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PEDUNCULOPONTINE TEGMENTAL NUCLEUS. PART I: CYTOARCHITECTURE, TRANSMITTERS, DEVELOPMENT AND CONNECTIONS

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The present review compiles data on the cytoarchitecture, transmitters, development, afferent and efferent connections of the pedunculopontine tegmental nucleus (PPN). PPN is a reticular formation nucleus, located in the pontomesencephalic tegmentum, closely associated with the ascending limb of the superior cerebellar peduncle. Its most typical cells are cholinergic and comprise the Ch5 neuronal group of Mesulam. It contains also glutamatergic neurons that may contain glutamate as a sole transmitter or as a co-transmitter of acetylcholine. The cholinergic neurons use also the gaseous transmitter nitric oxide, being the most prominent nitrergic neurons in the central nervous system (CNS). In aged animals, there is practically no cell loss but there are certain drastic changes in the somatodendritic morphology. PPN has an extremely rich afferent input. All basal ganglia send axons to PPN, the strongest connection being from the substantia nigra (SN), followed by pathways arising from the subthalamic nucleus (STN) and from both pallidal segments (PAL). PPN receives afferents also from the cerebral cortex, from areas of the limbic system and hypothalamus, from the cerebellum, from the brainstem - particularly serotoninergic axons from the raphe nuclei and noradrenergic axons from the locus ceruleus - as well as from the spinal cord. The efferent connections of PPN are extremely diverse, and some of them are carried out by axons that emit divergent collaterals to two different structures. The heaviest efferent pathway of PPN is destined to the thalamus, innervating virtually all thalamic nuclei, and especially the "nonspecific" intralaminar nuclei, that innervate broad ares of the cerebral cortex. All basal ganglia are innervated and in most cases the connection is bilateral. The most significant pathway innervates the dopaminergic neurons of SN, followed by a connection to STN and PAL. Other PPN efferent connections reach the cerebellum, the superior colliculus, nuclei of cranial nerves, the reticular formation, and the spinal cord. The reviewed connections of PPN suggest that it is involved significantly in the arousal systems, and is implicated in the disturbances of sleep and wakefulness. PPN is also involved in the motor functions of CNS, as well as in the movement disorders.
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INTRODUCTION

The nucleus tegmenti pedunculopontinus (pedunculopontine tegmental nucleus, PPN) was discriminated by Jacobsohn (1) in the human brain as a separate entity of the reticular formation (RF) in the tegmentum of the rostral pons and of the caudal mesencephalic tegmentum. Lateral to the crossing fibers of the ascending limb of the superior cerebellar peduncle (SCP) he described "…medium-sized, pointed-triangular or oblong-truncated cells … that appear larger than the majority of other reticular neurons… and that cluster themselves strongly together, so indeed they appear as one separate nucleus which I refer to as the nucleus tegmenti pedunculopontinus". For decades PPN attracted almost no attention, and its function was unknown until the significant observation of Moruzzi and Magoun (2) that the electrical stimulation of midbrain sites causes activation of the cortical electroencephalogram. Later, Shute and Lewis (3) described two ascending (dorsal and ventral) mesopontine tegmental pathways which were assumed to be cholinergic on the basis of acetylcholinesterase (AChE) histochemistry. The first evidence that PPN is wired also with extrapyramidal structures was presented by Nauta and Mehler (4). They demonstrated that the internal pallidum in the monkey sends its axons not only to the thalamus by means of ansa lenticularis, but that a more discrete pallidofugal bundle descends caudally and terminates in PPN. Presently, it is firmly established that PPN is a significant component of the "arousal systems" (5-10) and is interconnected - often bilaterally - with most of the basal ganglia (5,11-14).

We here present an updated review on the structure and connectivity of PPN, and add our results on transmitters, aging and branching efferent connections of PPN.

CYTOARCHITECTURE AND TRANSMITTERS OF PPN

As mentioned above, no attention was paid to PPN until the middle of the XX century. The nuclei of the mesencephalic reticular formation (MRF) have ill defined boundaries, the original data of Jacobsohn were forgotten, and the modern studies on the RF started with conflicting descriptions of the tegmental structures, as well as with a considerable confusion in the terminology (see 15 for painstaking review). Olszewski and Baxter (16) contributed much to our knowledge on the cytoarchitecture of the human brainstem. They described PPN as the third, most caudally located nucleus of MRF, curving around the decussation of SCP. The dorsal part of the nucleus is composed of densely packed medium-sized and large multipolar neurons, and was nominated "*pars compacta*". The ventral part of the nucleus, wedged between SCP and the medial lemniscus, contains loosely arranged cells, and was nominated "*pars dissipata*". By means of AChE histochemical staining the borders of the nucleus are more easily recognized, so that Paxinos *et al* (17) provided some corrections of the description of Olszewski and Baxter, mainly concerning the rostral borders of PPN. By means of classical cytoarchitectonic methods, PPN was identified more or less successfully also in animals: gorilla (18), cat (19) and rat (15). Subsequently, far more elaborated data were obtained by histochemical and immunohistochemical methods.

Shute and Lewis (3) suggested that the RF neurons, giving rise to ascending projection are cholinergic, since they stain for AChE. However, many other non-cholinergic populations contain AChE, including the dopaminergic neurons of the substantia nigra (SN) (reviewed in 11,17,20). Thus, a far more reliable marker for cholinergic neurons is the synthesizing enzyme of acetylcholine - choline acetyltransferase (ChAT). By means of immunohistochemical staining for ChAT it was firmly established in various species - from rat to man - that the vast majority of PPN neurons are cholinergic (15,21-44). PPN is very often identified as the Mesulam's cholinergic Ch5 group (22). There are certain species differences in the location of Ch5 and its borders with the noradrenergic neurons of *locus coeruleus* (LC). In rats (15,21,22,32,40), monkeys (25,26) and humans (24,42), cholinergic and noradrenergic neurons do not intermingle. However, the borders between PPN and LC are not clear in the guinea pig (37), ferret (28), cat (20,29,30,45) and dog (41). In the cat, the ChAT-positive PPN neurons and the tyrosine hydroxylase (TH)-positive LC neurons not only intermingle, but both neuronal populations invade also the parabrachial nuclei (20). On the other hand, the cholinergic PPN neurons in the rat reach a close proximity to SN, and some even invade its territory (20,27).

ChAT-positive neurons are seen also dorsomedially to the caudal PPN portion. This is the Mesulam's Ch6 group. Its neurons are located within the laterodorsal tegmental nucleus (LDTN) of Castaldi (46). LDTN is located in the rostral portion of the pontine central gray, immediately lateral to the dorsal tegmental nucleus of von Gudden, and medially to LC. In the human brain this structure is so unsightly that even the keen eyes of Olszewski and Baxter (16) failed to recognize it as a separate entity. Only the AChE and ChAT mappings of the human RF (17,24,31,44) helped to clearly identify LDTN in the human brain, and it is delineated in the present atlas of the human brainstem (47). In all species examined, ventrally from LDTN continues a loosely arranged group of cholinergic neurons. In most studies it is nominated "sublaterodorsal tegmental nucleus" (SLDTN), see Swanson (48). In the human brain, Paxinos and Huang (47) named LDTN "laterodorsal tegmental nucleus - ventral part". SLDTN loosely bridges the gap between LDTN and PPN, and the clear distinction between Ch5 and Ch6 might be artificial. Hence, the reference to the two groups together as a "PPN-LDTN complex" (31). Paxinos and Huang presented also stereotaxic parameters of both nuclei. LDTN extends for

a rostrocaudal length of 10 mm, from 24 to 33 mm rostral to the obex. The parameters of PPN are respectively +31 - +36. The rostrocaudal extent of PPN is shorter than that of LDTN, but its volume is larger, since it occupies a broader territory on transverse sections. According to Manaye *et al* (42), the average number of cholinergic cells in the human PPN and LDTN is approximately 20,000, with 30% of the neurons in PPN *pars compacta*, 57% - in the PPN *pars dissipata*, and 13% - in LDTN. Recently, another effective immunohistochemical marker for cholinergic neurons was introduced: the high activity choline transporter (49). In the monkey, the immunohistochemical demonstration of this protein corresponds closely to the ChAT-immunoreactivity, including the neurons of PPN and LDTN.

Along with the most abundant cholinergic neurons, PPN contains also non-cholinergic neurons (15,42,50-52). The studies reported by the Bruce Wainer's group (15,52-54) suggested that the term "PPN" should be limited to the cluster of cholinergic neurons that project to the thalamus, whilst the non-cholinergic neurons that are interconnected with the basal ganglia should be separately termed "the midbrain extrapyramidal area". However, tracing studies, combined with transmitter immunohistochemistry revealed that the basal ganglia receive also cholinergic pathways (see below). According to Spann and Grofova (50), the cholinergic neurons in the rat PPN are to be found in both *pars compacta* and *dissipata*. They are intermingled with non-cholinergic cells and do not respect the cytoarchitectural boundaries of the nucleus. The cholinergic neurons are large, with abundant cytoplasmic organelles. According to Spann and Grofova (50), these cells possess a scarce axosomatic synaptic input. There are two almost equally represented classes of non-cholinergic neurons: large and small. The large neurons are similar to the cholinergic ones, but they receive a rich synaptic input. Honda and Semba (51) also investigated the ultrastructural features of the cholinergic and non-cholinergic cells in PPN and LDTN of the rat. They report that the cholinergic neurons are larger by an average of 40%. Neurons in PPN tend to have more irregular shapes than those in LDTN. Somewhat at a difference to the previous report (50), Honda and Semba (51) claimed that the mean number of synapses received by cholinergic somata is greater, by about 70% compared to non-cholinergic perikarya.

There is a growing evidence that the excitatory transmitter glutamate is also present in PPN neurons. This was first suggested by Clements and Grant (55), and soon thereafter Clements *et al* (56) reported that glutamate-like immunoreactivity is present within cholinergic neurons of PPN and LDTN. This was confirmed by Lavoie and Parent (36), and several studies of the PPN efferent connections assured that the PPN terminals are glutamate enriched (57-62; see below). According to Ichinohe *et al* (63), 10% of the cholinergic neurons in PPN are glutamate-immunoreactive. However, cholinergic and glutamatergic neurons might represent also separate cell populations (50,64).

The inhibitory transmitter GABA plays a significant role in the afferent connectivity of PPN (see below). However, there are few studies on GABAergic neurons in PPN (9,65,66). Most of the RF GABAergic neurons are small interneurons that project locally onto the larger projection neurons of the reticular core but some groups of GABAergic neurons in PPN send long ascending projections (9). In the pontomesencephalic tegmentum, approximately 1% of the GABAergic neurons are retrogradely labeled with cholera toxin in the hypothalamus, and these neurons represent 6% of all hypothalamically projecting neurons in the pontomesencephalic tegmentum (66). Recently, Jia *et al* (67) reported that, surprisingly, 50% of the ChAT-immunoreactive neurons in PPN and LDTN also contain GABA. These somewhat unexpected results await further corroboration.

Nitric oxide (NO) is a gaseous neuromediator, apparently released as soon as synthesized, and not requiring a synaptic contact to exert its effects (68-74). The reduced nicotinamide dinucleotide phosphate diaphorase (NADPHd) was introduced 40 years ago as a histological marker that stains certain neurons with a Golgi-impregnation-like quality (75,76 and refs. therein). The function of this enzyme remained enigmatic, and considerably later came the discovery that NADPHd is a neuronal nitric oxide synthase (NOS) (77-80). Mappings of the brain distribution of NADPHd/NOS (81-95) consistently show that this enzyme appears in groups of neurons that cannot be defined by other anatomical and neurochemical criteria (perikaryal size, somatodendritic morphology, transmitters, neuronal connectivity, etc.). There is one notable exception. As early as 1983, when the first immunohistochemical mappings of cholinergic neurons were just appearing, and we knew nothing about NO as a neurotransmitter, Vincent *et al* (96) noticed that NADPHd is a selective marker for the RF cholinergic neurons, and that these cells are the most strongly stained for NADPHd) (see also 75,76). This was repeatedly confirmed in several species (40,84,97-102). Now, it is firmly established that all RF cholinergic neurons of PPN (Ch5) and LDTN (Ch6) simultaneously synthesize NO, but this is not valid for the other cholinergic populations in the central (CNS) and peripheral (PNS) nervous system (see especially 84).

On Fig. 1-3 we present serial sections of a rat brain stem, stained for NADPHd, according to Weinberg *et al* (76). As mentioned above, the NO-producing cholinergic neurons of LDTN and PPN display the strongest reaction for NADPHd/NOS in CNS (Fig. 2B). Followed from caudal to rostral, first appears LDTN (Fig. 1A). It is located in the most rostral part of the floor of the fourth ventricle, within the pontine central gray. Laterally to it are LC, and mesencephalic nucleus and

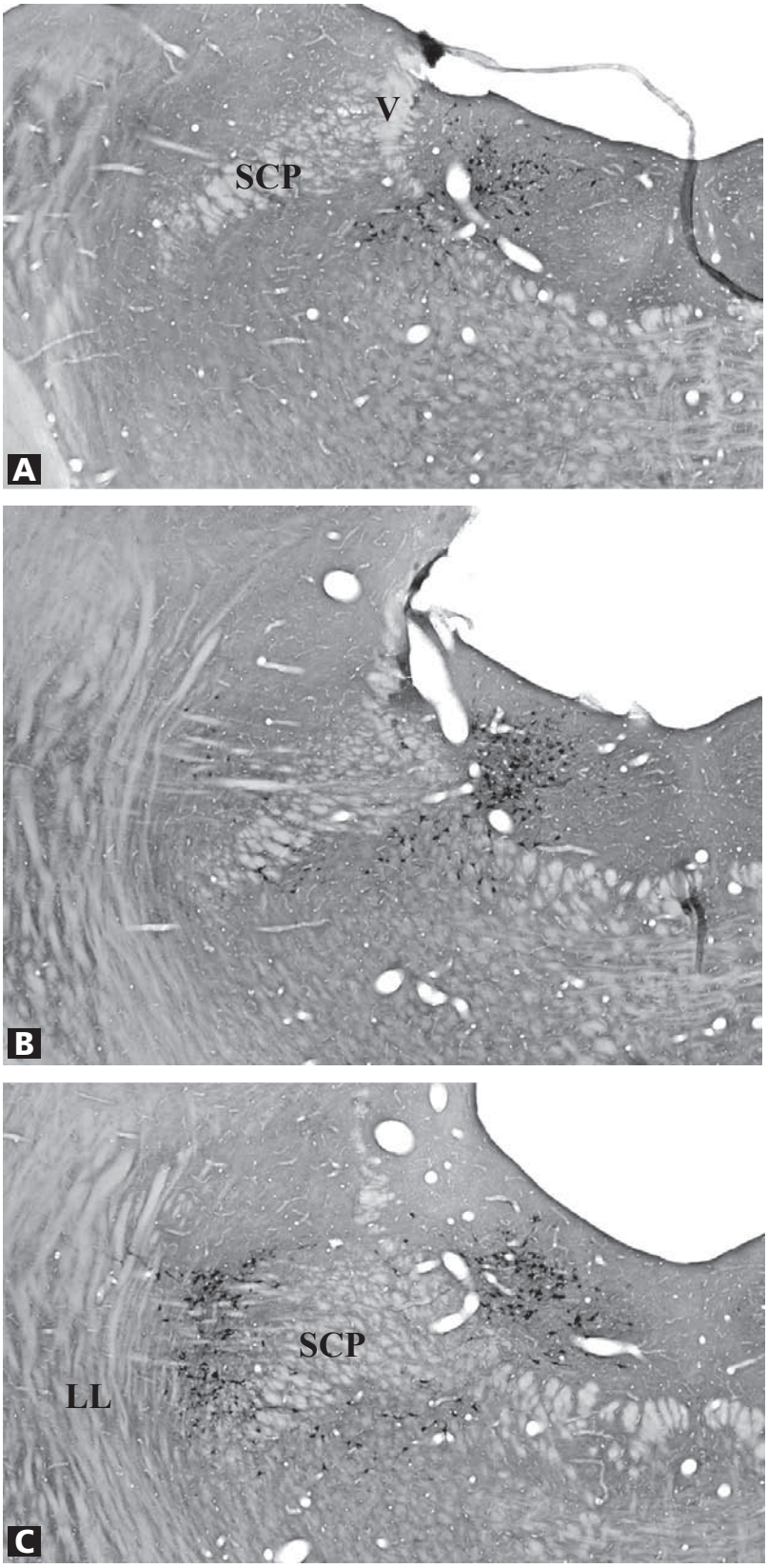

Figure 1. *Serial sections from caudal to rostral direction through LDTN and PPN, in the transitional zone between pontine and mesencephalic tegmentum. The sections are stained for NADPHd. Medial side is to the right.* ***(A)*** *Most caudally, within the pontine central gray is located LDTN. Ventrolaterally to LDTN and medially to SCP are the neurons of LDTNv. V - mesencephalic trigeminal tract.* ***(B)*** *LDTN enlarges. Laterally to SCP, within the fibers of the commissure of the lateral lemniscus, appear the most caudal neurons of PPN pars compacta.* ***(C)*** *LDTN reaches its maximum extent. Laterally to SCP, the number of neurons in PPN pars compacta increases. Ventromedially to SCP, the NADPHd-stained neurons are more loosely arranged - PPN pars dissipata. LL - lateral lemniscus. Magnification: x40.*

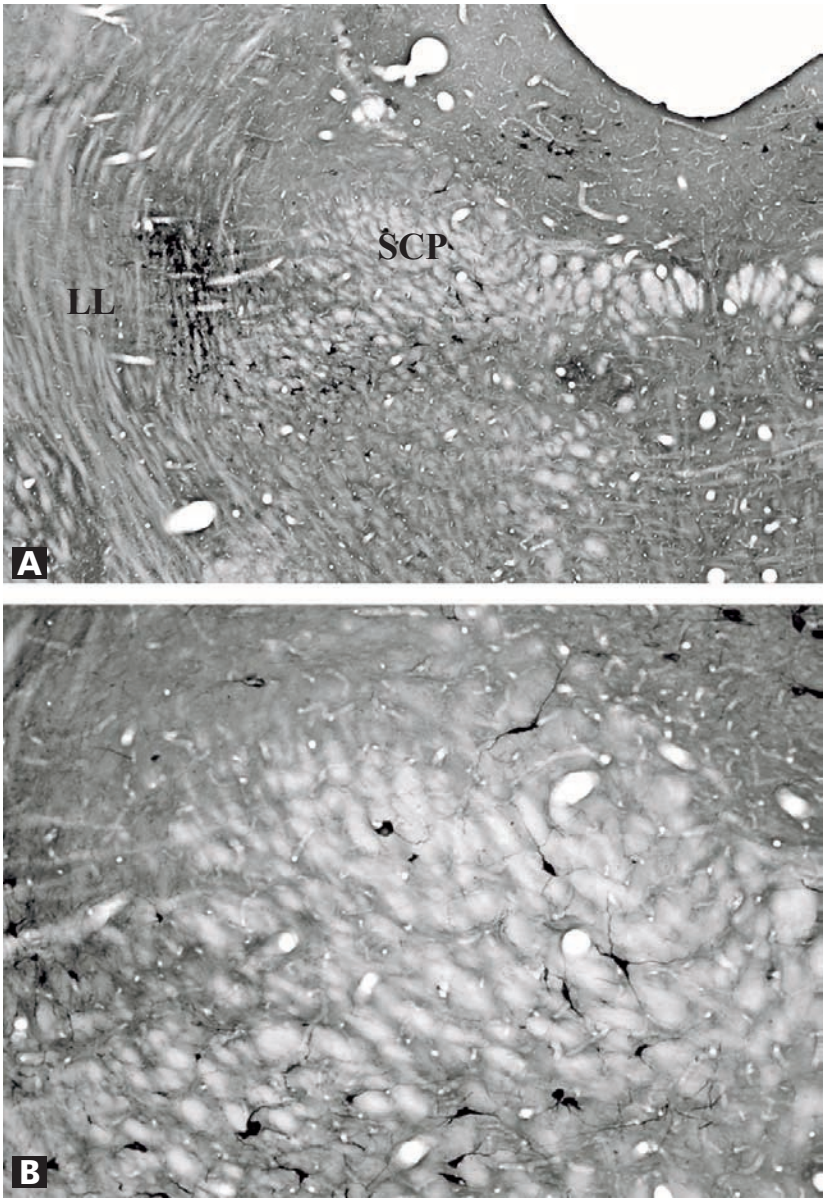

Figure 2. *Section through the caudal mesencephalic tegmentum, rostrally to the section presented on Fig. 1C.* ***(A)*** *LDTN reaches its rostral pole. PPN pars compacta is located laterally to SCP. The PPN pars dissipata is ventrally to SCP.* ***(B)*** *Detail from (A). Upper right - the rostral pole of LDTN, lower left - PPN pars dissipata. Within the fibers of SCP are scattered neurons that might be considered as the most rostral LDTNv, or as a dorsomedial extension of PPN pars dissipata. The NADPHd-stained neurons are medium to large, reaching 40 μm long diameter of the irregular multipolar perikarya. Magnification: A, x40; B, x100.*

tract of the trigeminal nerve. Medially is the dorsal tegmental nucleus of von Gudden. In the latter also occasional ectopic neurons of LDTN are to be found. Ventrolaterally to LDTN, a more loosely arranged group of NADPHd-positive neurons is seen: LDTN-ventral part (LDTNv). Immediately rostrally to this level (Fig. 1B) appear the most caudal PPN neurons, located within the axons of the commissure of lateral lemniscus. Further rostrally (Fig. 1C), the number of PPN neurons increases. The lateral part of the nucleus is composed of more densely arranged neurons, located laterally to the axons of the superior cerebellar peduncle (SCP). These cells might be the homologue of the human PPN *pars compacta*. Ventrally to the SCP fibers is the medial part of PPN. Its cells are more loosely arranged, and apparently correspond to the human PPN *pars dissipata*. Slightly more rostrally (Fig. 2A,B), the number of LDTN neurons starts to diminish and the neurons of PPN *pars dissipata* reach the neurons of LDTNv. In the caudal mesencephalon (Fig. 3A), the SCP axons continue to sink in the tegmentum, accompanied by PPN neurons. PPN *pars compacta* diminishes, and LDTN reaches is rostral pole, consisting of several loosely arranged neurons. At the level of the caudal border of the SCP decussation (Fig. 3B) PPN is represented only by the *pars dissipata*, that approaches the ventral part of the midbrain tegmentum, the region occupied

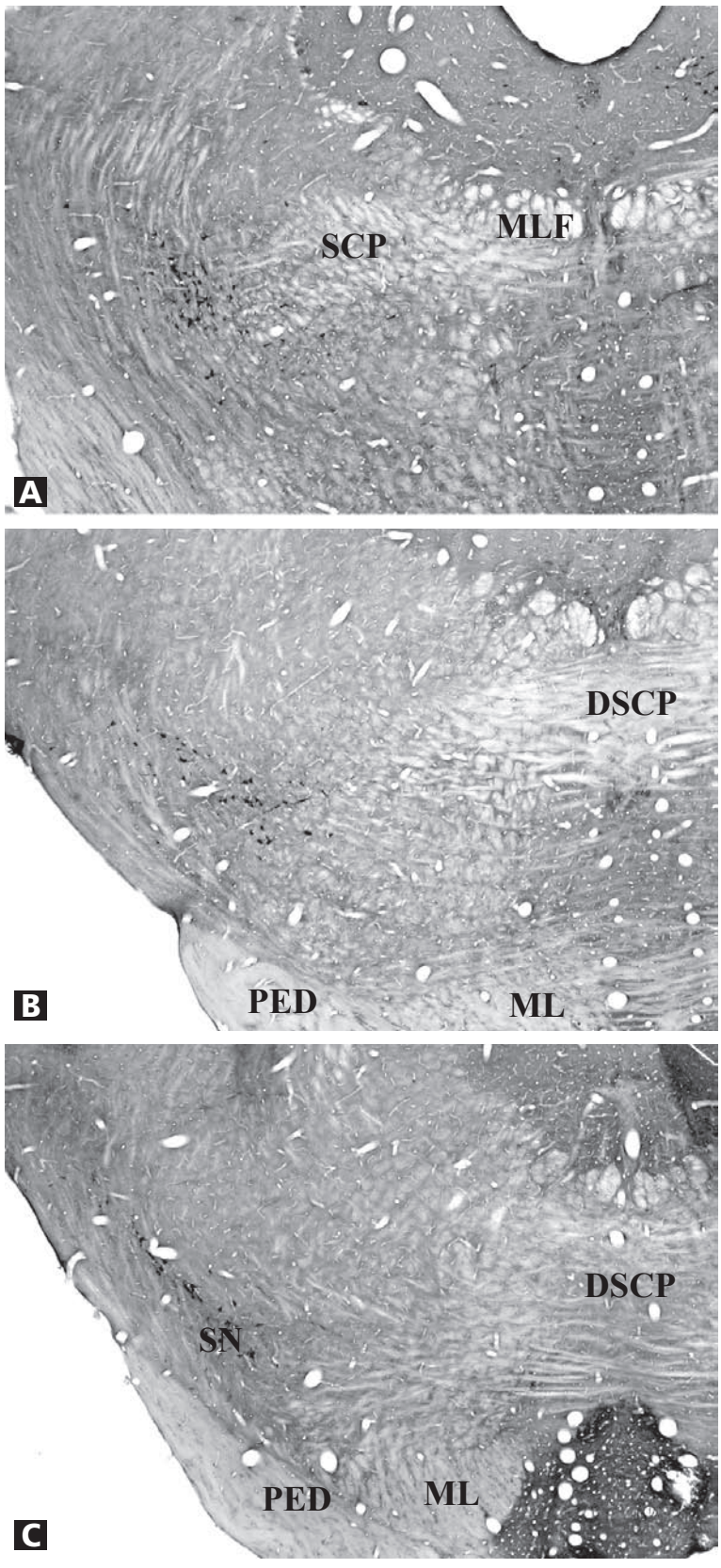

Figure 3. *Serial sections through the caudal mesencephalic tegmentum. The section, presented in* ***(A)*** *is rostral to those on Fig. 2. SCP starts to sink ventromedially. LDTN almost disappears and is presented by several neurons, PPN pars compacta diminsihes. MLF - medial longitudinal fasciculus.* ***(B)*** *Section through the most caudal part of the decussation of SCP (DSCP). PPN sinks deeply in the tegmentum and is presented by pars dissipata. ML, medial lemniscus; PED, cerebral peduncle.* ***(C)*** *The most rostral NADPHd-stained neurons of PPN pars dissipata invade the most caudolateral part of SN. Magnification: x40.*

by the dopaminergic neurons of the retrorubral area (the A8 group of Dahlstrom and Fuxe, 103). At the level of the caudal pole of SN (Fig. 3C) the most rostroventral PPN neurons are to be found. They abut the dorsal border of SN, and some even invade its territory.

The co-localization of the neuropeptide substance P in the ascending cholinergic reticular system was observed firstly by Vincent *et al* (104), and the co-localization with other peptides was also repeatedly confirmed (40,97,105-108). Approximately 30% of the cholinergic neurons in PPN and LDTN contain substance P, as well as corticotropin-releasing factor, bombesin/gastrin-releasing peptide, and atriopeptin. Recently, Kohlmeier *et al* (109) detected a substance P co-localization also in the PPN cholinergic neurons with descending connections.

The widely distributed through CNS calcium-binding proteins are present also in PPN and LDTN, but their co-localization in the cholinergic neurons differs significantly. In the squirrel monkey PPN, Cote and Parent (34) compared the distribution of cholinergic and calbindin D28k-immunoreactive neurons. The latter are more sparcely distributed and are smaller than the cholinergic neurons. No double-stained neurons were found, unlike the magnocellular basal nucleus of Meynert (Ch4 group of Mesulam), where 60% of the cholinergic cells display calbindin immunoreactivity. Geula *et al* (84) expanded this investigation, comparing the findings in rat, monkey, baboon and human. None of the cholinergic neurons in PPN and LDTN in any of the species examined were found to be calbindin-positive, unlike the Ch4 neurons. On the other hand, another calcium-binding protein, the calretinin is present in 8% of the cholinergic neurons in the monkey PPN and LDTN (110), and 12% of the calretinin-positive neurons are immunoreactive also for calbindin D28k, but these neurons are apparently non-cholinergic. Surprisingly, there are only very few and inconclusive data on the presence of a third calcium-binding protein (parvalbumin) in PPN. This lack (or paucity) of calcium-binding proteins in the cholinergic/NO-producing neurons may allow NOS in these cells to respond efficiently to increases in intracellular calcium (73). The neurons of human PPN and LDTNv are immunoreactive for corticotropin-releasing hormone (111).

NORMAL DEVELOPMENT AND AGING

Terada *et al* (112) studied the immunohistochemical localization of NOS in the developing rat brain. A few NOS-positive neurons in PPN and LDTN were first detected by E15, and the distribution pattern was fundamentally completed by E19. The neurons in PPN and LDTN stained very intensely as in adult rats. Skinner *et al* (99) used NADPHd histochemistry to localize cholinergic neurons in PPN of neonatal and adult rats. Measurements of perikaryal areas revealed an average area of approximately 200 μm^2 at birth, followed by a strong and rapid increase to 500 μm^2 by 2 weeks of age. Thereafter, there was a decrease in cell area so that by 5 weeks of age, the neurons had attained their adult size of about 300 μm^2. Kobayashi *et al* (113) reinvestigated these strange developmental changes and found that the transient hypertrophy was limited to cholinergic neurons, whilst non-cholinergic cells did not change significantly in size across this period. Carden *et al* (114) examined the postnatal development of the cholinergic innervation of the dorsal lateral geniculate nucleus in the cat. They found that the PPN neurons express ChAT at birth but the axons in the geniculate nucleus do not express ChAT until the end of the first postnatal week and cholinergic synaptic contacts are to be observed as early as the second week. The number of ChAT-positive axons increases slowly and reaches adult levels by 8^{th} postnatal week. Also in the cat, Kaiya *et al* (115) present somewhat different results, concerning the maturation of PPN projection axons. They established that although cholinergic neurons in PPN are present at birth, cholinergic fibers in the suprageniculate thalamic nucleus are present only after 7^{th} postnatal day, and they almost reach the adult level by postnatal day 28.

The volume of PPN and LTD and the number of NADPHd-positive neurons remained unaltered with advancing age. However, the cross-sectional area, maximum diameter and staining intensity of NADPHd-positive perikarya in this region are reduced in 26-month-old rats compared with 3-month-old rats (116). Furthermore, morphological alterations of the dendrites and a significant reduction in dendritic length are observed with aging, suggesting a mild, but continuous regression of the dendritic tree of the rat PPN and LDTN in normal aging (117). There is a moderate age-related loss of cholinergic neurons in the human PPN, and the cell loss is not linear (118). On Fig. 4A we present a NADPHd-stained neuron in the PPN of a young, 3-month-old rat. From the irregularly shaped perikaryon robust dendritic trunks emerge, that are free of spines and are usually only slightly varicose - typical isodendrites of the brainstem reticular core. The secondary dendrites are thin and varicose. In the aged 26-month-old rats (Fig. 4B) many neuronal perikarya are hypertrophic, with amputated dendrites, which often display swellings too.

AFFERENT CONNECTIONS OF PPN

From the cerebral cortex

The cerebral cortex is a source of moderate projection to PPN (119-125). The authors unanimously agree that it arises from the frontal lobe, and the projection is exclusively ipsilateral. In the monkey it arises from the primary motor cortex (area 4), whilst in the cat the premotor cortex (area 6) also gives rise to cortico-PPN axons. In the rat, the medial prefrontal cortex projects to the PPN (123,124) as well. Only recently a painstaking study concentrated on cortico-PPN projection

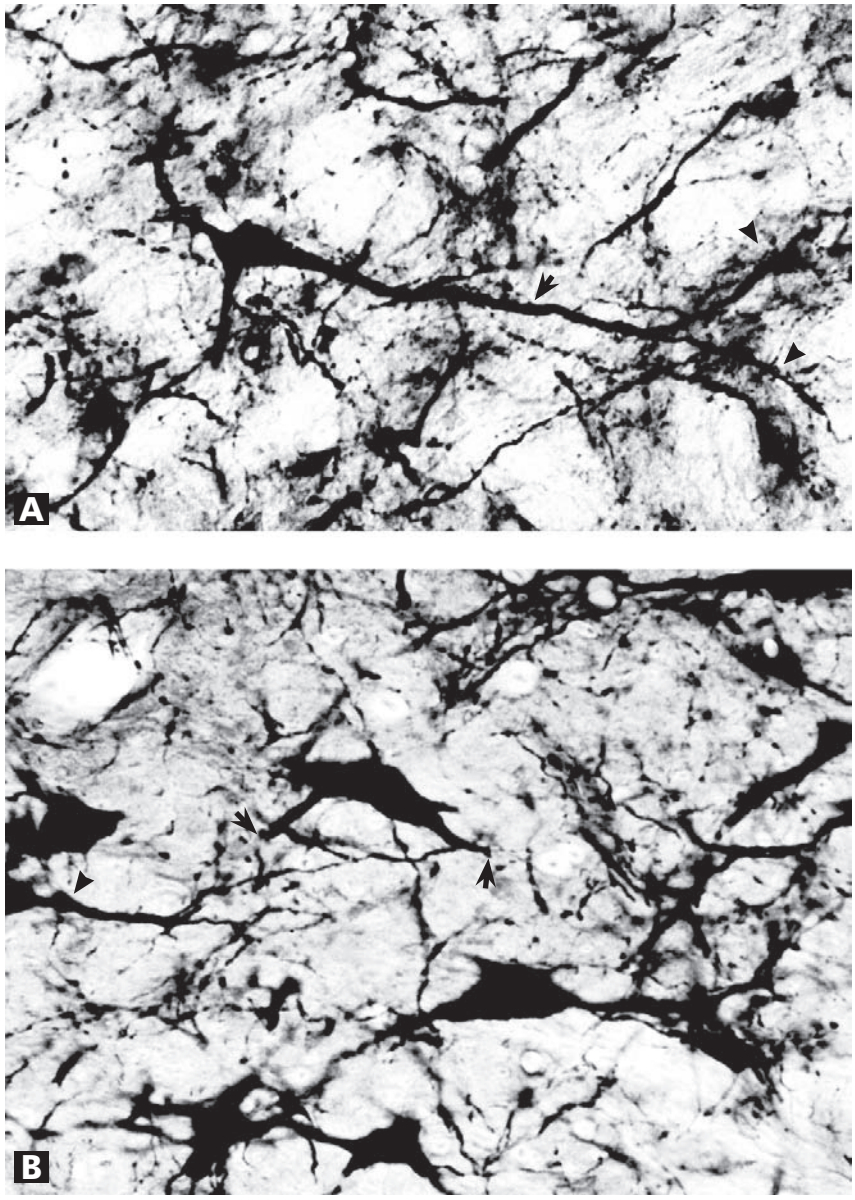

Figure 4. *NADPH-d-positive neuron in PPN pars dissipata of 3-month-old (A) and 26-month-old (B) rats.* ***(A)*** *The arrow points to straight primary dendrite and the arrowhead - to the secondary dendritic branches.* ***(B)*** *The arrows point to amputation stumps of the primary dendrites and the arrowhead shows to the local swelling of the dendrite. Magnification: x400.*

appeared (125). In the monkey, the corticotegmental inputs from orofacial, forelimb, and hindlimb representations of the primary motor cortex tend to be arranged orderly from medially to laterally in PPN, although the distribution areas overlap. The input zones from distal representations of the forelimb and hindlimb regions of the primary motor cortex are located medially to those from the proximal ones, and again an overlap is present. The terminal zones from the forelimb regions of the primary motor cortex, the supplementary and presupplementary motor areas, and of the premotor cortex overlap largely in the mediolaterally medial aspect of PPN, whilst the cortico-PPN input from the frontal eye field is scattered over PPN (125). The cortico-PPN projection is glutamatergic, excitatory, similar to all efferent connections of the cerebral cortex (reviewed in 12).

From the basal ganglia

Although the neostriatum (caudate nucleus, putamen and nucleus accumbens) is functionally closely related to PPN (14,126), there is no monosynaptic connection between the neostriatum (STR) and PPN. However, there is a very strong bisynaptic connection, the second neuron being located in the globus pallidus (pallidum, PAL) and in SN *pars reticulata* (reviewed in 11,12,127-130). There is a single report on monosynaptic striato-PPN connection (131). It arises from the rostral core of the nucleus accumbens (ventral, “limbic”

striatum), and from the ventromedial part of its caudal shell, and reaches the "midbrain extrapyramidal area" of Rye *et al* (15). For decades there was a firm belief that the lateral segment of the globus pallidus (PAL externum, PAL.e) emits only one but a very mighty efferent connection: to the subthalamic nucleus (STN) (reviewed in 11,12,127-129,132). It is presently known that PAL.e is a multiefferent structure, innervating the medial pallidal segment (PAL internum, PAL.i), SN *pars reticulata*, STR, and reticular thalamic nucleus. A faint connection reaches the ipsilateral PPN in cats and rats (15,133-135). However, Sato *et al* (136) were unable to trace axons from PAL.e to PPN in the monkey.

Far more significant is the afferent connection to PPN from the PAL.i, represented in the submammalian species by the entopeduncular nucleus (4,62,122,137-147). There are two types of projection neurons in the primate PAL.i (145). The abundant and centrally located type I neurons give rise to a long axonal branch that descends directly to the PPN, where it arborizes discretely. The axons of these cells arborize profusely and innervate also various thalamic nuclei (145,147). It was a classical belief that the projection neurons of PAL.i innervate ispsilateral thalamic and brainstem nuclei (reviewed in 12,127). Recently, however, Parent *et al* (62) demonstrated that in the monkey there are also pallidal axons that branch ipsilaterally as well as contralaterally to the thalamus and brainstem. They could play a crucial role in the functional organization of primate basal ganglia. Virtually all pallidofugal fibers course through Forel's field H, on their way to the thalamus and brainstem, although recent results (147) suggest that the separation of the ansa lenticularis and the lenticular fasciculus as distinct anatomical entities (as commonly believed) is largely overestimated. The pallidal endings in PPN, observed by Shink *et al* (144) are large boutons, containing pleomorphic, mainly elongated vesicles. They terminate by means of symmetric synaptic specializations upon the perikarya and proximal dendrites of the PPN neurons. They are practically identical with the pallidal endings in the cat thalamus (148), and in STN of cat and monkey (149,150). These ultrastructural features of inhibitory terminals correspond closely with the unequivocal data demonstrating that the neurons in both pallidal segments utilize GABA as neurotransmitter (11,12,127,128,151 and refs. therein). Recently Kha *et al* (152) suggested that the entopeduncular nucleus in the rat contains also cholinergic neurons. However, we feel that such neurons are most probably a "diaspora" from the cholinergic Ch4 group of Mesulam (see below, also ref. 136).

The basal forebrain is another area projecting to PPN (124,131,153,154). Injections of WGA-HRP in the cat PPN led to retrograde labeling of a moderate number of neurons in the lateral part of the ventral PAL, substantia innominata and preoptic area, only few of which displayed ChAT immunoreactivity. In monkeys, some axons, innervating PPN emit collaterals also to the reticular thalamic nucleus (153). In the basal forebrain of rats, the neurons projecting to PPN are usually segregated from the cortically projecting cholinergic neurons. Neurons of basal forebrain almost never project to the brainstem and only rarely have axon collaterals projecting to both cortex and brainstem (154).

The classical targets of the axons of neurons in STN are PAL.i and SN *pars reticulata* (12,127,128,151,155). Along the heavy innervation of these two structures, STN innervates with ascending axons PAL.e and STR, and its most caudal target is PPN, which receives a more modest projection (63,122,146,156-163). According to Parent and Smith (160), the projection from SN to PPN is at least 5-6 times stronger (see below). The STN axons branch significantly (155), but Takada *et al* (161) proposed that the STN-PPN projection arises from a separate neuronal type that does not project to the basal ganglia. The transmitter of STN efferent neurons was unknown, but the clinical speculations suggested, that it should be GABA (164). Therefore, the data that the STN neurons are glutamatergic (165,166), and that the STN-PPN projection is excitatory (167), were unexpected. Presently, the glutamatergic excitatory nature of the STN projection neurons is firmly established (168-172 and refs. therein).

One of the most significant afferent projection to PPN, rivaling the PAL.i-PPN connection, arises in SN (20,64,122,124,173-180). The SN-PPN connection arises in SN *pars reticulata*. Its cells are not pigmented (reviewed in 181) and utilize GABA as a transmitter (20,151,182,183). Thus, this pathway is GABAergic, inhibitory (124,184-189). The projection is bilateral but mainly ipsilateral (20,177,178,190). The neurons projecting to PPN in the monkey are scattered throughout the mediolateral extension of SN *pars reticulata*, but are more commonly located at middle to caudal levels (177). Garcia-Rill *et al* (191) established that slightly less than 10% of the SN neurons send their axons to PPN. A large proportion of SN *pars reticulata* (up to 60%) project collaterals to both thalamus and PPN (11,12,190,192). The most comprehensive investigation of the SN-PPN connection is carried out by Grofova and Zhou (64). They examined the projection by means of anterograde transport of *Phaseolus vulgaris* leucoagglutinin, combining it with immunohistochemistry and electron microscopy. According to them (64), although a small subpopulation of cholinergic neurons in PPN receives a direct synaptic input from SN, the primary target of the nigrotegmental fibers are the glutamatergic cells, located mainly in PPN *pars dissipata*. The SN-PPN boutons contain pleomorphic synaptic vesicles and terminate by means of symmetric specializations mainly upon proximal dendrites (20,64), e.g., they share common features with the pallidal terminals, as discussed above. Using the highly sensitive tracer biotynilated dextran amine, we

(193, in preparation) found a heavy bilateral connection from SN pars reticulata to PPN, but also a faint, ipsilateral connection following selective injections of the tracer in SN *pars compacta*. Thus, it is possible that the SN-PPN connection is dual, and along to the heavy GABAergic pathway, there is also a discrete dopaminergic projection.

From the limbic system, hypothalamus and zona incerta

Heavy projection to the cat's PPN from the medial division of the central amygdaloid nucleus was described by Moon Edley and Graybiel (122). The bed nuclei of the anterior commissure and of the stria terminalis project heavily to PPN in cats (153). The central nucleus of amygdala and the bed nucleus of stria terminalis project mainly to PPN, and to a lesser extent - to LDTN, whilst the lateral habenula projects mainly to LDTN (124). According to Steininger *et al* (143), the latter structure is a major source of afferent input to the "midbrain extrapyramidal area" of PPN. Several hypothalamic areas project to PPN. A heavy projection from the lateral hypothalamus was described by Semba and Fibiger (124) and Steininger *et al* (143). The descending axons of the posterior hypothalamic nucleus innervate more substantially LDTN than PPN (194). The multiefferent histaminergic neurons of the tuberomammillary nucleus innervate significantly the mesopontine tegmentum (195). Also, the broadly projecting hypocretin (orexin)-containing neurons in the dorsolateral, perifornical hypothalamus innervate LDTN and PPN by means of long, thick axons, with numerous boutons (196). Such PPN axons have overlapping distribution with noradrenergic axons (197). The sexually dimorphic areas, and especially the lateral one, project to PPN (198). Recently Heise and Mitrofanis (199) described a significant input to PPN from *zona incerta*. The projection is glutamatergic, excitatory.

From the cerebellum

Steininger *et al* (143) pointed out that the cerebello-PPN axons in the rat arise in the dentate and interpositus nuclei. The cerebello-PPN projection in the squirrel monkey was investigated by Hazrati and Parent (142). This connection is carried out by fine collaterals of the cerebellothalamic fibers, coursing in SCP. These collaterals arborize profusely within entire rostrocaudal extent of PPN. The cerebellofugal axons are long, slightly varicose and brake off into numerous shorter and thinner fibers whose terminal portions consist of a few large varicosities that are often closely apposed to dendrites and perikarya of PPN neurons. Some of the latter neurons are cholinergic. The ultrastructural analysis reveals that synapses formed by the cerebellar axons are of asymmetric type and occur predominantly on dendrites. The cerebello-PPN connection is excitatory, glutamatergic (200).

From the brainstem

Generally, the PPN afferents from the brainstem are less elucidated, and there are only few straightford studies. Moon Edley and Graybiel (122) briefly mentioned afferents to PPN in the cat from the RF nuclei pontis oralis, pontis caudalis, and parvocellularis, as well as few cells in the medullary reticular formation, the nucleus of the solitary tract, and the medial vestibular nucleus. Interestingly, in some cases Moon Edley and Graybiel noticed also heavy retrograde labeling of the superior olivary complex, but we feel that the latter projection should be verified. From the periaqueductal gray of the rat, a diffuse fiber bundle descends to the reticular formation, including PPN and LDTN (201). Recently the deep mesencephalic nucleus in the rat was identified as a significant source of afferent input to PPN (202).

By means of dopamine-β-hydroxylase immunohistochemical staining (197,203) noradrenergic axons from LC and nucleus subcoeruleus are directly traced to the adjacent LDTN and PPN, but it is still unclear if the more caudally located noradrenergic neurons also participate in the innervation of the cholinergic RF. The serotoninergic raphe nuclei project to the cholinergic RF (204-209). The dorsal raphe nucleus projects mainly to PPN (204,206,207), whilst the median raphe nucleus sends its axons mainly to LDTN (208). Not only unmyelinated but also myelinated serotoninergic axons are present in PPN and LDTN (205). However, the numerous serotoninergic terminals only occasionally made synaptic contacts with the cell bodies and proximal dendrites of the cholinergic neurons. During rat development, the serotoninergic responses in PPN switch from both excitatory and inhibitory to almost purely inhibitory at postnatal day 17 (209).

From the spinal cord

Inputs from the cervical and lumbar segments of the spinal cord to PPN have been shown in the rat (210,211) but very little is known about such a projection in primates (reviewed in 212).

EFFERENT CONNECTIONS OF PPN

To the thalamus

The most significant efferent projection of PPN is destined to the thalamus, reaching practically all thalamic nuclei (5,15,53,98,213-220). In the rat it has been estimated that 60% of cholinergic PPN neurons project to the thalamus, and that 90% of PPN inputs to the thalamus are cholinergic (221). Very similar results were reported also for LDTN (222). According to Steriade *et al* (98), associational and diffusely cortically projecting thalamic nuclei receive considerably stronger (3-8 times) projection from the cholinergic RF than the specific relay thalamic nuclei. There are detailed studies

on the projection to the ventrobasal thalamic complex (94), to the anterior thalamic nuclei (222), to the intralaminar nuclei (223,224), to the supragenicular nucleus (225), to the reticular thalamic nucleus (226-228), to the parafascicular nucleus (229), and to *zona incerta* (198,228). Almost all studies report a bilateral, mainly ipsilateral connection (see especially 94,222). It is already very well known that the PPN-thalamic connection is cholinergic, but there is also a smaller, non-cholinergic connection - up to 27% of all PPN neurons, projecting to the thalamus (94,223). The data on the mode of termination of PPN terminals in the thalamus are equivocal, and await further corroboration. Isaacson and Tanaka (224) observed the cholinergic PPN terminals in the canine *centrum medianum* and *nucleus parafascicularis* to arise from nonmyelinated axons. The terminals are small (0.5-0.7 μm) or medium-sized (up to 1.4 μm). The smaller terminals establish symmetrical or slightly asymmetrical synapses upon small dendritic profiles, and - strangely enough - the larger terminals contain pleomorphic vesicles and contact cell somata and proximal dendrites. Kuroda and Price (230) found that the terminals of the cholinergic RF in the dorsomedial thalamic nucleus are mostly large boutons (2-4.5 μm), with asymmetric synaptic specializations, and contain round vesicles - typical features of excitatory terminals. This is to be expected since such terminal type was encountered previously in the basal ganglia (see below). Whilst Kuroda and Price (230) regularly found the PPN boutons participating in the thalamic synaptic glomeruli, Hallanger *et al* (231) point out that the cholinergic terminals contact their targets in the extraglomerular neuropil. Moreover, the latter authors found also cholinergic terminals, participating in symmetric (presumably inibitory) synaptic specializations. Two recent investigations (229,232) reported that the PPN terminals in the thalamus are small with round vesicles. In addition, Oda *et al* (232) found PPN-thalamic symmetrical synapses.

To the cerebral cortex

The cerebral cortex is profusely innervated by cholinergic axons. It appears that the great majority of these fibers originates from the Mesulam's Ch4 group in the basal magnocellular nucleus of Meynert (22,31,233-236), while the corticopetal fibers arising in PPN are very scant. The anterograde tracing studies were practically negative (122,214,237), and according to Woolf *et al* (216) pontomesencephalic lesions produce no changes in the density of ChAT-immunoreactive axons. The retrograde tracing studies were more successful, and a tegmentocortical connection to the frontal lobe was briefly mentioned by Porrino and Goldman-Rakic (238), Saper and Loewy (239), Sloniewski *et al* (240), Newman and Liu (241). Following injections of fluorescent dyes in the motor cortex, we (240) observed few weakly fluorescing neurons in the ipsilateral PPN, that suggests that only a small number of terminal collateral branches of PPN axons reach the cerebral cortex. Approximately 10% of the PPN neurons innervating the reticular thalamic nucleus send collaterals to the frontal cortex (226). A projection from PPN and LDTN to area 17 of the visual cortex in the cat was traced by Higo *et al* (242). However, the latter authors consider peculiar cell populations in these nuclei. They describe that the cortically projecting neurons of PPN are exclusively noradrenergic, and in LDT about 20% of the corticopetal neurons are cholinergic, while the remaining neurons are noradrenergic. The unclear boundaries of the cholinergic and noradrenergic cell populations in the cat's dorsal pontomesencephalic tegmentum were reviewed above, hence the false delineations in the study of Higo *et al* (242).

To the basal ganglia

Similar to the corticopetal connections, the anterograde tracing studies of PPN afferents to STR were largely unsuccessful. Moon Edley and Graybiel (123) could not trace a single autoradiographically labeled axon on striatal territory, and we (237), by means of the most effective modifications of the Nauta and Fink-Heimer techniques, traced only very scant silver impregnated degenerating axons to the caudate nucleus and putamen following an electrolytic lesion of PPN in cats. Again, Woolf *et al* (216) found no decrease in the ChAT immunoreactivity in STR following a destruction of pontomesencephalic tegmentum. In primates, Parent *et al* (243) found numerous striatal afferent cells, located bilaterally in PPN. However, more recent investigations of Parent (217) demonstrated a rather moderate PPN-STR connection in the monkey. *Phaseolus vulgaris* leucoagglutinin-labeled fibers were traced as scattered axons in STR. They are abound in the peripallidal and ventral portions of the putamen, are more sparcely distributed in the remaining portion of the putamen as well as in the caudate nucleus, and are virtually absent in the ventral striatum.

On Fig. 5 we present a comparison between the connections of the rat PPN to the thalamus and to STR. In these structures, we injected stereotaxically two fluorescent tracers that are retrogradely transported from PPN terminals to PPN perikarya. In the thalamus we injected Fluoro-Gold, a tracer that by observation by a UV filter exhibits white colored labeled neurons, and in STR we injected Fluororubi, that by observation with a "green" filter exhibits red colored retrogradely labeled neurons. When the intralaminar thalamic nuclei are massively injected with Fluoro-Gold, PPN was labeled literally to the last neuron; the number of labeled neurons in the ipsilateral PPN was enormous (Fig. 5A). The number of retrogradely labeled neurons was significant also in the PPN contralateral to the thalamic injection. Even multiple voluminous injections of Fluororubi in STR lead to a retrograde labeling of only very low number of retrogradely

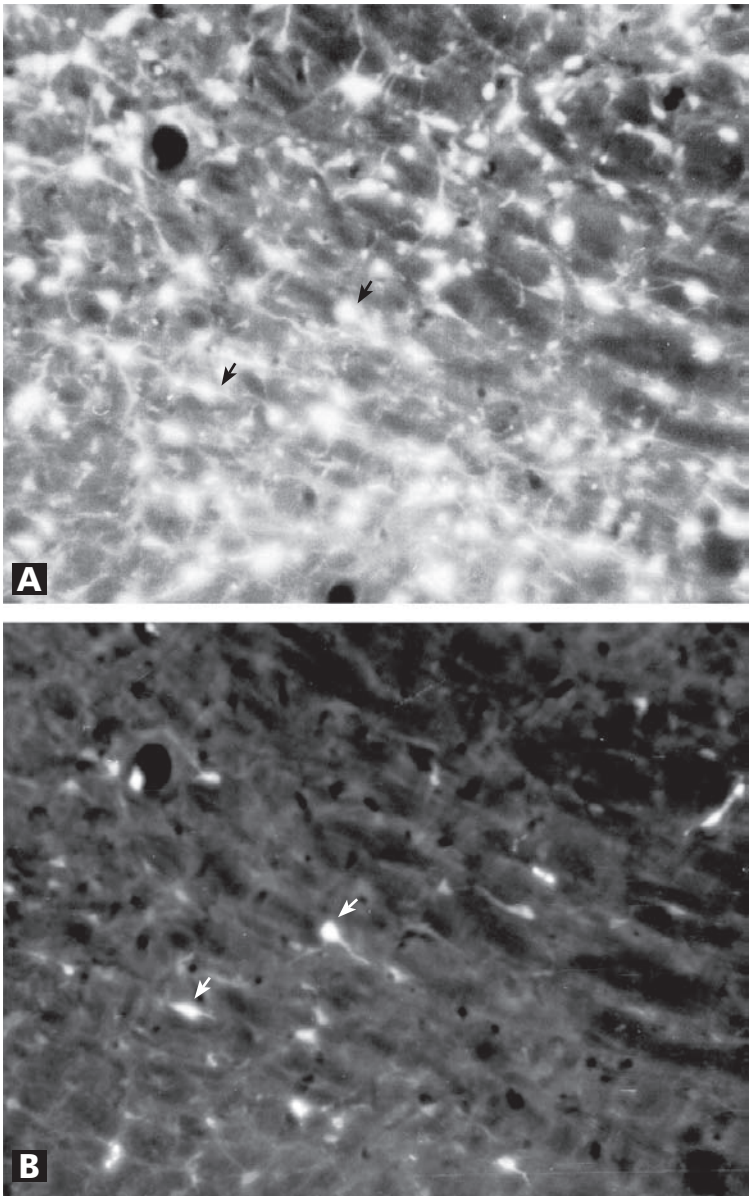

Figure 5. (A) *An enormous number of retrogradely labeled neurons, following an injection of Fluoro-Gold in the thalamus.* ***(B)*** *Only few retrogradely labeled neurons are seen following an injection of Fluororubi in STR. The neurons that are fluorescing by both filters are indicated by arrows. Such cells emit divergent axonal collaterals to both the thalamus and STR. Magnification: x200.*

labeled neurons in the ipsilateral PPN (Fig. 5B), and no retrogradely labeled neurons are to be observed in the contralateral PPN. Only a few PPN neurons emit divergent axonal collaterals to both the thalamus and STR. They are positive at observation by both filters, and on Fig. 5A and 5B are pointed by arrows.

The afferent input to PAL was consecutively established by retrograde transport of HRP (157,158,244), axonal degeneration (237), and histoautoradiography (122). Initially this projection was considered as moderate, and destined almost exclusively to PAL.i, but later studies (217) demonstrated a substantial PPN-PAL connection. The PPN axons arborize profusely in PAL, especially PAL.i. Several bundles of thick fibers oriented dorsolaterally in PAL give rize to thinner fibers that closely surround the soma and proximal dendrites of PAL neurons. Woolf and Butcher (213) claimed that the PPN-PAL pathway in the rat is cholinergic, but the group of Wainer reported that this tract arises from the non-cholinergic PPN cells (15,54). In the monkey, about 39% of cells projecting to PAL are cholinergic, and are located predominantly in PPN *pars dissipata* (245). The human PAL receives cholinergic axons not only from the basal forebrain, but also from the mesopontine tegmentum (246).

The basal forebrain (the largest accumulation of cholinergic neurons in CNS), receives an afferent input from PPN and LDTN (54,213,216,247-249). It is estimated to be moderate

(248) to strong (247). This connection is claimed to be largely non-cholinergic (213,214), although Losier and Semba (249) found also a cholinergic projection from PPN. Moreover, they state that approximately 8% of the total cholinergic population in PPN and LDTN of the rat emits axons with divergent collaterals that innervate simultaneously the basal forebrain and the thalamus.

There is a profuse projection from PPN to STN (38,63,122, 150,157,158,216, 217,250,251). The connection is bilateral, but mainly ipsilateral. Some of the PPN terminals in STN are collaterals of PPN efferents to PAL. The PPN-STN fibers are thin and appear to contact several neurons along their course (217). The PPN-STN endings are cholinergic, glutamate-enriched (58). We (250) identified the ultrastructural appearance of the PPN terminal boutons of the cat STN. Such boutons measure 1.5-3 μm, contain round synaptic vesicles and make asymmetrical axodendritic and axosomatic synaptic contacts with large (projection) STN neurons. There are very few contacts with the interneuronal vesicle-containing dendrites, and no contacts with the perikarya of the small STN neurons (putative interneurons).

The most significant PPN efferent connection, following the profuse innervation of the thalamus, is destined to SN, and this is the most prominent afferent connection of SN *pars compacta* (20). The connection is bilateral, predominantly ipsilateral, and arises in both *pars compacta* and *pars dissipata* of PPN, but especially in the latter (38,39,57,59,60-63,122, 158,178,213,216,217,219,237,252-258). The innervation of the ipsilateral SN *pars compacta* is extremely dense throughout its rostrocaudal and mediolateral extent, and the contralateral SN *pars compacta* is also substantially innervated (237). The innervation of SN *pars reticulata* is far more moderate, especially of contralateral one (217,237). In the monkey SN *pars reticulata*, only few PPN axons occur caudally, and their number progressively increases rostrally. These fibers are more abundant medially than laterally in the caudal part, whereas the inverse is observed in the rostral part (217). Within SN, the PPN axons are thin and varicose and often form pericellular baskets (20,57,217). According to Oakman *et al* (259), there is a strict seggregation of the cholinergic neuronal groups that innervate SN and the ventral tegmental area. We (252) presented the first ultrastructural description of the PPN terminals in the cat, providing the morphological criteria for an excitatory connection. The synaptic boutons are large, and contain round synaptic vesicles, measuring 46-48 nm, that are evenly distributed. The PPN boutons perform asymmetrical synapses mainly with larger dendrites, and fairly often contact also the neuronal perikarya. The synaptic active zone is single, relatively long, and is often associated with a row of subsynaptic bodies. Our results were confirmed also by other investigators (62,254,255,260). It is firmly established that the PPN-SN connection is cholinergic (213,216,245,253-256,258). However, there is growing evidence that PPN emits also glutamatergic axons to SN (36,57,59,60,62), and the glutamatergic endings account for 40-60% of PPN terminal boutons in SN (62,261). Thus, SN is excited by three types of PPN axons: cholinergic, glutamatergic, and terminals containing both transmitters. Quite unexpectedly, Charara *et al* (60) reported that 30-40% of the anterogradely labeled PPN terminals in SN and ventral tegmental area in the monkey display immunoreactivity for GABA and, in some cases, form symmetric synapses with dendritic shafts. Thus, they suggest that PPN is also a potential source of GABAergic inhibitory input to SN.

To the limbic system and hypothalamus

Hallanger and Wainer (214) investigated by means of anterograde and retrograde tracing methods the connections of PPN to various limbic structures. They traced PPN axons to the anterior, tuberal and posterior lateral hypothalamus, the dorsal and intermediate lateral septal nuclei, and to the central, medial and basolateral nuclei of the amygdaloid complex. Approximately 20% of the mesopontine cholinergic cells project to the hypothalamus. The vast majority (over 90%) of the connection to the septal nuclei is also cholinergic. On the other hand, Hallanger and Wainer (214) found out that the projection to the amygdala arises mainly from non-cholinergic neurons. There is a cholinergic connection to the suprachiasmatic nucleus from PPN, LDTN, and also - from the parabigeminal nucleus (262). Along the well known connection to the mammillary nuclei from the dorsal and ventral tegmental nuclei of von Gudden, the lateral mammillary nucleus receives also a cholinergic input, arising mainly from LDTN (108). A dense cholinergic innervation of the hypothalamic neurons producing melanin-concentrating hormone (located in the perifornical area), arising in PPN and LDTN was described by Bayer *et al* (263).

To the cerebellum

Along the mighty cholinergic innervation of the archicerebellar cortex from the vestibular nuclei (264,265), the cholinergic neurons of PPN and LDTN are origin of a moderate cerebellopetal connection. According to Woolf and Butcher (266) the projection arising in PPN is stronger than this one arising from LDTN. These authors found a pathway to the cerebellar nuclei, but not to the cerebellar cortex. The pathway to the fastigial nucleus was especially strong (267). Later, a projection to the cerebellar vermal cortex in the cat was also described (268). Jaarsma *et al* (265) present a careful comparison between the cholinergic fibers arising in the vestibular nuclei and in the mesopontine tegmentum. While the fibers from the vestibular nuclei are typical mossy fibers, the fibers originating from PPN, LDTN, the lateral paragigantocellular nucleus, and from the raphe nuclei, are beaded and

terminate in both the cerebellar nuclei and cortex by means of asymmetric synapses upon small and medium-sized dendritic profiles.

To the brainstem

The cholinergic innervation of the superior colliculus in the cat was studied by Hall *et al* (269). This heavy projection arises from PPN but also from the parabigeminal nucleus (Ch8 group of Mesulam, see 23). The latter nucleus innervates the superficial gray layer, whilst the deeper layers are innervated by PPN and LDTN. The PPN fibers terminate in patches. The cholinergic terminals contain densely packed, round vesicles, and form contacts with medium-sized dendrites that exhibit small, but prominent postsynaptic densities (e.g. asymmetric, presumably excitatory synapses). A few PPN terminals contact vesicle containing profiles (most probably - vesicle containing dendrites of interneurons). Hoshino *et al* (270) carried out double labeling experiments, injecting fluorescing tracers in the suprageniculate thalamic nucleus and the superior colliculus, and found out that a considerable population (35% of all labeled neurons in PPN) project by means of bifurcating axons to both the thalamus and the superior colliculus.

It is well known that the cells of the medial habenular nucleus are cholinergic (Ch7 group of Mesulam, see 23) and they are thought to be the source of a cholinergic projection through the fasciculus retroflexus to the interpeduncular nucleus. However, this pathway has reasonably been disputed by Woolf and Butcher (271). These authors claim that the cholinergic innervation of the interpeduncular nucleus derives primarily from PPN and basal forebrain, and the fibers having their origin in the medial habenula, if they exist, constitute a minor portion of the cholinergic input to the interpeduncular nucleus.

Aas *et al* (272) investigated the projection of PPN to the pontine nuclei in the cat by means of retrograde tracing combined with immunohistochemistry. Following injection of WGA-HRP in the pontine nuclei, more than 80% in PPN are also immunoreactive for ChAT. Notably, they found no projection from the cholinergic neurons in the basal forebrain, so that the cholinergic innervation of the pontine nuclei arises exclusively in the brainstem. The projection is significant and covers all parts of the pontine nuclei. According to Aas *et al* (272), all cells in PPN that project to the pontine nuclei, project also to the thalamus. Woolf and Butcher (275) traced PPN axons in the rat to the pontine nuclei, as well as to another important precerebellar station - the inferior olive.

There are several reports on the efferent connections of PPN to other nuclei of RF (179,273-279). Mitani *et al* (273) investigated the cholinergic projections from PPN and LDTN to the pontine gigantocellular tegmental field in the cat. In LDTN such connection arises from 10.2% of the ipsilateral and 3.7% of the contralateral neurons, while the projection from PPN is smaller: 5.2% of the ipsilateral cholinergic cells, and 1.3% from the contralaterally located ones. Woolf and Butcher (275) described cholinergic projections to the monoaminergic nuclei (LC, nuclei raphe dorsalis, and magnus), to the oral and caudal pontine nuclei, as well as to the medullary reticular nuclei. Iwasaki *et al* (278) traced projections from both PPN and LDTN to the region of the pontine RF in the rat that is reciprocally connected with the nucleus praepositus hypoglossi, and is considered to be the preoculomotor structure of horizontal gaze corresponding to the paramedian pontine RF in higher animals. The PPN is a major source of cholinergic axons terminating in the rostral ventrolateral medulla - structure implicated in the regulation of the blood pressure (277). More recently, a cholinergic connection from PPN was traced also to the nucleus magnocellularis in the ventromedial RF of cats, a stimulation of which produces changes in locomotion, muscle tone, heart rate and blood pressure (279). Semba *et al* (280) traced cholinergic, collateralizing projection from PPN and LDTN to the pontine RF and to the thalamus in the rat. The connection is ipsilateral, emitted from 5-21% of the cholinergic neurons, and these neurons represent the majority (45-88%) of neurons projecting to the pontine RF. Woolf and Butcher (275) traced cholinergic axons from PPN to the motor nuclei of the trigeminal, facial and hypoglossal nuclei, as well as to the spinal nucleus of the trigeminal nuclei, and to the vestibular nuclei. By means of transneuronal labeling with pseudorabies virus, Fay and Norgren (281-283) established, that the PPN innervation of the trigeminal, facial and hypoglossal nuclei is a significant component of multineuronal chains that coordinate the activity of the mandibular, oral and lingual movements.

To the spinal cord

There are relatively few data on the direct projections of PPN to the spinal cord (274,275,284-286). According to Rye *et al* (274), the spinally projecting axons of PPN and "midbrain extrapyramidal area" run through the ventromedial part of the tract of Probst. The cholinergic projection to the spinal cord is considerably smaller when compared with the connection to the medullary RF, and the spinal projections originate largely from non-cholinergic neurons of the "midbrain extrapyramidal area". Spann and Grofova (284) found out that the rostrally projecting PPN cells outnumber 5.4 times those projecting to the spinal cord. The spinally projecting neurons are located mainly in PPN *pars dissipata* and represent a separate population of PPN projection neurons. Two reports (275,285) insist that the PPN projection to the spinal cord is entirely non-cholinergic. Lakke (286) found out that the rat LDTN also projects to the spinal cord, and that this connection developes rapidly: as early as the 18th embryonic day the LDTN axons reach the lumbosacral spinal cord.

CONCLUSIONS

PPN and LDTN are crucial brainstem structures. The present review focuses on PPN, and suffice it to say that both nuclei share common morphological and neurochemical characteristics, and their connections are very similar, albeit not identical (125,219,259,287,288). PPN and LDTN possess morphological features of typical RF nuclei, concerning both the somatodendritic morphology (isodendritic neurons of the brainstem core) and the rich afferent and efferent connectivity. The bulk of the PPN neurons are cholinergic, but there are also glutamatergic neurons that may contain either glutamate as a sole transmitter or glutamate as a co-transmitter of acetylcholine. The cholinergic PPN and LDTN neurons use also the gaseous transmitter nitric oxide, being the most prominent nitrergic neurons in CNS. The age-related changes of PPN are relatively mild. There is practically no cell loss, although in very old animals there are certain drastic changes in the PPN perikarya and dendrites. PPN receives a strong afferent input from the basal ganglia: an extremely strong GABAergic inhibitory input from SN, followed by a glutamatergic excitatory input from STN, and a GABAergic input from PAL. If there is some monosynaptic input to PPN from STR, it should be very faint. The excitatory glutamatergic input from the cerebral cortex is moderate and arises mainly from the motor areas. Several limbic and hypothalamic structures project to PPN, most of them being destined also to LDTN. The cerebellar nuclei provide an excitatory input to PPN, and most probably the connection is carried out by collaterals of the cerebellothalamic axons. PPN receives also a serotoninergic input from the more rostrally located raphe nuclei, and a noradrenergic input from LC. There are also afferents from the non-monoaminergic RF nuclei as well as from the spinal cord. The efferent connections of PPN are extremely diverse. The heaviest efferent pathway innervates virtually all thalamic nuclei, and especially - the "non-specific" intralaminar nuclei, that innervate broad areas of the cerebral cortex. There is also a moderate monosynaptic connection to the cerebral cortex of the frontal lobe. All basal ganglia are innervated, and, with the exception of STR, the PPN efferents are distributed bilaterally. The heaviest pathway is destined to the dopaminergic neurons of SN *pars compacta*: this is its most significant afferent input. The innervation of the GABAergic neurons in SN *pars reticulata* is smaller, and is comparable to the innervation of the GABAergic neurons of PAL. There is a relatively moderate pathway to the cerebellum, mainly to the nuclei but also to the vermal cortex. The projection of PPN to the superior colliculus is heavy. PPN projects to the oromotor nuclei of the cranial nerves - both monosynaptically and by means of multisynaptic chains. There is only a moderate direct projection to the spinal cord but PPN sends axons to various supraspinal RF nuclei, thus enhancing its influence on the spinal cord mechanisms. The reviewed connections of PPN suggest that it is a structure involved in the arousal systems, as already promulgated by Moruzzi and Magoun (2), and thereafter repeatedly confirmed. It is implicated also in the disturbances in sleep and wakefulness (6-9,289). The heavy reciprocal connections with all basal ganglia, with the motor cortex, as well as with the cerebellum, the superior colliculus and the supraspinal RF nuclei clearly show that PPN is an important structure for the motor functions of CNS, and indeed it is involved in movement disorders (290,291). In a second part of our review on PPN (212, submitted for publication) we will concentrate on the functional considerations and on the strikingly broad pathology of PPN.

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