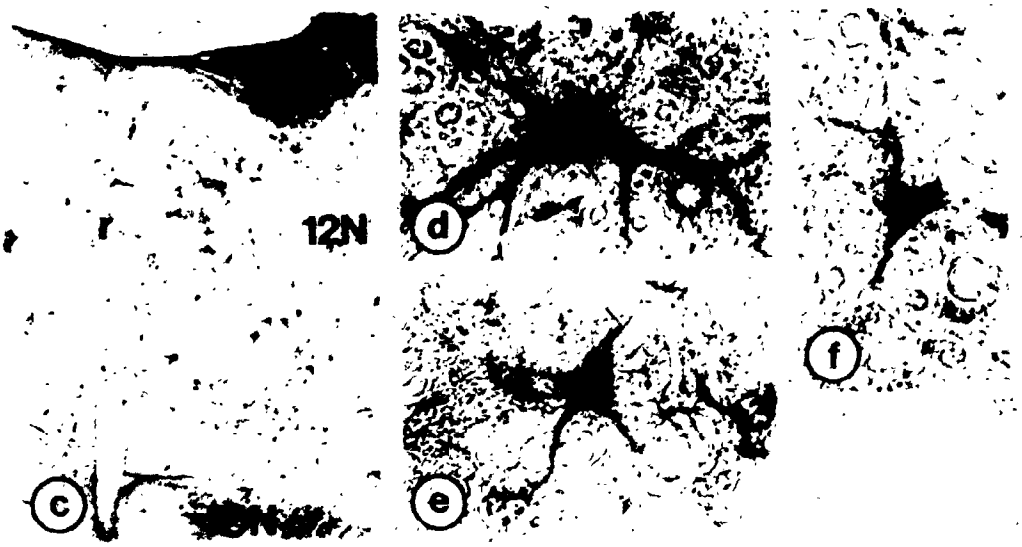

12N
c
d
e
f

FIGURE 25

Retrograde fluorochrome labeling of PRN neurones after injection of the spinal cord. o , FB single-labeled neurone; • , NY single-labeled neurone; * , FB-NY double-labeled neurone.

a. Transverse sections of the spinal cord showing the maximum extent of the injections in the region of the IML (shaded areas) of FB and NY in the T2 and T4 segments, respectively, and the resulting retrograde labeling of neurones in the PRN plotted on representative transverse sections of the brainstem.

b. The maximum extent of FB and NY injections (shaded areas) in the region of the IML in the T2 and T7 segments, respectively, and the resulting retrograde labeling of PRN neurones plotted on representative transverse sections of the brainstem.

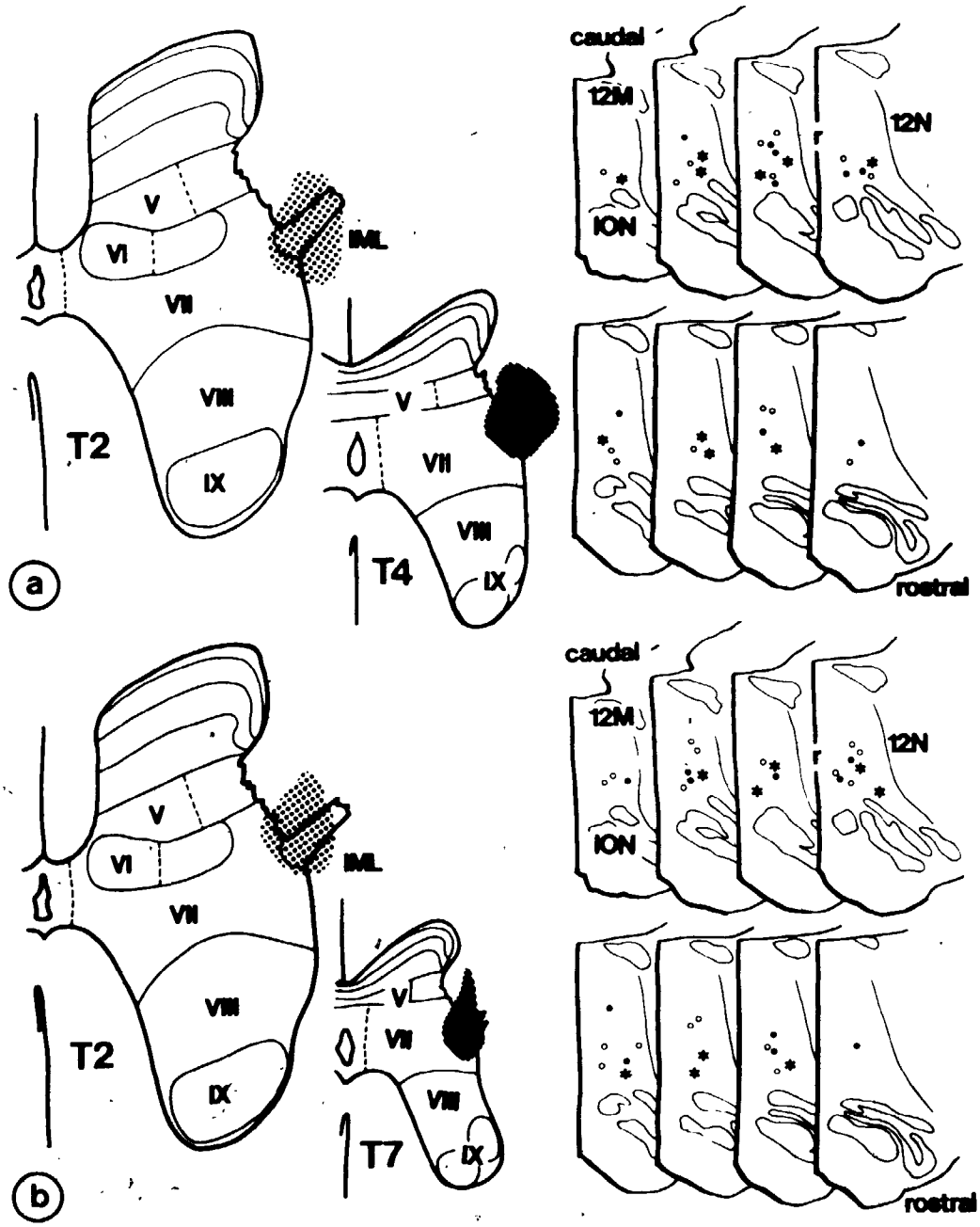

caudal
12M
12N
ION
rostral
V
VI
VII
VIII
IX
IML
T2
T4
T7
a
b

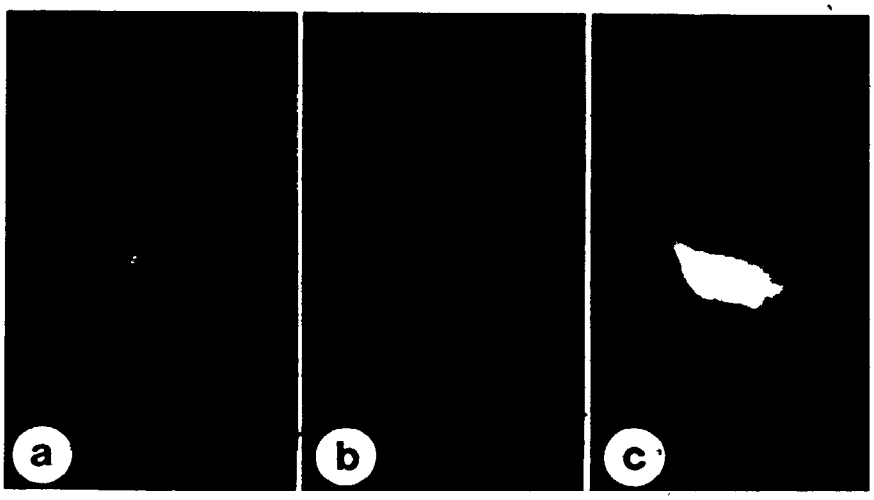
FIGURE 26

Photomicrographs (ultra-violet illumination) of fluorochrome-labeled neurones in the vPRN after injection of the spinal cord (x 420).

a. NY single-labeled neurone showing nuclear labeling.

b. FB single-labeled neurone showing cytoplasmic labeling and a nuclear shadow indicating the absence of nuclear labeling.

c. FB-NY double-labeled neurone showing both cytoplasmic and nuclear labeling.


a
b
c

4.3 Electrophysiological Identification of PRN Neurones Relaying Cardiovascular Afferent Input Directly to the Region of the IML

A. Electrophysiological Characteristics of Antidromically Activated PRN Neurones

Single units in the PRN which responded to electrical stimulation of functionally and histologically identified sites in the region of the ipsilateral IML (Figures 27a,28a) were assessed for antidromic activation using established criteria (Bishop et al., 1962; Fussey et al., 1970, Lipski, 1981): (a) constant latency of the evoked spike, (b) high following frequency, (c) duration of the evoked spike, (d) presence of an IS-SD spike configuration with induced failure of the SD component during high stimulation frequencies, and (e) collision of the evoked spike with a spontaneous spike. Not all antidromically activated single units could be evaluated for all criteria since 69% (43/62) of the units were not spontaneously active and could not be tested by the collision method. All spontaneously active units showed collision with antidromically evoked spikes. Seventy-four per cent of all antidromically activated single units showed two-component (IS-SD) spikes in which the SD spike could usually be induced to fail by a high frequency of stimulation.

In 43 electrode penetrations through the region of the PRN, 62 histologically verified single units were antidromically activated by electrical stimulation of functionally and histologically verified sites in the region of the ipsilateral IML (Figure 28a; Table III). Of these units, 19 (31%) discharged spontaneously (mean, 10.8 ± 3.2 spikes/s) and 43 were silent. Units responded with a mean latency of 3.0 ± 0.2 ms (range, 1.4 - 10 ms) and followed a mean rate of stimulation of 416 ± 31 Hz. The latencies of the antidromic responses corresponded to a mean conduction

FIGURE 27

Arterial pressure (AP) and heart rate (HR) responses elicited by electrical stimulation of the carotid sinus nerve (CSN), intermediolateral nucleus (IML), vestibular nuclear complex (VNC) and fastigial nucleus (FN). Stimulus applied between the two arrow heads. Calibration mark in A and B, 15 s.

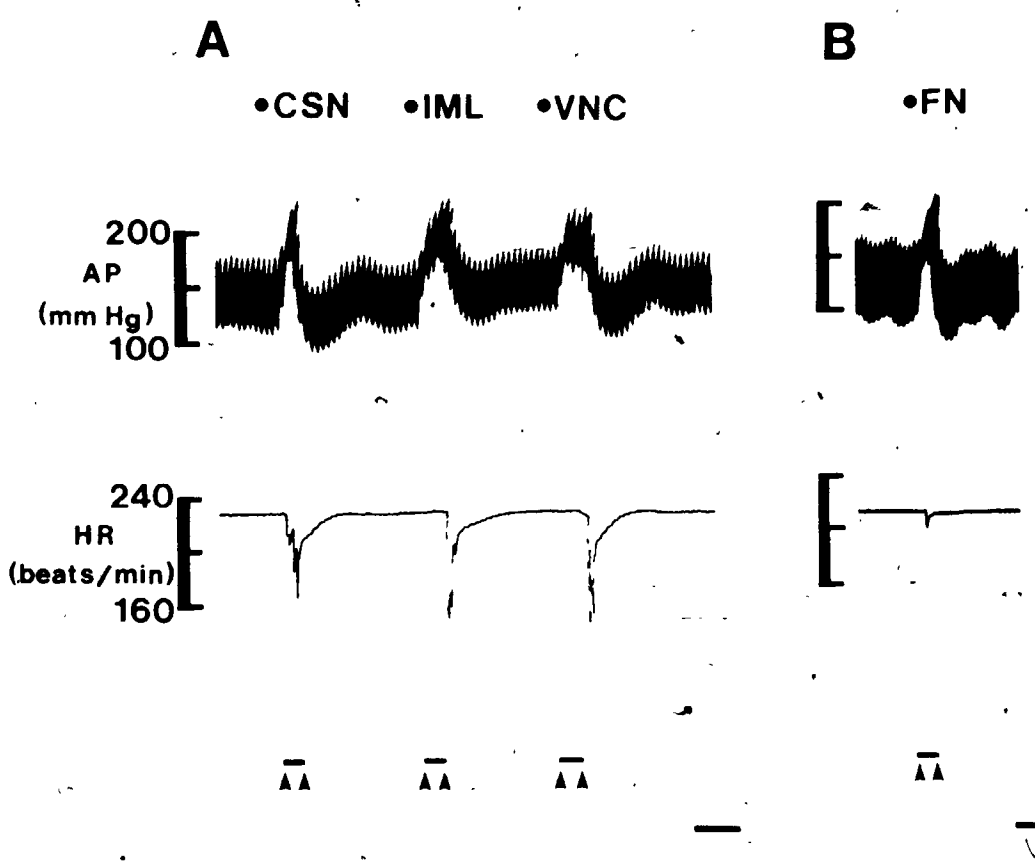

A
B
•CSN
•IML
•VNC
•FN
200
AP
(mm Hg)
100
240
HR
(beats/min)
160

FIGURE 28

Representative drawings of a transverse section of the spinal cord at the level of T2 (a) and of sagittal sections of the FN (b) showing the locations of histologically verified sites of stimulation.

a. Sites of stimulation in the region of the ipsilateral IML.

b. Sites of stimulation in the region of the contralateral FN (numbers indicate distances from the midline in mm according to Berman, 1968); scales in mm. CA, central autonomic area; DH, dorsal horn; LF, lateral funiculus; VH, ventral horn.

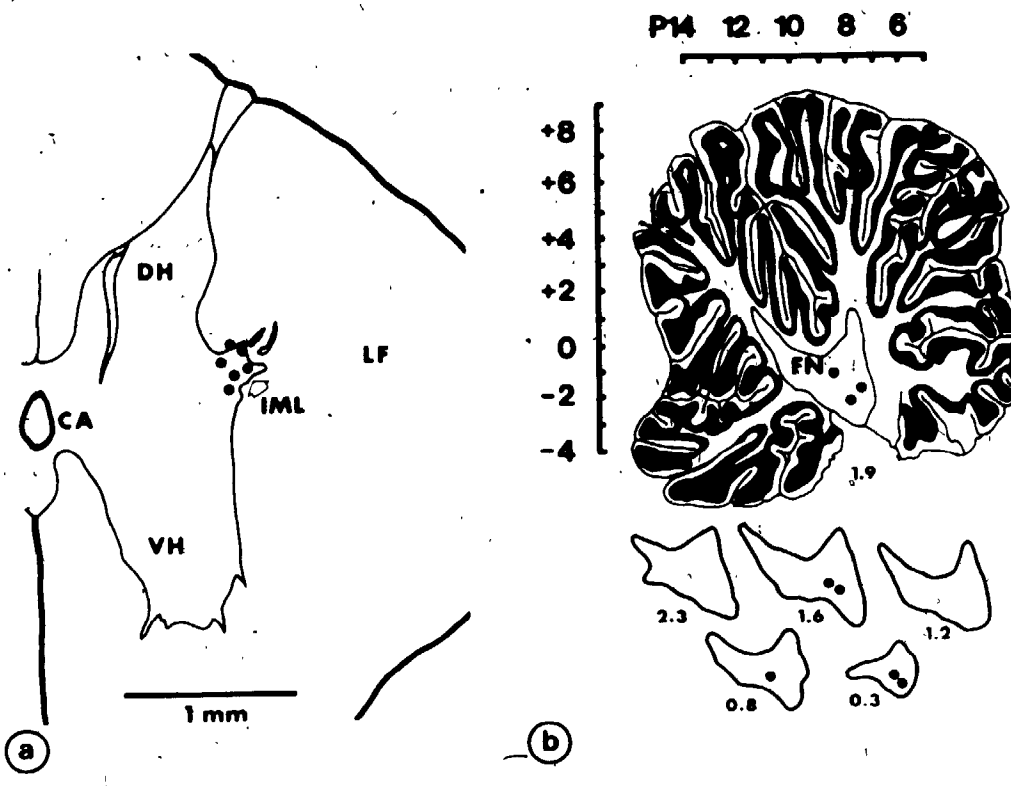

DH
LF
IML
CA
VH
1 mm
a
P14 12 10 8 6
+8
+6
+4
+2
0
-2
-4
FN
1.9
2.3
1.6
1.2
0.8
0.3
b

TABLE III

Electrophysiological characteristics of single units in the PRN antidromically excited by stimulation of the IML

Number of units:	62		
Number of spontaneously active units:	19		
Mean discharge rate:	10.8 ± 3.2	spikes/s	(0.2 - 60)
Mean latency:	3.3 ± 0.3	ms	(1.4 - 10)
Mean threshold:	484.0 ± 36.0	μA	(60 - 750)
Mean spike duration:	0.8 ± 0.04	ms	(0.2 - 1.6)
Mean following frequency:	462.0 ± 37.0	Hz	(100 - 900)
Mean conduction velocity of antidromic pathway:	36.4 ± 2.1	m/s	(10.0 - 75)
Mean conduction distance:	93.4 ± 2.6	mm	(85 - 106)

All values are means ± S.E. of the mean. Numbers in parentheses indicate the range of values for each parameter.

velocity of these descending fibers of 36.4 ± 2.1 m/s (range, 10 - 75 m/s; median 35.2 m/s) (Figure 29).

All units responded with a single spike (mean duration, 0.8 ± 0.04 ms) at threshold and suprathreshold stimulus intensities. The mean threshold stimulus intensity necessary to activate each unit antidromically when pulse durations of 0.1 - 0.3 ms were used was found to be 484 ± 36 μA. This stimulus intensity corresponded to a maximum stimulus spread to a sphere of tissue in the region of the IML with a radius of less than 0.5 mm (Bagshaw and Evans, 1976).

B. Cardiovascular Afferent Inputs to PRN Neurones

Twenty-five (40%) of the 62 antidromically activated units responded orthodromically to stimulation of the CSN and/or the FN (Figures 27b,c,28b). The remaining 37 antidromically identified units were non-responsive to the tested inputs. The evoked orthodromic response of these units was in the form of either a single spike or a burst of 2 - 3 spikes. Increasing the intensity of the stimulus usually decreased the latency of the responses and increased the number of spikes during a burst response. The anatomical distribution of the units antidromically activated by stimulation of the IML and orthodromically activated by stimulation of the CSN and/or the FN is shown in Figure 30. All units were confined largely to the caudal aspect of the vPRN with no distinct segregation of those units which were responsive to orthodromic stimulation from those that were not.

i. PRN Units Responding to CSN Stimulation

Of the orthodromically responsive units, 20% (5/25) were excited orthodromically by stimulation of only the CSN (mean latency,

FIGURE 29

Histogram of conduction velocities of 62 PRN single units antidromically activated by electrical stimulation of the IML. Hatched area represents the conduction velocities of antidromically activated units which were also excited by stimulation of the CSN and/or the FN.

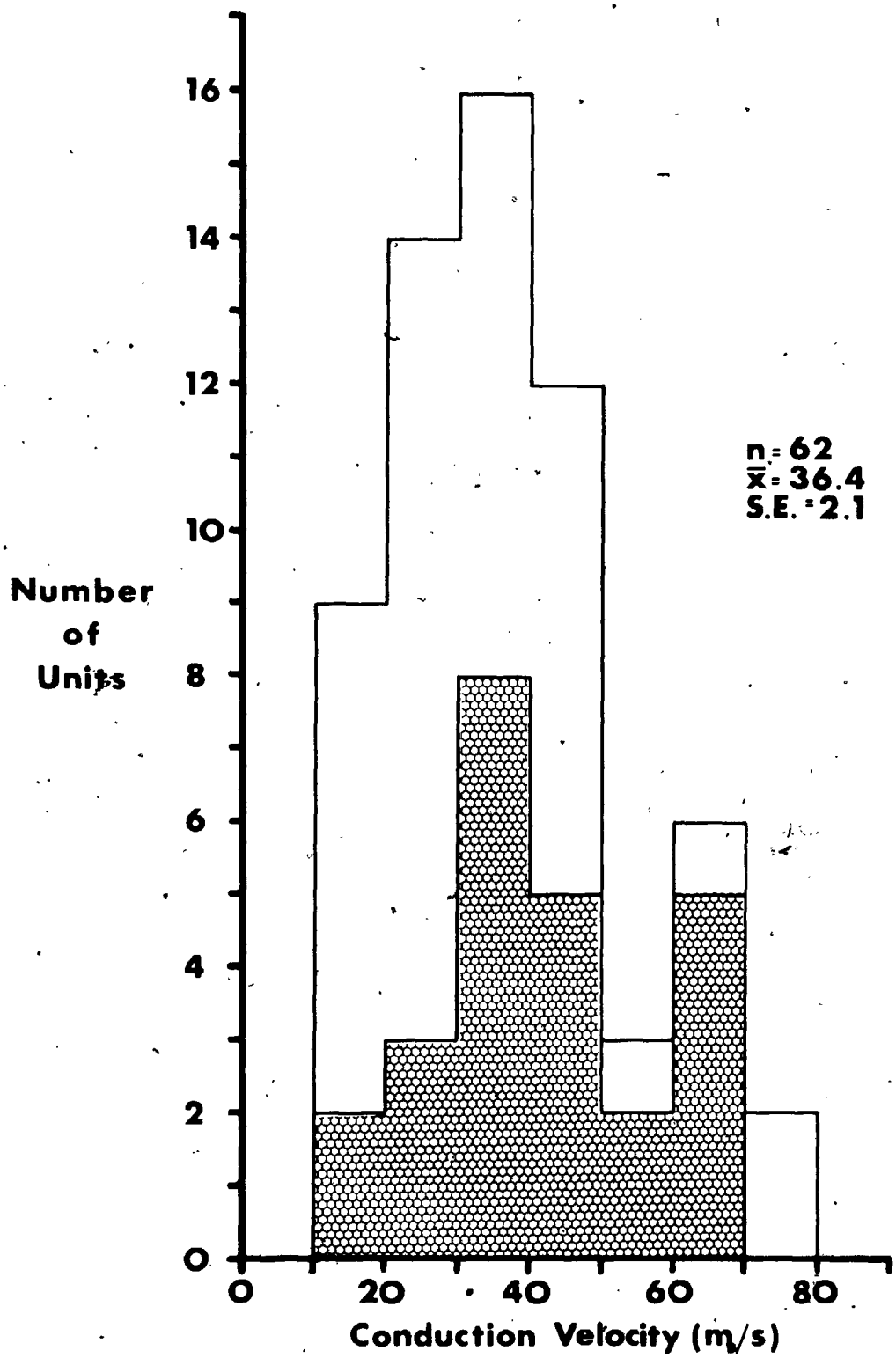
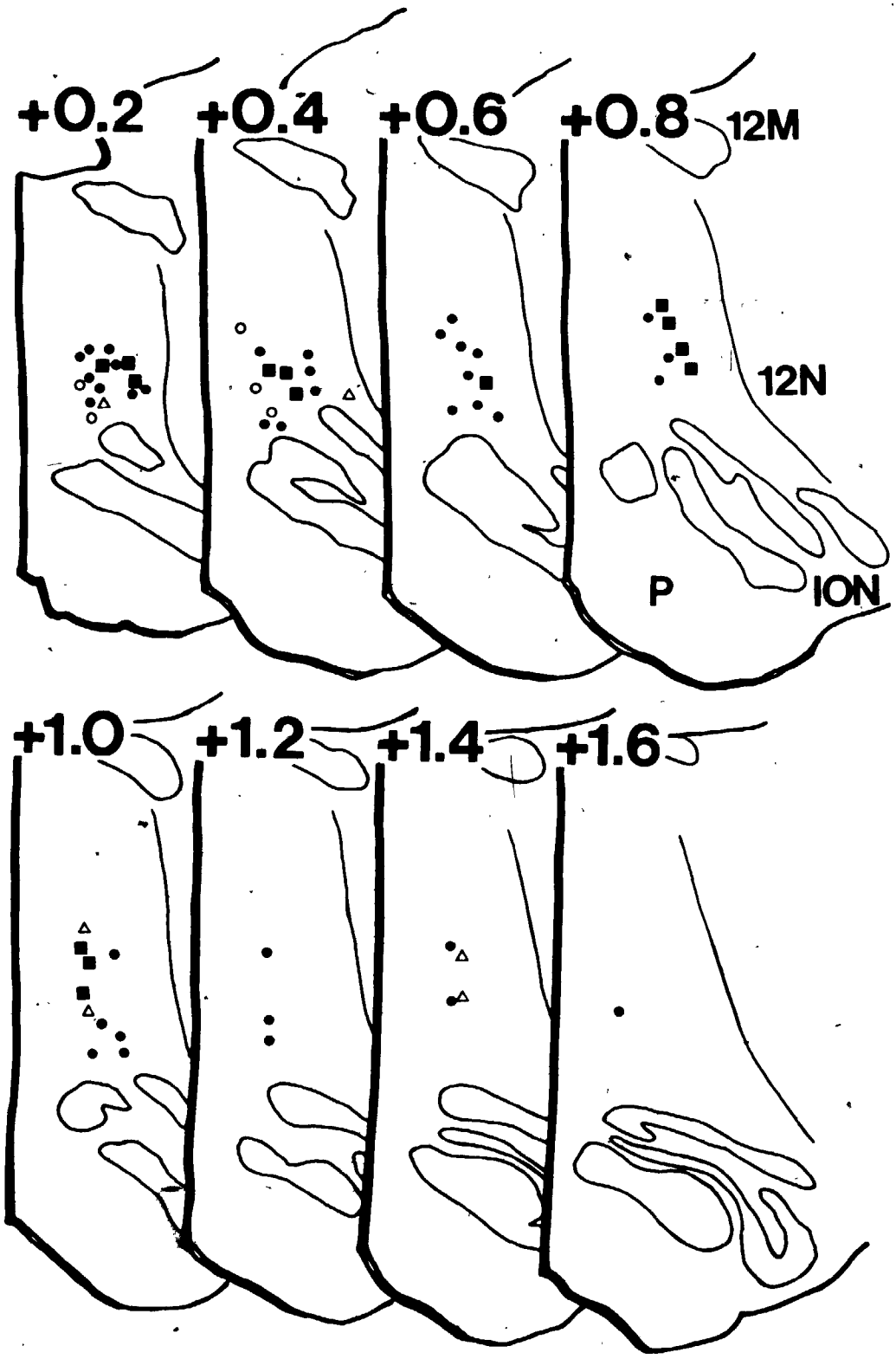

16
14
12
10
8
6
4
2
0
Number of Units
n = 62
x̄ = 36.4
S.E. = 2.1
0
20
40
60
80
Conduction Velocity (m/s)

FIGURE 30

Representative drawings of transverse hemisections of the medulla showing the location of single units in the right PRN antidromically excited by stimulation of the ipsilateral IML, and responding orthodromically to stimulation of the CSN and the FN. Numbers indicate levels in mm rostral to the obex. ● , units unresponsive orthodromically to stimulation of the CSN and the FN; ○ , units activated only by CSN stimulation; △ , units activated only by FN stimulation; ■ , units activated by CSN and FN stimulation.


+0.2
+0.4
+0.6
+0.8
12M
12N
P
ION
+1.0
+1.2
+1.4
+1.6

18.3 ± 9.9 ms). Their latencies of antidromic activation corresponded to a mean conduction velocity of 41.5 ± 6.6 m/s (Figure 31; Table IV). Fifty-six per cent (14/25) of the units were found to be excited orthodromically to stimulation of both the CSN and FN with mean latencies of 12.3 ± 2.9 ms and 8.4 ± 1.0 ms, respectively (Figure 32; Table IV). These units were activated antidromically with latencies corresponding to a mean conduction velocity of 43.4 ± 4.1 m/s. The mean conduction velocities of the reticulospinal projections of units responding to stimulation of the CSN only and of those responding to stimulation of both the CSN and FN did not differ significantly ($p < 0.01$).

ii. PRN Units Responding to FN Stimulation

Twenty-four per cent (6/25) of the units responded with excitation to stimulation of only the FN (mean latency, 7.0 ± 1.7 ms). Their latencies of antidromic activation corresponded to a mean conduction velocity of 41.5 ± 5.9 m/s (Figure 33; Table IV). The mean conduction velocities of the reticulospinal projections of units responding to stimulation of the FN only and to stimulation of both the CSN and FN did not differ significantly ($p < 0.01$).

iii. PRN Units Unresponsive to CSN and FN Stimulation

Single units orthodromically excited by stimulation of the FN and/or CSN responded antidromically to stimulation of the IML with latencies corresponding to a mean conduction velocity of 42.6 ± 2.9 m/s (range, 11.4 - 66.3 m/s) whereas the mean conduction velocity of the units non-responsive to the test inputs was 32.4 ± 2.7 m/s (range, 10 - 75 m/s) (Figure 29). Although the ranges of conduction velocities of the two groups of units overlap, the mean conduction velocities for the two groups were significantly different ($p < 0.01$).

FIGURE 31

Response of a single unit in the PRN to electrical stimulation of the ipsilateral IML (a-c) and CSN (d). Each record is 5 superimposed sweeps and the stimulus artifact is marked by a dot. Calibrations, 1 ms and 50 μV (a-c) and 5 ms and 50 μV (d).

A. Antidromic response of a PRN unit showing constant latency to stimulation of the IML at 0.5 Hz.

B. Action potentials evoked with 2 stimuli applied at 333 Hz.

C. Action potentials evoked with 2 stimuli applied at 667 Hz showing occasional failure of the antidromic spike.

D. Orthodromic response of the same unit to stimulation of the CSN.

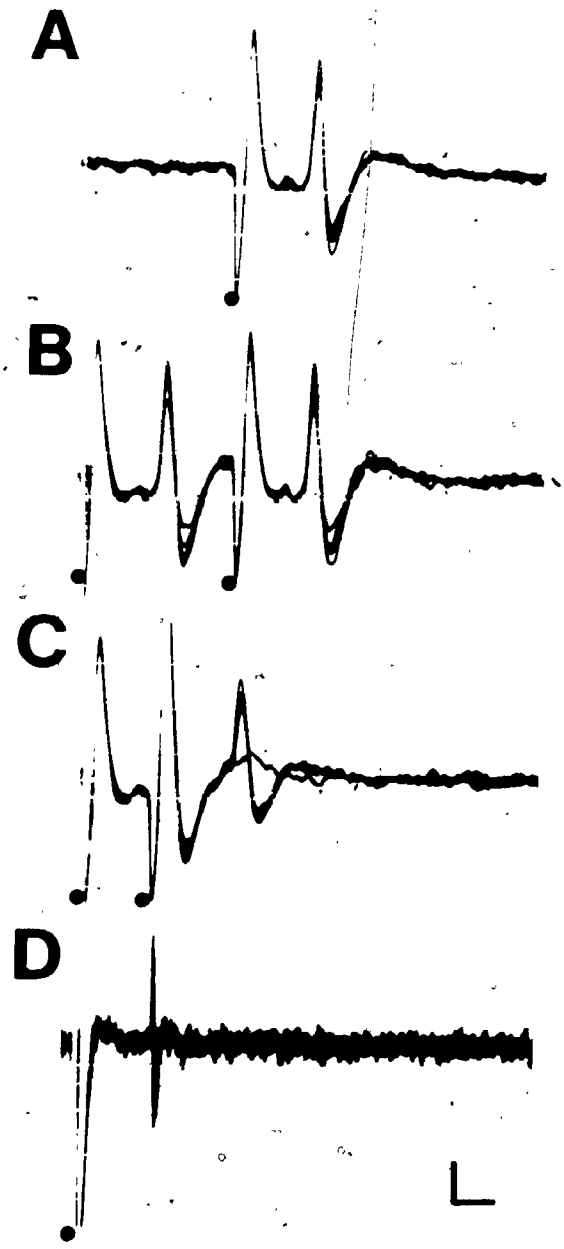

A
B
C
D
L

TABLE IV

Electrophysiological characteristics of orthodromically responsive units in the PRN which project to the IML

Inputs	Number of units	Conduction velocity of antidromic pathway (m/s)	Latency of orthodromic response (ms)
CSN only:	5 (3)	41.5 ± 6.6	18.3 ± 9.9
FN only:	6 (4)	41.5 ± 5.9	7.0 ± 1.7
CSN / FN:	14 (8)	43.4 ± 4.1	-
CSN:	-	-	12.3 ± 2.9
FN:	-	-	8.4 ± 1.0

All values are means ± S.E. of the mean. Numbers in parentheses indicate the number of spontaneously active units. CSN, carotid sinus nerve; FN, fastigial nucleus; IML, intermediolateral nucleus; PRN, paramedian reticular nucleus.

FIGURE 32

Response of a single unit in the PRN to electrical stimulation of the ipsilateral IML (b,c) and CSN (d) and the contralateral FN (e). Each record is 5 superimposed sweeps unless otherwise stated and the stimulus artifact is marked by a dot.

A. Spontaneous activity of a single unit; single sweep; calibration, 10 ms and 67 μV.

B. Antidromic response of the unit showing constant latency to stimulation of the IML at 0.5 Hz. calibration, 1 ms and 67 μV.

C. Action potentials evoked with 2 stimuli applied at 200 Hz, calibration, 1 ms and 67 μV.

D. Orthodromic response of the unit to stimulation of the CSN, calibration, 10 ms and 67 μV.

E. Orthodromic response of the unit to stimulation of the FN, calibration, 2 ms and 67 μV.

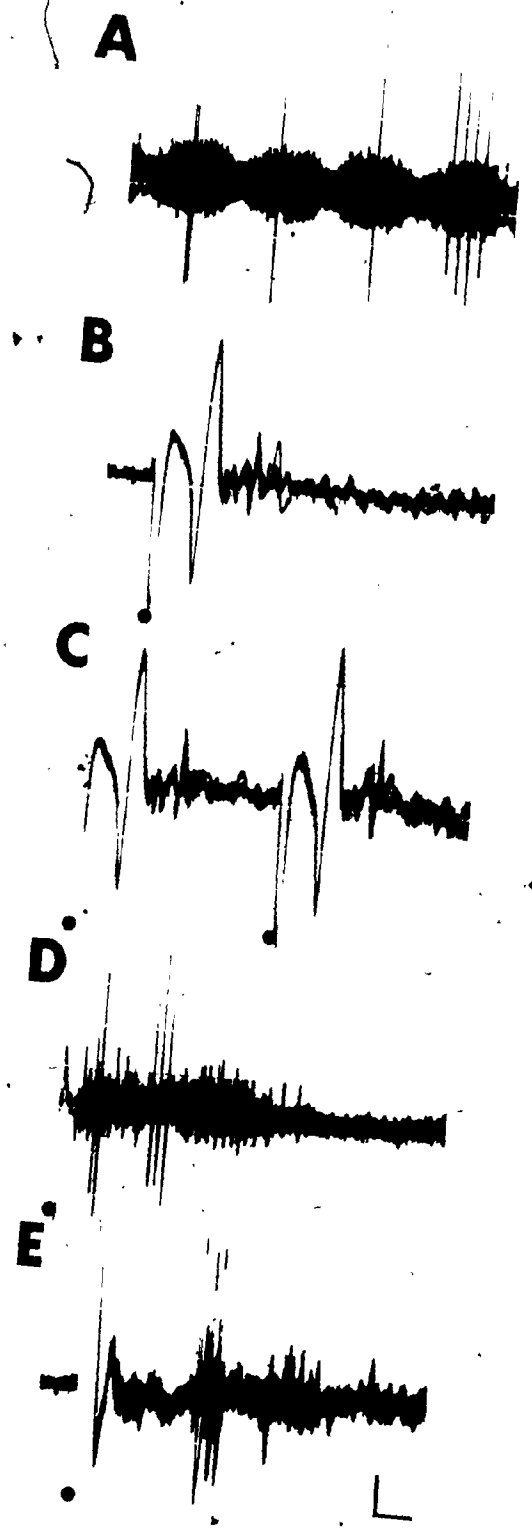

A
B
C
D
E

FIGURE 33

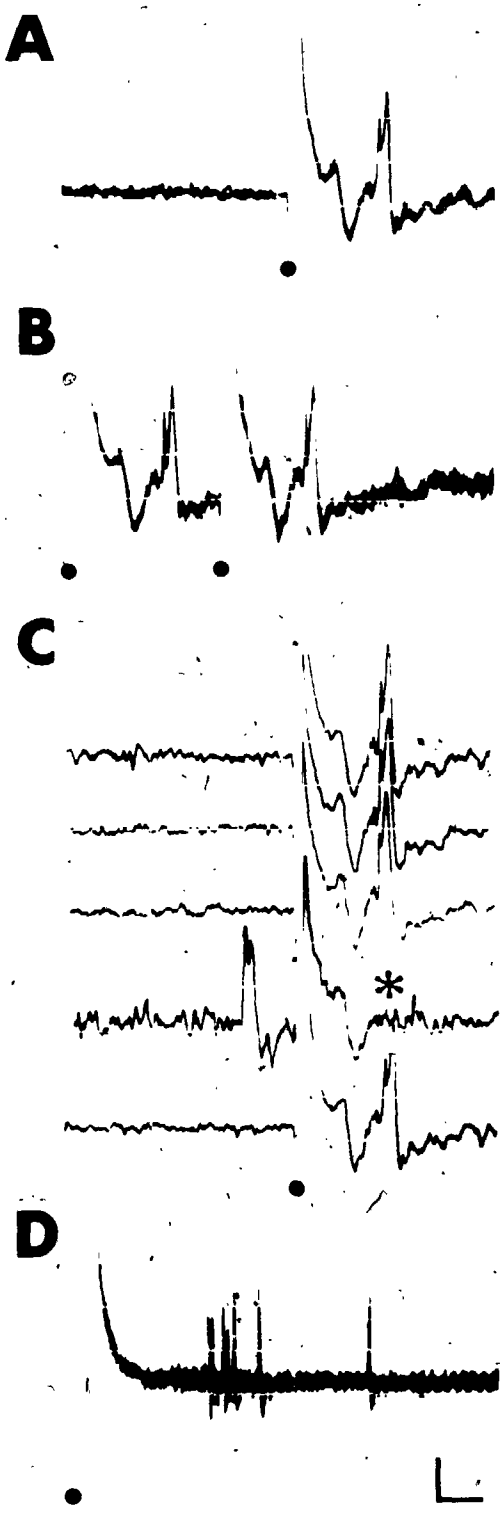
Response of a single unit in the PRN to electrical stimulation of the ipsilateral IML (a-c) and contralateral FN (d). The stimulus artifact is marked by a dot. Calibrations, 1 ms and 60 μV (a-c) and 5 ms and 60 μV (d).

A. Five superimposed sweeps showing a two component (IS-SD) spike and the constant latency of the antidromic response to stimulation of the IML at 0.5 Hz.

B. Five superimposed sweeps showing the response of the unit to stimulation of the IML at 333 Hz.

C. Five successive sweeps showing cancellation of the antidromic spike (*) evoked by stimulation of the IML by spontaneous spike.

D. Ten superimposed sweeps showing the response of the same unit to stimulation of the FN.


A
B
C
D
*

4.4 Electrophysiological Identification of PRN Neurones Relaying Vestibular Afferent Input Directly to the Region of the IML

A. Electrophysiological Characteristics of Orthodromically Activated PRN Units

In order to eliminate the possibility of stimulating fastigioreticular fibers to the PRN during stimulation of the vestibular nuclear complex (VNC), lesions of the fastigial nuclei were made and the fibers allowed to degenerate. The extent of these cerebellar lesions is shown schematically in Figure 34. In all cases the midline lesions extended rostrally to the anterior lobe vermis and ventrally to the fourth ventricle and included at least the medial aspects of both fastigial nuclei. Therefore, the crossed midline fastigioreticular fibers were cut by the lesions in all preparations.

A total of 27 electrode penetrations were made through the region of the PRN. Forty-seven histologically verified single units in the PRN were orthodromically activated by stimulation of functionally and histologically identified sites in the VNC (Figures 27,35b). Of these units, 20 (43%) were discharging spontaneously (mean discharge rate, 12.5 ± 1.2 spikes/s) and 27 (57%) were silent. Of the spontaneously active units, 3 (15%) were inhibited and 3 (15%) were excited and followed by inhibition of spontaneous activity by VNC stimulation. Of the 47 units, 44 (94%) were excited. Units responded with excitation with a mean latency of 6.3 ± 0.6 ms (range, 1.4 - 19 ms). Thirty-nine (83%) of the 47 units responded with latencies of under 10 ms (Figure 36). The excitatory response consisted of either a simple spike (29 units) or a burst of 2 - 5 spikes (18 units). Increasing stimulus intensities over a 3 to 5 fold range, decreased the latency of the response but did not increase the number

FIGURE 34

Extent of cerebellar ablations to remove the fastigioreticular input to the PRN.

a. Bright-field photomicrograph of a neutral red counterstained transverse section of the rostral cerebellum and medulla oblongata (case K1) showing central ablated area extending ventrally to the roof of the fourth ventricle and laterally to involve the medial fastigial nuclei bilaterally (x 6).

b. Representative drawings of transverse sections of the cerebellum (cases K5,6) showing schematically the extent of ablated tissue (shaded area).


V4

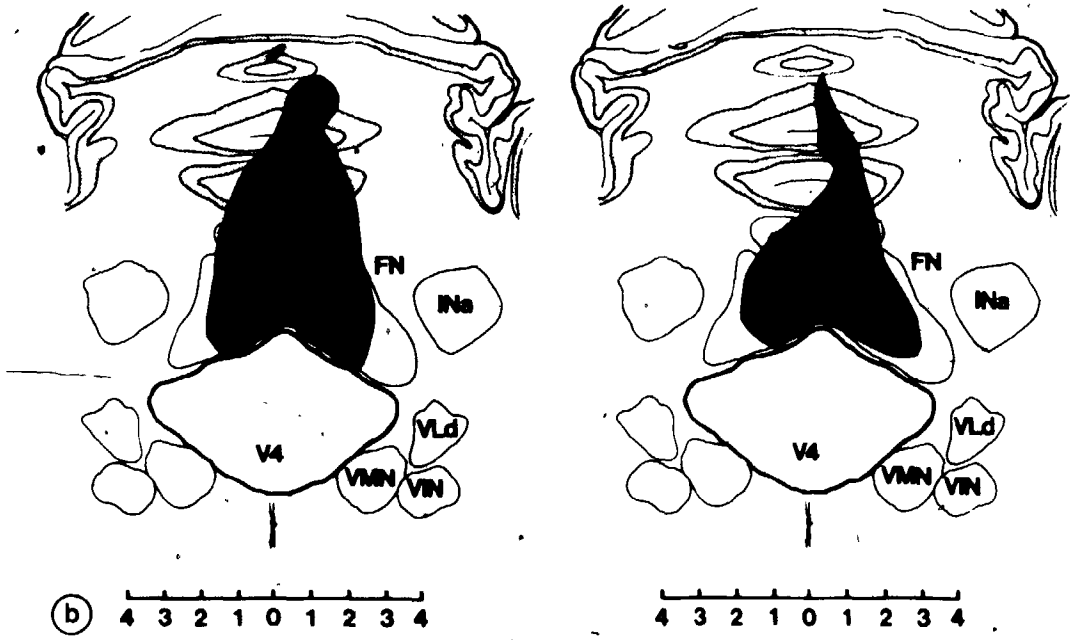
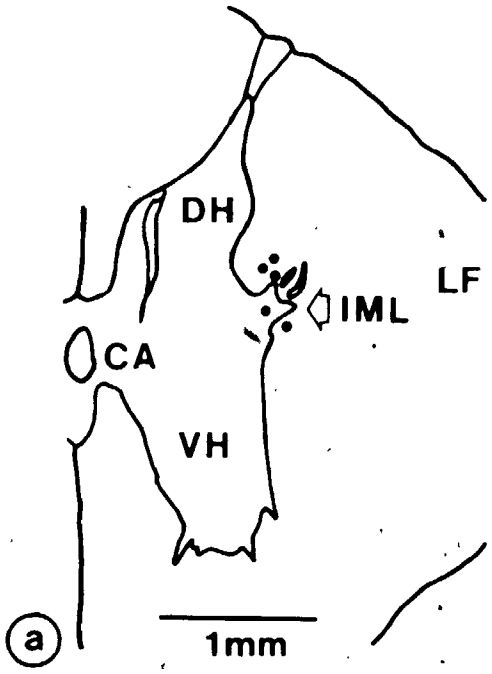

FN
INa
V4
VLd
VMN
VIN
FN
INa
V4
VLd
VMN
VIN
b
4 3 2 1 0 1 2 3 4
4 3 2 1 0 1 2 3 4

FIGURE 35

Representative drawings of transverse sections of the spinal cord at the level of T2 (a) and of the medulla oblongata in the region of the VNC (b).

a. Sites of stimulation of the ipsilateral (right) DTL.

b. Sites of stimulation in the region of the ipsilateral (right) VNC at 7.7 and 7.1 mm caudal to the interaural line (scales in mm).


DH
LF
IML
CA
VH
a
1mm

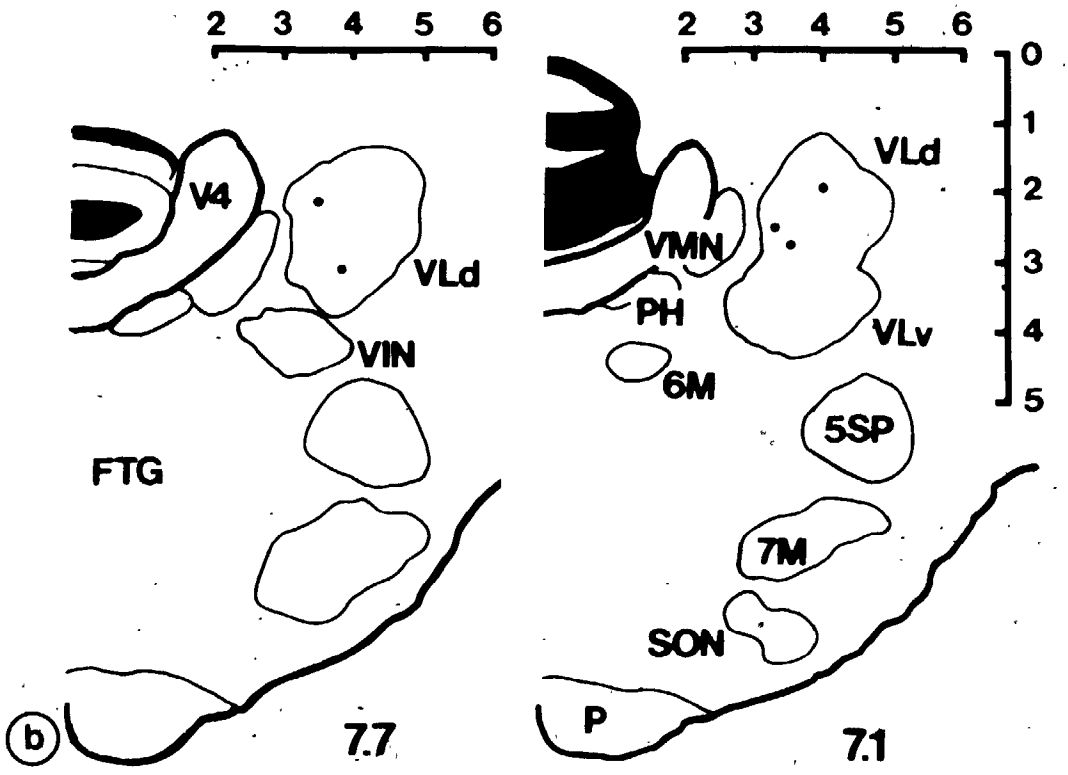
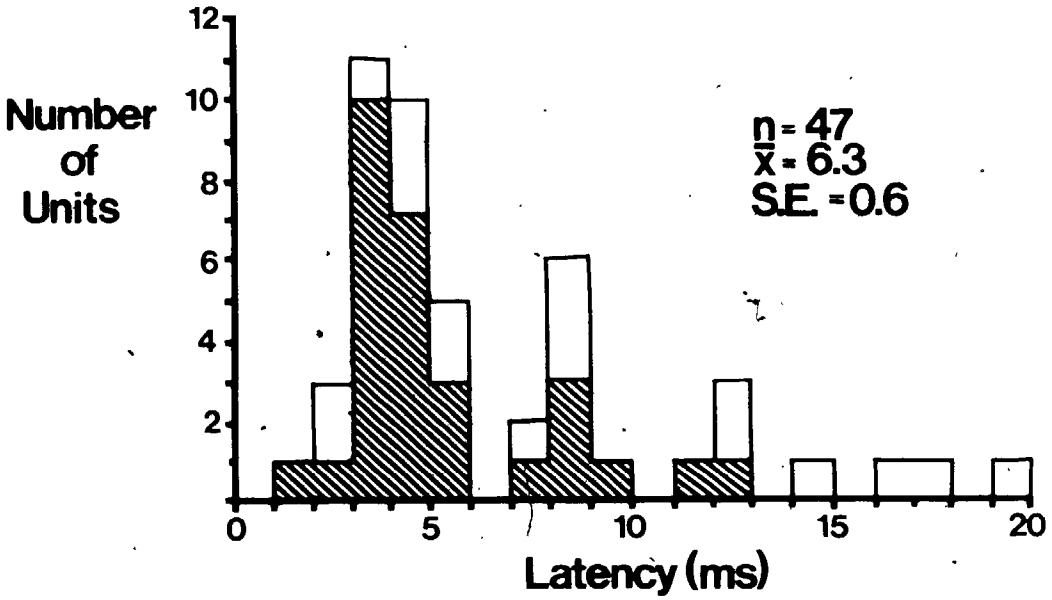

2 3 4 5 6
V4
VLd
VIN
FTG
b
7.7
2 3 4 5 6
0
1
2
3
4
5
VLd
VMN
PH
VLv
6M
5SP
7M
SON
P
7.1

FIGURE 36

Histogram of latencies of orthodromic responses of PRN single units to stimulation of the ipsilateral vestibular nuclear complex (VNC). Hatched area represents the latencies of orthodromically activated units which were also activated antidromically by stimulation of the IML.


Number of Units
12
10
8
6
4
2
0
5
10
15
20
Latency (ms)
n = 47
x̄ = 6.3
S.E. = 0.6

of spikes in a simple spike response. On the other hand, an irregular recruitment of spikes occurred with increases in stimulus intensity during a burst response. A characteristic orthodromic response of a PRN unit during stimulation of the VNC is shown in Figure 37. The threshold current to evoke these orthodromic responses in PRN units ranged from 100 to 760 μm (mean, 320 ± 24 μA when using a pulse duration of 0.2 ms).

B. Electrophysiological Characteristics of Antidromically Activated PRN Units

The criteria used to assess for antidromic activation have been previously stated (section 4.3A). Not all antidromically activated single units could be evaluated for all criteria since 57% (27/47) of the units were not spontaneously active and could not be tested by the collision method.

Of the 47 orthodromically identified units, 29 (62%) were antidromically excited by stimulation of functionally and histologically identified sites in the region of the ipsilateral IML (Figures 27,35a). Units responded with a mean latency of 2.0 ± 0.2 ms (range, 0.9 - 3.2 ms) which was constant for any one unit and followed rates of stimulation of 220 - 890 Hz (mean, 399 ± 30 Hz). The latencies of the antidromic responses corresponded to conduction velocities of these reticulospinal fibers of 48.6 ± 3.5 m/s (Figure 38). The majority (27/29; 93%) of PRN units activated antidromically by stimulation of the IML region responded with latencies of under 10 ms to stimulation of the VNC, whereas only 7% of the PRN units with latencies of over 10 ms to VNC stimulation were antidromically activated by stimulation of the IML. The range of threshold currents evoking these antidromic spikes was 200 to 820 μA (mean, 429 ± 30 μA when using a

FIGURE 37

Response of a single unit in the PRN to electrical stimulation of the region of the IML (a) and VNC (b). Each record is five superimposed tracings. Stimulus was delivered at the time indicated by the dot. Calibration, 2 ms and 100 μV (a) and 5 ms and 100 μV (b).

A. Note the constant latency of the antidromic potential and the inflexion point (arrow) on the rise phase of the potential indicating the separation of the IS-SD components of the antidromic spike.

B. Note the relatively long duration excitatory orthodromic response to stimulation of the VNC.

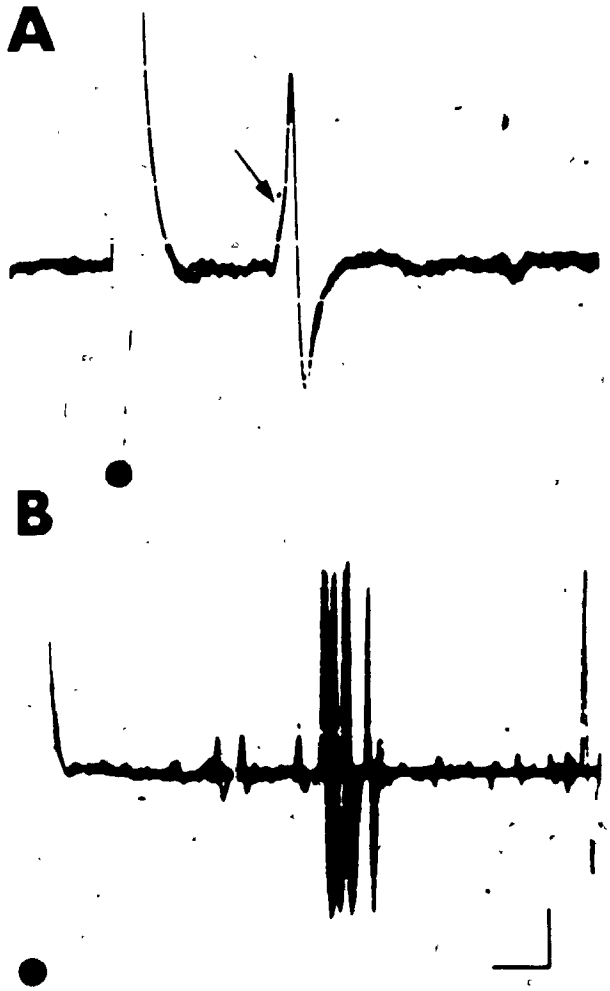
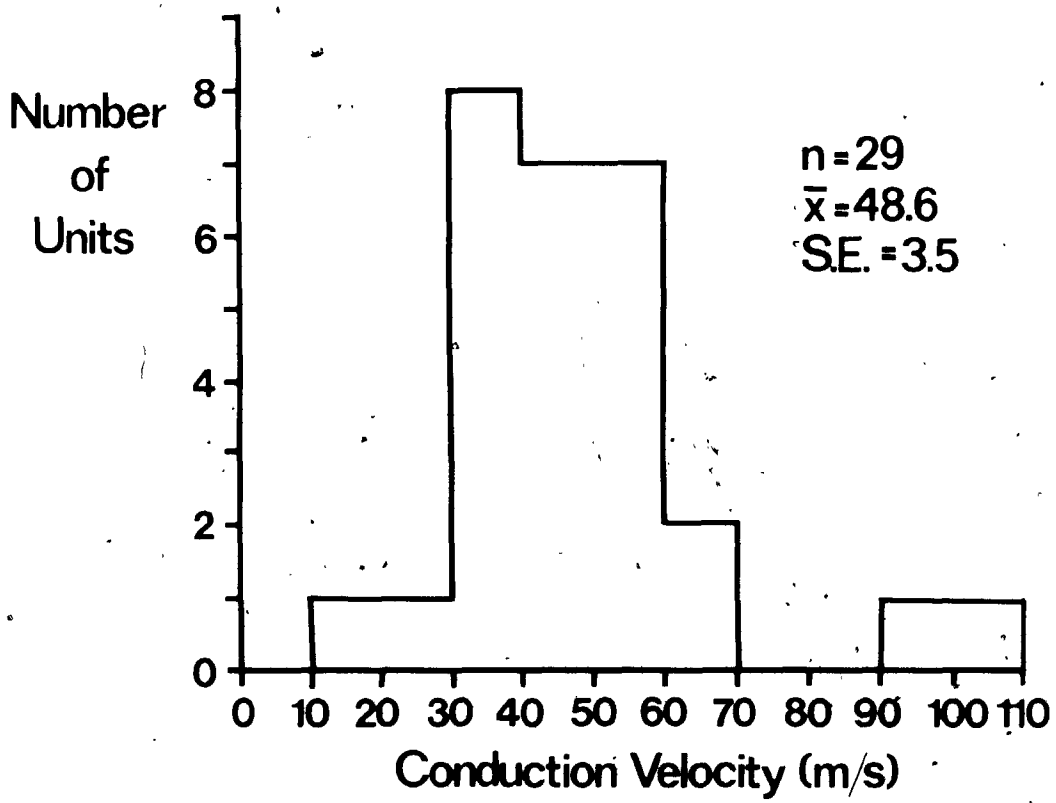

A
B

FIGURE 38

Histogram of conduction velocities of 29 PRN single units antidromically activated by electrical stimulation of the IML which had previously responded orthodromically to stimulation of the VNC.


Number of Units
8
6
4
2
0
n=29
x̄=48.6
S.E.=3.5
0 10 20 30 40 50 60 70 80 90 100 110
Conduction Velocity (m/s)

pulse duration of 0.2 ms). All units responded with a single spike at threshold and suprathreshold stimulus intensities. Fourteen (48%) of the antidromically activated units showed two-component spikes (IS-SD) of which the SD component could be induced to fail by a high frequency of stimulation in some cases. Figure 37 shows a PRN unit which was excited orthodromically by VNC stimulation and antidromically to stimulation of the IML. None of the 47 orthodromically responding units in the PRN to VNC stimulation responded orthodromically to stimulation of the ipsilateral CSN.

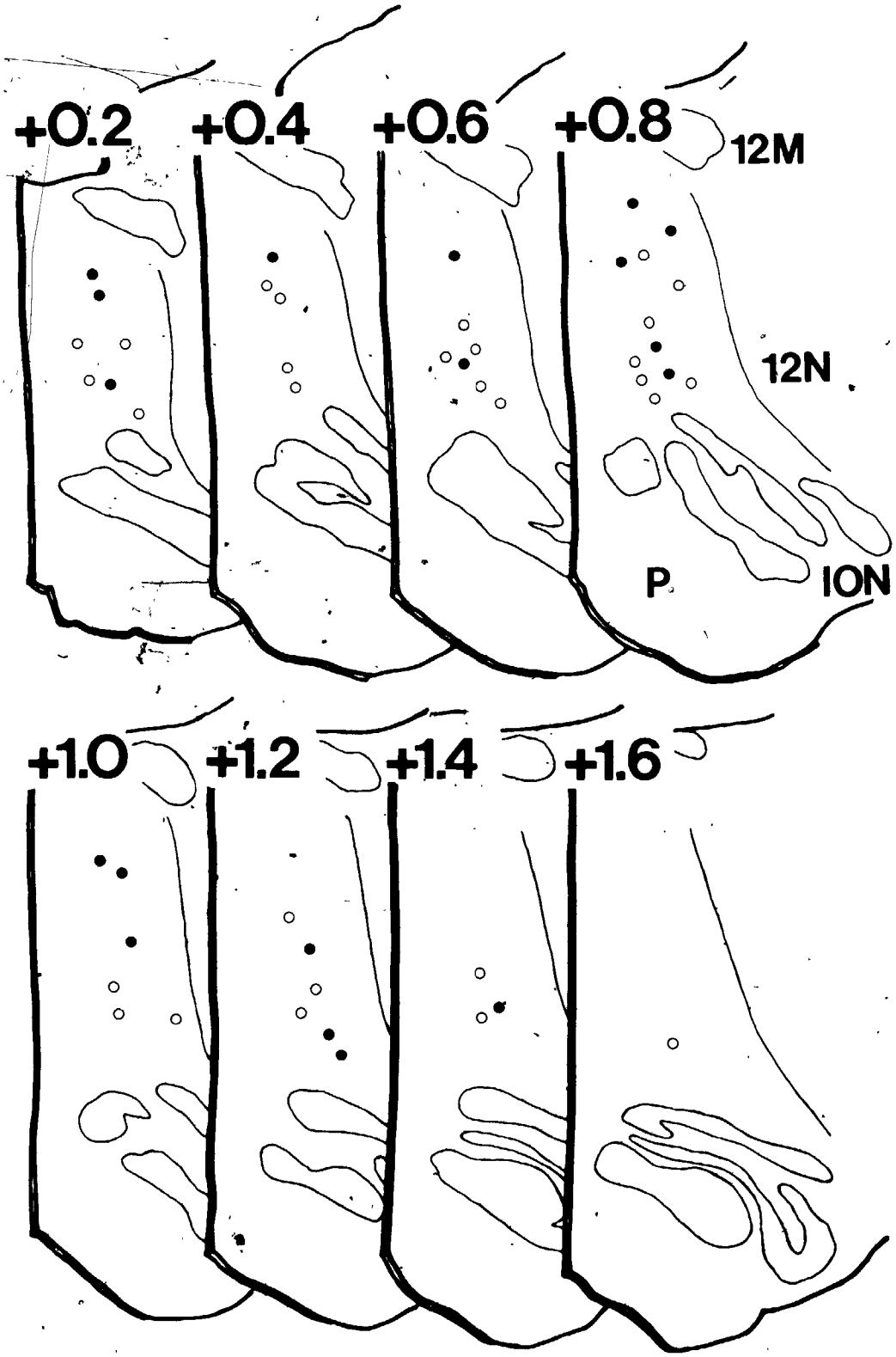
C. Anatomical Distribution of PRN Units Responsive to Vestibular Stimulation

The anatomical distribution of units in the PRN activated orthodromically by stimulation of the VNC is shown in Figure 39. PRN units activated by stimulation of the VNC were found in both the dPRN and vPRN. Units which responded antidromically to IML stimulation and orthodromically to VNC stimulation were confined largely to the vPRN while a few appeared within the ventral portion of the dPRN. The majority of units (20/47) were found in the caudal half of the vPRN extending from 0.2 to 0.8 mm rostral to the obex.

The location of stimulations in the VNC is shown in Figure 36. All stimulation sites were localized to the lateral vestibular nucleus.

FIGURE 39

Location of PRN units orthodromically activated by electrical stimulation of the VNC and antidromically activated by stimulation of the IML plotted on representative transverse sections of the PRN (0.2 to 1.6 mm rostral to the obex). ● , units activated only by VNC stimulation; ○ , units activated by stimulation of both the VNC and IML.


+0.2
+0.4
+0.6
+0.8
12M
12N
P
ION
+1.0
+1.2
+1.4
+1.6

CHAPTER 5 - DISCUSSION

The central neural regulation of the cardiovascular system is inherently linked with the regulation of other systems. Cardiovascular adjustments are often observed to be appropriate to the needs of one or a variety of these systems. An orderly functional organization of numerous sites within the central nervous system is, of course, a necessary prerequisite for the coordinated responses of these systems. Our understanding of this organization comes largely from a variety of electrophysiological experiments done in the past two decades which have elucidated the effects of stimulation of various brainstem centers upon arterial pressure and heart rate. One such center, the paramedian reticular nucleus (PRN), has been studied in this manner; however, its internal functional organization and central connectivity, particularly with spinal sympathetic vasoconstrictor and cardioacceleratory neurones of the intermediolateral nucleus, are not well known.

The data from the present investigation provide anatomical and electrophysiological evidence of afferent and efferent connections of the PRN and identify neurones in the PRN involved in relaying cardiovascular and vestibular afferent information directly to spinal sympathetic centers in the region of the intermediolateral nucleus. The study demonstrates that: (1) primary afferent projections to the PRN originate in nuclei involved with postural (motor) and/or cardiovascular control; (2) neuronal

cell bodies in the PRN receive cardiovascular and vestibular afferent inputs and their axons project directly to sympathetic centers of the thoracic cord; (3) PRN neurones send collateral axonal branches to the cerebellar cortex as well as to the region of the intermediolateral nucleus of the thoracic cord and; (4) subpopulations of neurones in the PRN are likely to be involved in separate bulbospinal pathways mediating cardiovascular and vestibulosympathetic activity. The following discussion analyzes in detail the findings of the present study and draws conclusions regarding the connections of the PRN and their role with regard to the central control of the cardiovascular system.

5.1 Topographical Anatomy of the Origins of Afferent Projections to the PRN

Afferent projections to the dorsal and ventral subdivisions of the PRN differ somewhat in the origin and relative magnitudes of their inputs with some terminating in only one of the two divisions. The areas of termination of these inputs, however, largely coincide and may be summarized as in the following.

A. Deep Cerebellar Nuclei

A cerebelloreticular contribution to the PRN was first described using the silver impregnation method of Glees and was shown to be moderate in amount and bilateral with some predominance of ipsilateral projections (Brodal and Gogstad, 1957). Degenerating terminal fibers were seen in both the dPRN and vPRN. Since then, restricted lesions of the deep cerebellar nuclei and improved tracing techniques have refined our knowledge of their efferent distribution further.

i. Fastigial Nucleus

Bilateral fastigiobulbar projections to the PRN in the cat were shown using the Marchi (Carpenter, Brittin, and Pines, 1958) and Nauta (Thomas, Kaufman, Sprague, and Chambers, 1956; Carpenter, 1959) methods. Terminal degeneration in the medullary reticular formation was chiefly medial and contralateral exceeding that found in both the lateral reticular nucleus and perihypoglossal nuclei. Degeneration within the dPRN exceeded that of the vPRN after complete destruction of the contralateral FN (Walberg, Pompeiano, Westrum, and Hauglie-Hanssen, 1962). Slightly less intense degeneration was found ipsilaterally and this may have resulted from damage of the neighbouring uncinate fasciculus carrying fibers of the contralateral FN. Autoradiographic studies performed in the monkey later showed fastigioreticular fibers to be almost entirely crossed, passing via the uncinate fasciculus and arising from all rostrocaudal levels of the FN except for its extreme rostral pole (Batton, Jayaraman, Ruggiero, and Carpenter, 1977). Labeled fibers within the ipsilateral juxtarestiform body were not found to terminate in the PRN. Rostral parts of the FN and, in particular, dorsolateral regions of the rostral half of the FN, appeared to contribute all of the fibers destined to the PRN. Earlier work in the opossum using the Fink-Heimer method following lesions confined to the posteromedial FN revealed terminal degeneration in the PRN (Martin, King, and Dom, 1974). This contrasted with the results obtained after lesions of the anterolateral FN in which no terminal degeneration was found in the reticular formation. Electrical stimulation of either FN produced excitatory postsynaptic potentials with monosynaptic latencies in cells within the area of the PRN (Ito, Udo, Mano, and Kawai, 1970). Furthermore, the presence of the PRN appeared necessary in mediating the fastigial pressor response upon stimulation of the ventromedial portion of the rostral

half of the FN in the cat (Miura and Reis, 1970). Bilateral electrolytic lesions of the PRN involving both the dPRN and vPRN or of the inferior cerebellar peduncles completely abolished the response whereas unilateral ablation, either ipsilateral or contralateral to the stimulated FN reduced the amplitude of the response by one-half (Miura and Reis, 1969a, 1970).

The results of the present study are in partial agreement with previous anatomical work in that a bilateral afferent contribution to the PRN from the FN is evident in the cat. The fastigial contribution to the dPRN is predominantly contralateral and found dorsomedially with a posterior concentration in the FN similar to that in the opossum (Martin et al., 1974). A similar pattern is found in the case of the vPRN although relatively more fastigial fibers originate anteriorly within the nucleus. The ventromedial portion of the anterior half of the FN contributes the majority of fibers to the ipsilateral PRN and may coincide with the location of cells responsible for the fastigial pressor response mediated through the PRN (Miura and Reis, 1970, 1971). The findings differ somewhat with those found in the monkey (Batton et al., 1977) and this may perhaps be resolved by species differences and the lower sensitivity of the autoradiographic technique in the case of the sparser ipsilateral FN projection.

ii. Interposed and Dentate Nuclei

A common mammalian pattern has been shown in the overall distribution of cerebellofugal fibers forming the contralateral descending pathway of the brachium conjunctivum as evidenced in the rat (Chan-Palay, 1977), opossum (Martin et al., 1974; Martin, Henkel, and King, 1976; Yuen, Dom, and Martin, 1974), cat (Brodal and Szikla, 1972; Graybiel, Nauta, Lasek and Nauta, 1973) and monkey (Chan-Palay, 1977). Evidence of

projections from the dentate (DN) and interposed (IN) nuclei to the PRN in the cat has been conflicting (Cohen, Chambers, and Sprague, 1958; Carpenter and Nova, 1960). Injection of tritiated leucine confined to the DN and IN produced some labeling in the PRN (Graybiel et al., 1973) however, application of HRP into the PRN in the opossum failed to retrogradely label cells in the DN and IN (Martin et al., 1976). Terminal degeneration in the PRN of the rat was noted using the Fink-Heimer technique after destruction of the contralateral brachium conjunctivum (Faull, 1978).

A significant afferent projection to the PRN from the DN is confirmed in this study. No difference in the topographical pattern of afferent input from the DN to the dPRN and vPRN is evident. This disagrees with earlier findings of terminal degeneration found mainly about cells of the vPRN after lesions of the DN in the cat (Carpenter and Nova, 1960). The PRN is also shown to be one of the most caudal sites of termination of afferent fibers from the IN. In particular, a marked afferent input to the vPRN is noted relative to that destined for the dPRN. The findings are in agreement with previous electrophysiological work describing a projection from the IN to the PRN in which neurones in the IN were activated antidromically from the contralateral PRN (Murakami, Ozawa, Katsumaru, and Tsukahara, 1981). The present study indicates that most of these neurones are likely to be found in the INp and to project to the vPRN.

B. Vestibular Nuclear Complex

Lesions of various portions of the vestibular nuclear complex in the cat produced terminal degeneration mainly within the ipsilateral vPRN and less markedly in the dPRN (Brodal and Gogstad, 1957;

Carpenter, 1960; Ladpli and Brodal, 1968). Most lesions were not limited to particular vestibular nuclei and few conclusions could be made as to whether all or only some of the vestibular nuclei projected to the PRN. In the monkey, lesions of the medial vestibular nucleus produced modest preterminal degeneration in the PRN with an ipsilateral predominance (McMaster, Weiss, and Carpenter, 1966). Definite conclusions regarding the vestibuloreticular projection have been hampered by concomitant destruction of fastigioreticular fibers which are known to course through the lateral (VLN), medial (VMN) and inferior (VIN) vestibular nuclei (Batton et al., 1977). The present study, while avoiding the latter difficulty, confirms the existence of a bilateral vestibuloreticular projection with an ipsilateral predominance. The ventrolateral position of the lateral vestibulospinal fibers within the medulla avoids the problem of inadvertent injury of fibers of passage during injection of the PRN. The same cannot be said for the descending fibers of the VMN and VIN which course medially within the medulla although their projection to the PRN appears to be in excess of their relatively sparse spinal projections. Examination of cervicothoracic spinal cross-sections revealed no anterograde label in the sites of termination of medial vestibulospinal fibers (i.e. laminae VII and VIII), supporting the premise that very few if any medial vestibulospinal fibers had been damaged in the penetration. There is no evidence for an afferent input from the superior vestibular nucleus to the PRN. A topographical pattern appears to be present and is of interest in the functional organization of the PRN. In particular, mediation of extensor tone of the limbs by the VLN (Pompeiano, 1972a) may have some bearing upon the involvement of the PRN in postural control. Neurones of the PRN have been shown to respond to lateral tilt of the head and trunk in a pattern similar to that seen amongst neurones of the

vestibular nuclei and the FN (Spyer, Ghelarducci, and Pompeiano, 1973; Ghelarducci, Pompeiano, and Spyer, 1974a). Likewise, the VMN may have similar influences as it has been shown to project via the medial vestibulospinal tract onto the motor neurones of the upper cervical cord (Wilson and Yoshida, 1969). The demonstration of afferent input from the VIN establishes a descending supraspinal projection from this nucleus. Projections of the VIN to both fastigial nuclei (Carpenter, 1960) may have some bearing on afferent input from the latter to the PRN as has been shown in the present study.

C. Accessory Oculomotor Nuclei

In previous silver impregnation studies, extensive lesions of the pontomesencephalic area have produced considerable terminal degeneration within the PRN (Brodal and Gogstad, 1957). The extent of degeneration in these cases exceeded that obtained after large frontoparietal cortical ablations. This suggested to earlier authors that other sources of supranuclear afferents existed. Although these lesions were not purely unilateral, some cases in which a close approximation was obtained indicated a preponderance of degeneration ipsilaterally. However, subsequent orthograde degeneration studies revealed only modest projections from the ipsilateral interstitial nucleus of Cajal (Carpenter, Harbison, and Peter, 1970) and contralateral superior colliculus (Kawamura, Brodal, and Hoddevik, 1974) in the cat. These results were coupled with equally modest projections from the sensorimotor cortex (Sousa-Pinto, 1970) and the contralateral deep cerebellar nuclei (Faull, 1978; Elisevich, Hrycyshyn, and Flumerfelt, 1983).

Caudally projecting fibers of the interstitial nucleus of Cajal have been shown to descend in the dorsomedial part of the ipsilateral

MLF to the PRN and to spinal levels (Carpenter et al., 1970). A contribution from the contralateral interstitial nucleus has not been previously identified following lesions of either the interstitial nucleus (Carpenter et al., 1970) or the MLF rostral to the abducens nucleus (Carpenter and Hanna, 1962; Carpenter and Strominger, 1965). A bilateral projection with an ipsilateral predominance from the interstitial nucleus to the dPRN is shown in the present study. Neighbouring reticular neurones of the central tegmental field contribute to this projection and also show an ipsilateral predominance. A very sparse distribution of preterminal label confined to the ipsilateral cervical cord indicated negligible damage to interstitiospinal fibers in the region of the PRN. An equally abundant projection to the ipsilateral vPRN from the interstitial nucleus was also identified. A contralateral projection, however, was not evident from the interstitial nucleus or the surrounding reticular formation indicating that the micropipette penetration past the region of the dPRN failed in most cases to label terminals in the latter area.

A sparse bilateral projection to the dPRN from the nucleus of the posterior commissure could not be demonstrated consistently in all cases and is of questionable significance. These spurious results may indicate the occasional uptake by either intact or damaged fibers of passage destined for the spinal cord (Bucher and Bürgi, 1952). The nucleus of Darkschewitsch has not been previously shown to project to the PRN. The present findings indicate a significant afferent projection from the ipsilateral nucleus of Darkschewitsch to the vPRN with a negligible contribution to the dPRN. Although a previous study has indicated that a descending spinal projection from the nucleus of Darkschewitsch (Savas, 1954) may exist, more recent reports (Nyberg-Hansen, 1966; Carpenter et al.,

1970) have shown no degenerating fibers within the spinal cord following ablations of the nucleus. The present data show that a segregated input to the vPRN from the nucleus of Darkschewitsch exists which does not appear to involve the dPRN. Some fibers destined for the medial accessory and principle olivary nuclei (Loewy and Saper, 1978) from the neurones in the region of the interstitial nucleus and nucleus of Darkschewitsch may have been damaged by the penetration and injection of the vPRN although the origin of these fibers has been rather ill-defined and modest in extent (Carpenter et al., 1970; Loewy and Saper, 1978).

It is difficult to discuss separately each nucleus of the accessory oculomotor group of nuclei functionally. The topographic differences in the projections to the dorsal and ventral subdivisions of the PRN must await further physiological study to delineate their significance. The accessory oculomotor nuclei are known to influence head and ocular position and the presence of a projection from these nuclei to the PRN underlines the importance of the PRN in mediating postural influences as it has also been shown physiologically to respond to macular stimulation (Ghelarducci et al., 1974a). Moreover, similar patterns of response to head tilting have been noted in the vestibular nuclear complex, rostral fastigial nucleus and the PRN (Spyer et al., 1973; Ghelarducci et al., 1974a). The PRN has been shown anatomically to receive direct afferent inputs from the vestibular nuclear complex (Elisevich et al., 1983) and the FN (Thomas et al., 1956; Batton et al., 1977; Elisevich et al., 1983).

D. Superior Colliculus

The descending tectoreticular fibers form an integral part of the MLF immediately adjacent to the inferior central nucleus of the

raphe (Altman and Carpenter, 1961). Degenerating fiber terminals in the PRN of the cat were described following both large and small lesions of the contralateral superior colliculus (Kawamura et al., 1974). The projection was felt to be modest. No distinctions were made regarding the exact regions of termination in the PRN. A similar projection in the opossum was not identified (Martin, 1969). The present findings indicate a relatively selective input to the vPRN from the contralateral superior colliculus. Most fibers in this projection arise from neurones in the intermediate layer of the superior colliculus throughout its rostrocaudal extent. The presence of labeled tectoreticular neurones throughout most of the intermediate layer of the superior colliculus accounts for a previous inability to obtain a topographical order in the tectoreticular projection with small lesions of the deeper portions of the superior colliculus (Kawamura et al., 1974). A rather restricted efferent projection to the vPRN exists however, based on the present findings. A variable pattern of anterograde terminal labeling in lamina VII of the upper cervical cord in 2 of the 4 cases indicated that tectospinal fibers passing through the region of the PRN may have taken up a small amount of HRP label. The remaining 2 cases demonstrated no anterograde label and showed essentially the same pattern of retrograde neuronal labeling in the superior colliculus.

The superior colliculus is important in orienting responses involving integrated eye, head and body movement (Ingle and Sprague, 1975). It is not surprising therefore to identify an input mediating postural adjustments to the PRN. The PRN may further help to coordinate these adjustments by its rather extensive bilateral projection to the cerebellum (Somana and Walberg, 1978; Kotchabhakdi, Hoddevik, and Walberg, 1980).

E. Solitary Nuclear Complex

The demonstration of a distinct afferent projection from the caudal solitary nucleus (NTS) supports earlier findings of preterminal fiber degeneration in the PRN after lesions of the NTS in the cat (Cottle and Calaresu, 1975). Other previous work has failed to demonstrate a similar projection using the Fink-Heimer method (Palkovits and Zaborszky, 1977) and autoradiography in the rat (Norgren, 1978). It is noteworthy that Humphrey (1967) recorded long latency evoked potentials from the region of the PRN during stimulation of the carotid sinus nerve (CSN), a buffer nerve which projects mainly to the NTS, and attributed the delay of afferent input to synaptic transmission through the NTS. More recently, recordings by intracellular methods of mono- and paucisynaptic potentials from neurones within the PRN have been made upon electrical stimulation of the CSN showing synaptic activation to occur largely in the dorsolateral quadrant of the PRN (Miura and Kitamura, 1979). Some topographical differences in the connections of the NTS with each of the dPRN and vPRN are apparent in this study. Whereas the contralateral ventrolateral solitary nucleus (Svl) projects mostly onto the dPRN, the vPRN receives a lesser input from both the contralateral and ipsilateral Svl. In addition, however, it receives an input from an area immediately ventral to the NTS. This latter site of efferent projection to the PRN corresponds to another terminal field of projection of the CSN (Ciriello, Hrycyshyn, and Calaresu, 1981a) implying a second source of relayed baroreceptor and/or chemoreceptor information to the PRN. Collaterals of cerebellar afferents from the NTS may project to the PRN. Of interest is the finding of similar terminal fields in the anterior lobe vermis of the efferent projections of both the PRN (Brodal and Torvik, 1954; Somana and Walberg, 1978) and the caudal NTS (Somana and Walberg, 1979). This may implicate the PRN in a modulatory circuit involving the NTS and cerebellum.

F. Nucleus Intercalatus (of Staderini)

A significant bilateral efferent input from the nucleus intercalatus of Staderini (INT) to the PRN has not been previously reported. It shows no particular topographical arrangement and originates throughout most of the rostrocaudal extent of the nucleus. The INT itself receives projections from the VMN and VIN (Mergner, Pompeiano, and Corvaja, 1977) some of which may themselves be collaterals of fibers from the latter two nuclei to the PRN. The medial part of the NTS also is known to project to the INT (Palkovits and Zaborszky, 1977) and a similar arrangement may exist. These interconnections illustrate the complexity of the circuitry involving primary or collateral projections to the PRN at the level of the caudal medulla suggesting by itself a possible modulatory role for the PRN at the precerebellar and prespinal level.

G. Reticular Formation, Raphe Nuclei and Periaqueductal Gray

The presence of an afferent input from the lateral tegmental field (FTL) is of some interest as it lies within the cardiovascular pressor area of the medulla (Alexander, 1946) and a monosynaptic input from the CSN to the FTL has more recently been demonstrated (Miura and Kitamura, 1979). The magnitude of the projection from the FTL cannot be definitely stated because of the certain interruption of some of its efferent fibers traversing the PRN to other destinations. A bilateral input from the gigantocellular (FTG) and magnocellular (FTM) tegmental fields has not been previously reported. The projection is somewhat heavier ipsilaterally particularly that to the vPRN. Of interest is an efferent projection from the NTS to the FTG (Palkovits and Zaborszky, 1977) along which the PRN may be a collateral station.

Some efferent projections of the caudal raphe are likely to have been interrupted as they traversed the PRN at the site of injection of HRP. The present study in part confirms previous autoradiographic evidence of an afferent contribution from the postpyramidal nucleus of the raphe (nucleus raphe pallidus) to the vPRN (Bobillier, Seguin, Petitjean, Salvert, Touret, and Jouvet, 1976). The efferent projections of the inferior central nucleus of the raphe have been little studied and their terminations are not yet accurately stated. The results of the present study suggest that the PRN is possibly one of these either as the recipient of a primary input or a collateral input of the prominent ascending projection of this nucleus. Autoradiographic studies (Bobillier et al., 1976; Taber Pierce, Foote, and Hobson, 1976) have identified neurones in the superior central nucleus of the raphe in addition to the nuclei raphe dorsalis, magnus and pontis projecting to the PRN. Most projections were described as being low density, however a significant raphe-reticular projection to the PRN of high density was described in the case of the superior central nucleus (Bobillier et al., 1976). The present findings confirm a light projection of fibers from raphe neurones in all of the previously mentioned nuclei in addition to the inferior central nucleus to both the dPRN and vPRN. Neurones in the neighbouring medial reticular formation belonging to the gigantocellular, paralemniscal and central tegmental fields were often more extensively labeled particularly ipsilateral to the site of injection. It is conceivable that labeling of medial reticular neurones occurred during injections of autoradiographic tracer into the superior central nucleus. This would explain the apparent discrepancy in the density of projection of this nucleus to the PRN. Raphe projections to the cat cerebellar cortex (Taber Pierce, Hoddevik, and Walberg, 1977) appear to be coextensive with those of the PRN and it is

possible that some of the former may be collaterals of projections to the PRN.

A projection from the ventrolateral aspect of the ipsilateral periaqueductal gray in the region of the interstitial nucleus of Cajal and nucleus of Darkschewitz to the vPRN is suggested in this study. Previous reports have not indicated a descending projection from the periaqueductal gray to this region.

H. Spinal Cord

A moderate spinal projection to the PRN traversing the dorsal funiculi has previously been reported (Brodal and Gogstad, 1957). Convincing evidence of a relay in the dorsal column nuclei of spinal impulses influencing the PRN has not been forthcoming (Brodal and Gogstad, 1957; Hand and van Winkle, 1977; Berkley and Hand, 1978; Asanuma, Thach, and Jones, 1983). Neither can it be said to occur in the present study. Stimulation of cutaneous mechanoreceptors of the forelimb and hindlimb in the cat has excited neurones in the PRN (Eccles, Nicoll, Schwarz, Táboříková, and Willey, 1976). The present work identifies neurones primarily in the cervical cord projecting to the PRN and establishes in part the anatomical substrate for the previous findings. Projections to the vPRN begin further caudally within the thoracolumbar cord whereas those to the dPRN are almost entirely confined to the cervical cord. These connections imply differences in the receptive fields of the two regions in the PRN and may have some bearing on their respective roles in modulating postural information at the precerebellar stage.

I. Cerebral Cortex

Bilateral cortical projections to the PRN have previously

been reported in the cat (Brodal and Gogstad, 1957; Kuypers, 1958; Sousa-Pinto, 1970) using orthograde degeneration techniques. In particular, fibers originating in the sensorimotor cortex were shown to terminate mainly in the vPRN. Sousa-Pinto (1970) identified projections from the anterior sigmoid and ventral coronal gyri (first somatomotor area) which terminated in the vPRN and a smaller input from the anterior sigmoid gyrus which terminated in the dPRN. Scanty projections to both the dPRN and the vPRN from the posterior sigmoid and dorsal coronal gyri (first somatosensory area) and area 6 of Hassler and Muhs-Clement (1964) in the medial wall of the anterior sigmoid gyrus were also described. Projections from the parietal cortex (middle suprasylvian and lateral gyri) could not be found to terminate within the PRN (Mizuno, Mochizuki, Akimoto and Matsushima, 1973).

In the present study, cortical projections to the PRN originating from outside of the sensorimotor cortex are not observed. All fibers belong to neurones of layer V. The largest contribution of fibers appears to arise from the first somatomotor cortex with a projection to the dPRN from the ventral coronal gyrus and to the vPRN from the anterior sigmoid and ventral coronal gyri. The dPRN also receives a scanty projection from the lateral aspect of the anterior sigmoid gyrus, area 6 of Hassler and Muhs-Clement and the posterior sigmoid gyrus (first somatosensory area). Cortical fibers passing to the adjacent perihypoglossal nuclei may have been labeled during penetration of the dorsal medulla and injection of the PRN. Some labeling of cortical neurones in this instance may have been due to uptake by these fibers. The vPRN receives fibers from both lateral and medial portions of the anterior sigmoid, the designated forelimb and hindlimb regions of the motor cortex, respectively. Additional scanty projections arise from the posterior sigmoid gyrus and area 6 of Hassler and Muhs-Clement.

A bilateral input from both head and limb regions of the motor cortex is confirmed. Although the cortical area of origin is not as extensive as for the corticopontine projections (Kawamura and Chiba, 1979) a similar contribution to the inferior olive (Walberg, 1956) and the lateral reticular nucleus (Brodal, Marsala, and Brodal, 1967; Hrycyshyn and Flumerfelt, 1981) has been previously identified. Of interest, is a recent report of olivary afferents from the PRN in the cat (Walberg, 1982). Whether these PRN neurones themselves receive inputs from the cerebral cortex, vestibular nuclear complex or the deep cerebellar nuclei must await further study. Internuclear integration of postural information at the precerebellar stage may serve to refine subsequent impulse propagation to the cerebellum. Stimulation of cutaneous mechanoreceptors of the limbs in the cat has also excited neurones orthodromically in the region of the PRN (Eccles et al., 1976). It would be of interest to see whether cortical stimulation inhibits test responses evoked by stimulation of spinal afferent fibers to the PRN much as it does in the case of the lateral reticular nucleus, another precerebellar relay nucleus (Bruckmoser, Hepp-Reymond, and Wiesendanger, 1970).

The present data suggest that the PRN is probably involved in the control of movement since it receives information from the cerebral motor cortex as well as from peripheral mechanoreceptor organs. In its role as a precerebellar relay nucleus, the PRN may then transmit data to the cerebellum regarding the effectiveness of the cerebrocortical influence on the final motor pathway.

5.2 Collateral Efferent Projections of PRN Neurones

A. Projections to the Cerebellum

In keeping with the results of Somana and Walberg (1978)

and Kotchabhakdi et al. (1980), a widespread projection area of cerebellopetal fibers from the PRN is seen. Collateral axonal branching is also widespread throughout the cerebellum and originates from cells within both the dPRN and vPRN. The aPRN is shown in this study not to send axonal collaterals to a wide area of the cerebellum. This finding could be the result of rather marginal labeling of lobule I (lingula), a major terminus for cerebellopetal fibers from the aPRN and a reported general absence of projection to the remaining anterior lobe, lobulus simplex and crus I and crus II (Somana and Walberg, 1978). Conflicting reports of the presence or absence of efferent projections of the PRN to the lobulus simplex appear to be due to differences in the size of the injection field, the tracer material used and the species of animal. Connections have been shown in the cat (Batini, Buisseret-Delmas, Corvisier, Hardy, and Jassik-Gerschenfeld, 1978; Somana and Walberg, 1978) and monkey (Somana and Walberg, 1978) with HRP although not in the rat (Payne, 1983) after using fluorescent compounds. The injection sites in the present study exceeded the limits of the lobulus simplex to include crus I and crus II.

No topographical organization is observed in the cerebellopetal collateral projections of the PRN. The pattern of double-labeling appears to reflect the magnitude of projection to a particular site and the contribution made to the site by either the dPRN or the vPRN. Slightly more neurones appeared to project to the ipsilateral cerebellar cortex in agreement with the findings of others (Somana and Walberg, 1978). About half of the axonal projections to the ipsilateral cortex of the anterior lobe appeared to have collateral projections to the corresponding contralateral cortex. Somewhat fewer axonal projections to the ansiform lobule (crus I and crus II) and the lobulus simplex had collateral projections to the contralateral cortex. Neurones giving rise to collateral axonal

projections to opposing sides of the cerebellar cortex were more abundant in the dPRN than in the vPRN, a reflection perhaps of the relative densities of the cerebellopetal projections of the two divisions.

The results of this study have some bearing upon the functional aspects of the connections of the PRN and cerebellum. Simultaneous efferent projections to opposing sides of the cerebellum may coordinate postural information relayed through the PRN from a number of previously mentioned sources (section 5.1 B, C, D, H, I). Such connections would ensure the simultaneous activation of Purkinje axons on either side of the midline necessary for coordinated bilateral motor adjustments for the maintenance of various postures.

B. Projections to the Spinal Cord

The finding of labeled neurones in the PRN after an HRP deposit in the region of the IML is in agreement with previous anatomical (Miura, Onai, and Takayama, 1983) and electrophysiological data (Elisevich and Ciriello, 1984). In addition, the present study has demonstrated that the majority of PRN neurones projecting to the region of the IML at different thoracic segmental levels were found in the vPRN. A relatively minor component of this descending pathway was also found to originate in the ventral part of the dPRN. Approximately 40% of the neurones in the PRN which projected to the region of the IML at or caudal to the T2 level distributed collateral axons to the IML region at and caudal to the T4 or T7 levels. Using similar methods of fluorochrome histochemistry, Huisman, Kuypers, and Verburgh (1982) have shown that in the cat, 55 - 60% of raphe cervical neurones and 20% of rubrocervical neurones send collateral axonal branches to more caudal segments of the spinal cord. In an

electrophysiological study, Peterson, Maunz, Pitts, and Machel (1975) found that 66% of reticulocervical neurones distributed collaterals to segments caudal to the L1 segment of the spinal cord.

It may be argued that in the present study the demonstration of double-labeled neurones in the PRN resulted from labeling of a single axon that did not give off collateral branches to different spinal segments but rather was located within the injection site at different segmental levels (Sawchenko and Swanson, 1981). Although this possibility cannot be totally excluded, this is unlikely as Henry and Calaresu (1974c, 1974d) have shown that autonomic fibers from the PRN projecting to the IML course in the ventral funiculus of the cord. The ventral funiculus was never involved in an injection site. Furthermore, projections to spinal motor nuclei from the medial pontomedullary reticular formation have been shown to descend in the ventromedial or ventrolateral funiculus of the spinal cord (Nyberg-Hansen, 1966; Ito, Udo, and Mano, 1970; Petras, 1967; Peterson et al., 1975). These reticulospinal projections would also not have been interrupted by the micropipette penetrations nor involved by the injection site.

These data demonstrate at least a dual collateral projection to the region of the ipsilateral IML from PRN neurones. On the basis of the similar topological patterns of labeled neurones in the PRN after combined injection of the T2 - T4 and T2 - T7 segmental regions with fluorochromes, it is possible that even more axonal collaterals to various levels of the IML region exist. Finally, the approximately even distribution of these PRN projections to the IML region at various segmental levels suggests that the PRN does not preferentially alter the activity of spinal autonomic neurones innervating either different vascular beds or visceral organs.

5.3 Electrophysiological Identification of PRN Neurones Relaying Cardiovascular Afferent Input Directly to the Region of the IML

These data have provided electrophysiological evidence in the cat for the existence of a direct pathway from neurones in the PRN to the region of the IML of the upper thoracic cord which mediate cardiovascular afferent information from the carotid sinus nerve (CSN) and pressor sites in the fastigial nucleus (FN). The finding of neurones in the PRN which project directly to the region of the IML is in agreement with recent anatomical studies in the cat using the retrograde transport of HRP (Miura et al., 1983). The distribution of antidromically identified single units in the PRN in the present study corresponds closely to that of the retrogradely labeled neurones, suggesting that the electrical activity recorded from the single unit most likely originated in cell bodies and not in fibers of passage. This suggestion is further supported by the observations that 74% of the antidromic spikes had a clear two-component spike in which the somatodendritic component could usually be induced to fail with high frequency of stimulation (Lipski, 1981), that all units had spike configurations and durations similar to those evoked from somata (Fussey, Kidd, and Whitman, 1970; Lipski, 1981), and that the evoked antidromic spike could be recorded over a dorsoventral distance of greater than 100 μm.

The latencies of the antidromically activated units corresponded to an estimated mean conduction velocity of 36.4 $\pm$ 2.1 m/s. Previous estimates of conduction velocities of bulbospinal pathways thought to be involved in cardiovascular regulation have been reported as 3 - 7 m/s (Gootman and Cohen, 1971; Gebber, Taylor, and Weaver, 1973; Coote, Macleod, Fleetwood-Walker, and Gilbey, 1981), 19 m/s (Caverson, Ciriello, and

Calaresu, 1983), 29 m/s (Lipski and Trzebski, 1975) and 63 m/s (Henry and Calaresu, 1974a,d). Although this value is higher than most previous estimates of conduction velocities of these bulbospinal pathways, it should be noted that in the present study, axons conducting in the previously reported low and high ranges were found. Seventy-five per cent of the axons in this study conducted within the range of 10 - 60 m/s overlapping considerably the findings of other studies using similar electrophysiological techniques (Henry and Calaresu, 1974a,d, Lipski and Trzebski, 1975; Caverson et al., 1983). In those studies which reported slower conduction velocities (Gootman and Cohen, 1971; Gebber et al., 1973; Coote et al., 1981), mass evoked activity was recorded in peripheral nerves during stimulation of a central site and an approximate conduction velocity for the bulbospinal system was estimated without taking into account the number of synapses being activated or the electrophysiological properties of spinal interneurones interposed between the descending pathways and the preganglionic sympathetic neurones. In the present study, antidromic activation of single units by direct electrical stimulation of the IML region avoided synaptic delay which would bias the results toward slower conduction velocity ranges. The absence of conduction velocities lower than 10 m/s in this study in part reflect the use of a different electrophysiological technique. The functional significance of such a fast conducting bulbospinal pathway is only conjectural. However, since the PRN has been implicated in mediating the cardiovascular responses to postural adjustments (Reis, 1972), it may be suggested that this pathway mediating cardiovascular information may serve to compensate in time that which is lost in relaying sensory and motor information related to postural changes through extensive connections the PRN has with the vestibular and deep cerebellar nuclei (Ito et al., 1970; Ghelarducci et al., 1974; Elisevich et al., 1983).

All antidromically activated units were tested for their orthodromic responses to electrical stimulation of the CSN and pressor sites in the FN. The finding that 40% of these units responded orthodromically to stimulation of at least one of these inputs was not totally unexpected. Miura and Reis (1971) have previously reported single units in the PRN which altered their firing frequency to stimulation of the CSN and FN, although the output pathways of these were not investigated. On the basis of the long and variable latency of the orthodromic response to stimulation of the CSN, it is likely that the PRN units were activated polysynaptically through a relay in the nucleus of the solitary tract (NTS), the primary site of termination of cardiovascular afferent fibers (Crill and Reis, 1968; Ciriello and Calaresu, 1981). As the response of these units to selective activation of baroreceptor and chemoreceptor afferent fibers was not examined, it is not possible to assign a specific function to them. It is not unreasonable, however, to suggest that PRN units excited by stimulation of only the CSN are relaying baroreceptor afferent information to spinal inhibitory interneurones (McCall, Gebber, and Barman, 1977) antecedent to sympathetic preganglionic neurones, as lesions of the PRN have been shown to abolish the depressor response to stimulation of the CSN (Miura and Reis, 1971) and stimulation of the PRN has been demonstrated to elicit a cardiac slowing which is due to inhibition of the sympathetic input to the heart (Calaresu and Thomas, 1971). With regard to the majority of units which were excited by stimulation of both the CSN and pressor sites in the FN, it would seem likely that they function as sites of interaction between baroreceptor and FN inputs to produce an integrated output signal transmitted directly to spinal sympathetic areas for the control of arterial pressure and heart rate. However, the possibility cannot be excluded that units excited by stimulation of both the CSN and

FN are responding to activation of peripheral chemoreceptors as it seems unlikely that a neurone relaying excitatory information from pressor sites in the FN would also relay an inhibitory input from baroreceptors.

A small population of antidromically activated units were also found to respond to electrical stimulation of only pressor sites in the FN. These units responded with latencies suggesting a monosynaptic input. In support of this suggestion, a direct pathway between the FN and PRN has been demonstrated using the autoradiographic technique (Batton et al., 1977). These units likely function as part of descending sympathoexcitatory pathways from the FN to spinal sympathetic areas.

In summary, these data provide electrophysiological evidence of a direct pathway which relays cardiovascular afferent information from the CSN and FN via neurones in the PRN to the region of the IML. As the FN has been implicated as the final output station of a cerebellar loop for vestibular influences on postural tone (Doba and Reis, 1972) and both vestibular and cardiovascular inputs to the PRN have been demonstrated (Elisevich et al., 1983; Miura and Reis, 1971), these data suggest that the PRN neurones projecting directly to the IML region serve as a functional coupling between postural changes and the appropriate cardiovascular adjustments to the motor movements.

5.4 Electrophysiological Identification of PRN Neurones Relaying Vestibular Afferent Input Directly to the Region of the IML

These data have provided electrophysiological evidence in the cat of a direct pathway from neurones in the PRN relaying vestibular information to the region of the IML of the upper thoracic cord. The vestibular input from the region of the lateral vestibular nucleus to the

PRN has been shown electrophysiologically to have a rather long conduction time. A broad range (1.4 - 19 ms) of latencies for this pathway was identified although the majority of recorded latencies measured under 10 ms. Considering a measured distance of 7 to 12 mm from the site of stimulation in the VNC to the site of recording in the PRN, a mean latency of 6.3 ± 0.6 ms indicates a relatively slow transfer of activity from the VNC to the PRN. A paucisynaptic pathway from the VNC to the PRN may partly explain this finding. In support of this suggestion, a direct caudal projection from the medial medullary reticular formation to the PRN has recently been shown using the HRP technique (Elisevich et al., 1983) and the lateral vestibular nucleus has been shown to project to this part of the brainstem (Ladpli and Brodal, 1968). It has previously been shown that a similarly slow conduction time ranging from 0.6 to over 3 ms existed in a direct pathway from the VNC to the contralateral pontomedullary reticular formation, in particular, the gigantocellular tegmental field found immediately rostral to the PRN (Peterson and Abzug, 1975). Straight line conduction distances between the reticular and vestibular nuclei measured 4 to 9 mm in the latter experiments providing a minimum estimate of conduction velocity ranging from 2.0 to 14 m/s (Peterson and Abzug, 1975). Both excitation and inhibition of PRN neurones was elicited in the present study in agreement with previous intracellular recordings which indicated a widespread direct excitatory and inhibitory action on medial reticular neurones by the VNC (Peterson and Abzug, 1975).

All orthodromically activated units from electrical stimulation of the VNC were tested for their antidromic responses to electrical stimulation in the region of the IML and orthodromic responses to electrical stimulation of the CSN. Almost two-thirds (62%) of the PRN units activated orthodromically to stimulation of the VNC were

antidromically activated by stimulation of the region of the IML. The finding of a relay of vestibular activity through the PRN directly to sympathetic centers in the spinal cord was not unexpected. Vestibulosympathetic activity has been recorded in splanchnic nerve and stimulation of the VNC has been shown to elicit both elevation and depression of arterial pressure (Megirian and Manning, 1967; Cobbold, Megirian, and Sherry, 1968. Tang and Gernandt, 1969. Uchino, Kudo, Tsuda, and Iwamura, 1970). Furthermore, vestibular evoked sympathetic responses of long latency were found to depend on the functional integrity of supraspinal structures (Cobbold et al., 1968). Most PRN units projecting directly to the region of the IML had orthodromic response latencies to VNC stimulation of less than 10 ms. The latencies of the antidromically activated units ranged from 0.9 to 3.2 ms and corresponded to a mean conduction velocity of 48.6 ± 3.5 m/s. Conduction velocities of this direct pathway again exceeded most previous estimates of conduction velocities of bulbospinal pathways thought to be involved in cardioregulatory and vasomotor control and projecting to the IML (see section 5.3). Eighty-three per cent of the PRN units in the present study conducted within the range of 15 - 60 m/s overlapping considerably the findings of other studies using similar electrophysiological techniques for other cardioregulatory bulbospinal systems (Henry and Calaresu, 1974a,d, Caverson et al., 1983). The mean conduction velocity in the present study exceeds that obtained in the previous study which examined the projection of PRN neurones to the region of the IML mediating cardiovascular afferent information (Elisevich and Ciriello, 1984). These relatively fast conduction velocities may serve to compensate in time that which is lost in the relatively slow conduction pathway from the VNC to the PRN. Furthermore, it is well known that the vestibuloreticular system

is only partially activated by afferent input from the vestibular labyrinth (Peterson and Abzug, 1975). It is likely therefore that a number of vestibular neurones relay activity from other sources such as the cerebellum (Batton et al., 1977) or spinal cord (Pompeiano, 1972b) adding to the number of synapses encountered in mediating autonomic or motor responses and increasing the conduction time of the bulbar neural circuitry. The location of PRN neurones relaying vestibular afferent information to the region of the IML largely within the vPRN coincides with that of another population of PRN neurones found to relay cardiovascular afferent information from the ipsilateral CSN and pressor sites in the contralateral FN directly to the region of the IML (Élisevich and Ciriello, 1984). The findings of the present study suggest, however, that very few of the PRN neurones transmitting vestibulosympathetic activity are influenced by CSN stimulation and that therefore they must largely comprise a separate subpopulation of neurones in the vPRN mediating sympathetic activity. It is possible that some units would respond to stimulation of both the VNC and FN but the ablation of fastigioreticular inputs in the present experimental model precluded the testing of this possibility. In support of this, pressor responses have been elicited through electrical stimulation of both the vestibular apparatus (Tang and Gernandt, 1969) and fastigial nucleus (Miura and Reis, 1969a).

Sites of stimulation within the VNC appeared to be largely confined to the lateral vestibular nucleus (VLN). Threshold stimulus intensities used to activate PRN units corresponded to a maximum stimulus spread to a sphere of tissue with a radius of less than 0.5 mm (Bagshaw and Evans, 1976). Hence, the likelihood of stimulating the medial (VMN) and inferior vestibular nuclei was minimized.

It has been suggested that the VMN is responsible for sympathetic responses mediated through bulbar reticular neurones (Uchino et al., 1970). Ablations of this nucleus have attenuated vestibulosympathetic responses achieved by stimulation of the vestibular nerve. Although the present data are not entirely inconsistent with this, ablations of the VMN in previous experiments appear likely to have involved varying portions of the VLN. In addition, the VLN has been shown both electrophysiologically (Peterson and Abzug, 1975) and anatomically (Elisevich et al., 1983) to project to medial medullary reticular neurones.

In summary, these data provide electrophysiological evidence of a projection from the VNC to the PRN and the relaying of vestibular information from the PRN directly to sympathetic centers in the spinal cord. Previous work indicating that vestibulosympathetic activity alters vasoconstrictor activity to produce either elevation or depression of arterial pressure lends support to the suggestion that vestibular afferent information relayed through the PRN to the region of the IML is likely involved in cardioregulatory and/or vasomotor activity.

5.5 Conclusions

Involvement of the region of the PRN in the reflex regulation of cardiac and vasomotor activity is well known (Wang and Ranson, 1939; Alexander, 1946; Humphrey, 1967, Crill and Reis, 1968; Miura and Reis, 1969b; Homma, Miura and Reis, 1970; Miura and Kitamura, 1979). Stimulation of the PRN has been shown to elicit responses in arterial pressure and heart rate and to mediate the cardiovascular responses to stimulation of the CSN and FN (Miura and Reis, 1971; Calaresu

and Thomas, 1971). This study has shown that the cardiovascular afferent information from the CSN and FN is relayed directly to the region of the IML in the upper thoracic cord which is known to contain sympathetic preganglionic neurones. Stimulation of the rostral FN (fastigial pressor area) has been shown to elicit cardiovascular responses closely resembling reflex cardiovascular responses evoked by the assumption of an upright posture (Doba and Reis, 1972). As both macular (Spyer et al., 1973; Ghelarducci et al., 1974) and cardiovascular inputs to the PRN have been demonstrated, a similar functional coupling between postural changes and cardiovascular adjustment has been proposed (Reis, 1972; Calaresu, Faiers, and Mogenson, 1975). The findings of the present study support this postulate further by providing detailed evidence for an appropriate neuroanatomical circuitry for the transmission of this activity and establishing electrophysiologically the existence of a relay through the PRN for cardiovascular and vestibular information directly to the region of the IML. In addition to their obvious role in motor regulation, projections of the motor cortex, accessory oculomotor nuclei, superior colliculus and bulbar reticular formation to the PRN may also influence sympathetic activity through its connections with the IML. In support of this, stimulation of the primate motor cortex has been shown to alter the mean blood flow through muscle groups in a topographical fashion in paralyzed limbs (Clarke, Smith, and Shearn, 1968). The present data show that the vPRN receives most of the motor cortical output representing the limb regions (anterior sigmoid gyrus) and that it projects directly to sympathetic centers in the spinal cord. Therefore, the PRN would be a suitable candidate for the mediation of these corticosympathetic (vasomotor) responses. Its extensive collateralized input to the region of the IML might serve to coordinate the responses of numerous vascular

beds simultaneously to bring about an appreciable response in systemic arterial pressure.

The PRN has been shown to be a major center of convergence for a large number of inputs from a variety of sources which sends collateral axonal branches mainly to the cerebellum and spinal cord. Its extensive connections and functional autonomic relations suggest a central role in mediating orthostatic reflex activity.

CHAPTER 6 - SUMMARY

1. The topographical organization of afferent input to the paramedian reticular nucleus (PRN) of the cat was studied by the horseradish peroxidase (HRP) method of retrograde neuronal labeling following selective injection of the enzyme into each of its dorsal (dPRN) and ventral (vPRN) subdivisions.

2. Labeled neurones were found within all three contralateral deep cerebellar nuclei and the ipsilateral fastigial nucleus (FN) following injections of HRP into either the dPRN and vPRN. Small or medium-sized labeled perikarya were concentrated posteriorly in the dorsomedial portion of the contralateral FN and in the ventromedial portion of the anterior half of the ipsilateral FN following HRP injections of the dPRN. Injection of the vPRN resulted in cell-labeling predominantly within the anteromedial aspect of the contralateral FN and in the ventromedial portion of the ipsilateral FN.

3. The majority of cells labeled in the interposed nuclei of the cerebellum resulted from HRP injection of the vPRN and consisted largely of medium-sized and multipolar neurones in the anterodorsal portion of the posterior subnucleus. Labeled perikarya in the dentate nucleus were however found throughout the anteroposterior extent of the dentate nucleus following HRP injection of either the dPRN or the vPRN.

4. The majority of vestibular afferent fibers to the PRN arise in both lateral vestibular nuclei (VLN). Injection of HRP into either the dPRN or the vPRN resulted in cell-labeling in both dorsal and ventral subnuclei of the VLN in addition to the medial and inferior vestibular nuclei on both sides of the brainstem.

5. Major mesencephalic sources of afferent projection of the PRN include the accessory oculomotor nuclei and the superior colliculus. Injection of HRP into the dPRN resulted in dense labeling of medium-sized and multipolar neurones in the interstitial nucleus of Cajal bilaterally in addition to neurones in the neighbouring medial reticular formation and periaqueductal grey. A similar pattern of cell-labeling occurred in the ipsilateral interstitial nucleus following HRP injection of the vPRN. Additionally, a high concentration of labeled cells was found in the ipsilateral nucleus of Darkschewitz and the intermediate layer of the contralateral superior colliculus after injection of the vPRN.

6. Afferent projections to both the dPRN and the vPPN originate from the caudal half of the solitary nuclear complex, in particular, the contralateral ventrolateral subnucleus. A relatively significant number of labeled perikarya were also found in the reticular formation immediately ventral to the solitary complex following injection of HRP into the vPRN. Another medullary source of afferent projections to the dPRN and the vPRN is the nucleus intercalatus (of Staderini) in which labeled neurones appeared in approximately equal numbers bilaterally when either the dPRN or the vPRN was injected with HRP.

7. Diffuse neuronal labeling of the raphe and the medial reticular formation of the brainstem occurred following injection of HRP into both the dPRN and the vPRN. The most consistently labeled raphe neurones appeared within the inferior and superior central nuclei of the raphe whereas others were found in the nuclei raphe magnus, pontis and dorsalis. Labeled reticular neurones appeared scattered bilaterally within the lateral gigantocellular, magnocellular, paralemniscal and central tegmental fields.

8. Neurones of the spinoreticular pathway to the PRN originate within laminae VII and VIII of the cervical cord bilaterally. In addition, a concentration of labeled neurones in the medial aspect of lamina VI of the upper contralateral cervical cord was found following injection of HRP into the vPRN.

9. A major source of cerebrocorticoreticular fibers to the PRN was shown by the HRP method to be confined largely to layer V of the sensorimotor cortex, in particular, the head and limb regions of the motor cortex. Labeled cortical neurones appeared predominantly within the ventral coronal gyrus following injection of the dPRN and within the anterior sigmoid and ventral coronal gyri following injection of the vPRN. A somatotopical relationship in this pathway is suggested.

10. Collateral axonal projections of PRN neurones to the cerebellum and spinal cord were studied using fluorescence histochemistry. Various portions of the cerebellar cortex and segmental levels of the cord in the region of the intermediolateral nucleus (IML) were injected

with the fluorescent compounds nuclear yellow (NY) and fast blue (FB) following which retrogradely labeled PRN neurones were examined for the presence of single or double label.

11. Approximately half of the axonal projections of the PRN to the ipsilateral cortex of the anterior lobe of the cerebellum have collateral projections to the corresponding contralateral cortex Fewer axonal projections from the PRN to the ansiform lobule and the lobulus simplex have collateral projections to the corresponding contralateral cerebellar cortex. Neurones in the PRN giving rise to collateral axonal projections to opposing sides of the cerebellar cortex are more abundant in the dPRN than in the vPRN, a reflection perhaps of the relative densities of the cerebellopetal projections of the two divisions.

12. Approximately 40% of the neurones in the PRN which project to the region of the IML at and caudal to the T-2 level distribute collateral axons to the region of the IML at and caudal to the T-4 or T-7 levels. The patterns of distribution of single- and double-labeled neurones in the PRN appeared similar and showed no topological variation among cases injected at either the T-2, T-4 or T-7 levels.

13. In an additional series of experiments, the region of the PRN was systematically explored for single units antidromically activated by electrical stimulation of the IML in nine chloralosed, paralyzed and artificially ventilated cats. These antidromically identified units were then tested for their responses to electrical stimulation of the carotid sinus nerve (CSN) and fastigial nucleus (FN).

14. Sixty-two single units in the PRN were antidromically activated with latencies corresponding to a mean conduction velocity of 36.4 ± 2.1 m/s to stimulation of the IML. Of these units, 40% (25/62) responded orthodromically to stimulation of the CSN and/or the FN. Of the responsive units, 20% (5/25) were excited by stimulation of only the CSN, 24% (6/25) were excited by stimulation of only the FN and 56% (14/25) were excited by stimulation of both the CSN and FN.

15. All PRN units antidromically activated by electrical stimulation of the IML were confined largely to the caudal half of the PRN within the vPRN and the ventral aspect of the dPRN. No distinct anatomical segregation of those units which were responsive to orthodromic stimulation from those that were unresponsive to the tested inputs was found.

16. In a final series of experiments involving five chloralosed, paralyzed and artificially ventilated cats, the region of the PRN was systematically explored for single units orthodromically activated by electrical stimulation of the vestibular nuclear complex (VNC) following chronic surgical ablation of the fastigioreticular input to the PRN. These orthodromically identified units were then tested for their responses to electrical stimulation of the IML and the CSN.

17. Forty-seven single units in the PRN were orthodromically activated with a mean latency of 6.3 ± 0.6 ms to stimulation of the VNC. Of these units, 43% (20/47) were discharging spontaneously. Amongst the latter, 15% (3/20) were inhibited by VNC stimulation, 15%

(3/20) were excited and subsequently inhibited whereas of the initial 47 units, 94% (44/47) were excited.

18. Twenty-nine of the 47 orthodromically identified units (62%) were antidromically activated with latencies corresponding to a mean conduction velocity of 48.6 ± 3.5 m/s to stimulation of the IML. None of the orthodromically identified PRN units responded to CSN stimulation.

19. Units activated by stimulation of the VNC were found in both the dPRN and vPRN whereas the units which responded antidromically to IML stimulation and orthodromically to VNC stimulation were confined largely to the vPRN and the ventral aspect of the dPRN with the majority found in the caudal half of the vPRN. All stimulation sites in the VNC were localized to the lateral vestibular nucleus (VLN).

20. Extensive afferent connections with the PRN from a variety of central sources are established in the present study. The latter include, in particular, the fastigial nucleus, vestibular nuclei, solitary nuclei, accessory oculomotor nuclei, superior colliculus and the sensorimotor cerebral cortex. In addition, a partially collateralized efferent projection from the PRN to the cerebellar cortex and the IML region of the thoracic cord is demonstrated. This neuroanatomical circuitry supports previous assumptions of a central role for the PRN as a functional integrator between postural changes and cardiovascular adjustments appropriate to these changes. Electrophysiological evidence of direct pathways from neurones in the PRN to the region of the IML which mediate cardiovascular afferent information from

the GSN and pressor sites in the PN as well as vestibular information predominantly from the VLN further supports the premise that through its extensive connections and functional autonomic relations, the PRN subserves a central role in mediating orthostatic reflex activity.

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