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THE PEDUNCULOPONTINE NUCLEUS: Towards a Functional Integration with the Basal Ganglia

Juan Mena-Segovia, Hana M. Ross, Peter J. Magill, and J. Paul Bolam¹

1. INTRODUCTION

The pedunculopontine tegmental nucleus (PPN) is a neurochemically heterogeneous structure located in the rostral brainstem that forms part of two important regulatory systems of behaviour: the *reticular activating system* and the *mesencephalic locomotor region*. The modulation of the activity of the forebrain and the brainstem underlie the PPN's roles in diverse behavioural functions. The long axonal projections of its neurons reach a wide variety of targets, from the frontal cortex to the thoracic segment of the spinal cord. In addition to these projections, the PPN has a high degree of interconnectivity with the basal ganglia. The anatomical, physiological and behavioural evidence of this close relationship suggests that the PPN and the basal ganglia could be considered as part of the same functional circuit. The aim of this chapter is to present some of the information supporting this notion, focusing on the importance of this relationship for a deeper understanding of basal ganglia function.

2. THE CHALLENGES POSED BY CELLULAR DIVERSITY IN THE PPN

The PPN is composed of a wide variety of cell types. The cholinergic cell population, the best described cell group, only accounts for about 50% of the neurons in the PPN (Manaye et al., 1999). The location of this population has traditionally been used to delineate the boundaries of the PPN. Little is known about the other cell types and whether their cell bodies contribute to the boundary of the PPN as clearly as the cholinergic cells do. It is thus apparent that one of the main difficulties in the study of the PPN lies in the definition of the limits of its extent. Clements and Grant (1990), who first demonstrated the presence of glutamatergic cells in the PPN, showed that the limit of the area occupied by these cells does not correspond to the limits of the cells labelled for

¹ MRC Anatomical Neuropharmacology Unit, University of Oxford, Mansfield Road, Oxford, OX1 3TH.

nicotinamide adenine dinucleotide phosphate (NADPH), which also co-express choline acetyltransferase (ChAT), the synthetic enzyme selectively enriched in cholinergic neurons. By tracing the projections from the PPN and labelling the cholinergic cells, Semba and colleagues (1990) observed that some of the projection cells, which were negative for ChAT, were located dorsal to the population that were positive for ChAT. In other words, classical targets of the PPN receive axons from cells that are located outside of the cholinergic cell population of the PPN. Recent functional studies showed that non-cholinergic cells, whether within or dorsal to the PPN (as defined by the location of ChAT-positive neurons), were activated under the same experimental conditions (Mena-Segovia et al., 2004b; Mena-Segovia and Giordano, 2003).

The cellular diversity of the PPN region further complicates the situation and any possible future redefinition of PPN boundaries. Even amongst the cholinergic cells, it has been shown that differences exist in terms of physiology, connectivity, receptor expression, and neurochemistry. For instance, in terms of physiological membrane properties, three classes of cholinergic neurons have been described in brain slices: cells with low-threshold spikes (LTS type), cells with transient outward current (A-current type), and cells with both characteristics (A+LTS type) (Saitoh et al., 2003). With respect to the receptors expressed, one third of the cholinergic cells express α_{1A} adrenoceptors, whereas half of the cells express α_{2A} adrenoceptors (Hou et al., 2002). The connectivity of each cell type in the PPN also varies according to the input: the GABAergic input from the substantia nigra pars reticulata (SNr), for example, innervates a smaller proportion of cholinergic cells than non-cholinergic cells (Grofova and Zhou, 1998). In terms of neurochemistry, cholinergic cells co-express NADPH (Vincent et al., 1983), and, to varying extents, glutamate (Lavoie and Parent, 1994b) and GABA (Jia et al., 2003). Furthermore, some of the cholinergic cells also express calbindin (Cote and Parent, 1992), while a small sub-population co-express calretinin (Fortin and Parent, 1999). Parvalbumin is also expressed in the PPN (Dun et al., 1995), albeit at lower levels, as are enkephalin and substance P, although their levels of expression in cholinergic cells remains to be determined. This list of heterogeneities of the cholinergic cells is not exhaustive, but has far-reaching implications for the functions of the PPN. Furthermore, a similar degree of heterogeneity exists for the non-cholinergic cell groups.

The non-cholinergic components of the PPN have been less well-studied, even though the glutamatergic and GABAergic neurons were identified more than 10 years ago (Clements and Grant, 1990; Ford et al., 1995). A significant number of non-cholinergic neurons are positive for some peptides, and it is likely that there are populations of neurons hitherto undescribed. The lack of a clear definition of the neurochemical natures of the non-cholinergic cells is partly explained by the difficulties in obtaining reliable immunocytochemical signals in the PPN. It is clear, however, that some of these neurons share some characteristics, at least in terms of physiology and connectivity, with the cholinergic cells. Thus, it has been described that approximately 40% of the A-current cell type, and an even higher proportion of LTS and A+LTS cell types, are non-cholinergic (Saitoh et al., 2003). Furthermore, the PPN sends both GABAergic and glutamatergic projections, along with the cholinergic projection, to the subthalamic nucleus (STN; Bevan and Bolam, 1995).

As alluded to above, and despite the large degree of morphological and neurochemical heterogeneity of neuronal populations in the PPN, the physiological characteristics of PPN neurons show a limited number of patterns. *In vitro* studies in brain slices have identified three groups of neurons, one of which is partly made up by

cholinergic cells (60%). This group of cells fires rhythmically and spontaneously (without any distinction between cholinergic or non-cholinergic cells) and has two different characteristics; they possess either short duration action potentials, slow axonal conduction times and high frequencies of discharge, or long action potentials, fast conduction and slow frequencies of discharge. The other two groups consist predominantly of non-rhythmically firing, non-cholinergic neurons (Takakusaki and Kitai, 1997). No other correlation between intrinsic physiological activity and any other neurochemical marker has been described. *In vivo* studies have mainly described two types of activity in PPN neurons; fast, bursting activity and slow, regular activity (Scarnati et al., 1987), both of which are related to cortical activation (Steriade et al., 1990). In addition to these, we have observed tonically-active, fast-firing neurons that do not modify their activity in relation to the activation of the forebrain and cortex (unpublished results). These physiological categories do not represent the entire population of PPN, as some cells remain silent in different conditions (Siegel and McGinty, 1976). Thus, it may be argued that the great cellular diversity in the PPN continues to challenge a ready characterisation of the roles of different PPN neurons in brain function. The questions that arise at this point are whether these physiological characteristics and activity patterns represent different populations of neurons, and whether they are correlated with the wide variety of functions subserved by the PPN.

3. CORRELATION BETWEEN MORPHOLOGY AND PHYSIOLOGY

The problem of cellular heterogeneity of the PPN, coupled with the absence of any apparent sub-nuclei boundaries, is a major issue. The most direct approach to address this is to correlate directly the morphological characteristics, neurochemistry and physiological properties of PPN neurons. The use of juxtacellular recording and labelling allows us to correlate these characteristics at the single-cell level, and thus, to define different sub-groups of cells at different functional levels, including their connections.

Cholinergic neurons have medium to large somata, ranging from 20 to 40 µm in their longest axis. Larger cells tend to be fusiform, triangular or multipolar, and give rise to 3-6 thick primary dendrites, whereas medium-sized cells are round or oval and give rise to 2-3 thinner dendrites (Ichinohe et al., 2000; Rye et al., 1987). In contrast, glutamatergic and GABAergic cells usually have a smaller cell body (10-20 µm) and, in the case of the glutamatergic cells, give rise to 2-4 thin dendrites. Some of the glutamatergic and GABAergic cells, however, have larger cell bodies, and this seems to be in accordance with the co-expression of acetylcholine (Clements and Grant, 1990; Ichinohe et al., 2000; Jia et al., 2003). From this evidence, one can assume that larger cells (>20 µm) are cholinergic and smaller cells (<20 µm) are non-cholinergic, a proposal that is in agreement with our observations of juxtacellular labelling combined with immunofluorescence. Figure 1 shows basal firing rates and responses to cortical activation associated with sensory stimulation (hindpaw pinch) of identified PPN neurons in relation to the dimensions of the cell bodies of the recorded cells. The neurons with larger cell bodies, which are presumably cholinergic (some of which were confirmed to be so by immunofluorescence for ChAT) had slower basal firing rates, and most of them increased their activity in relation to cortical activation, a function closely associated with the reticular activating system. Interestingly, some cells with smaller cell bodies, which had faster basal firing rates, also increased their activity in response to cortical activation.

This could indicate that the function of a population of non-cholinergic cells is related to cortical activation, and therefore, that cholinergic and non-cholinergic cells are involved in common PPN functions. The fact that some neurons in the PPN are not activated in response to the sensory stimulation is also of importance. Whether these cells have a modulatory function within the PPN, or have projections associated with different functions or brain states is still undetermined, but supports the notion of functional heterogeneity in the PPN.

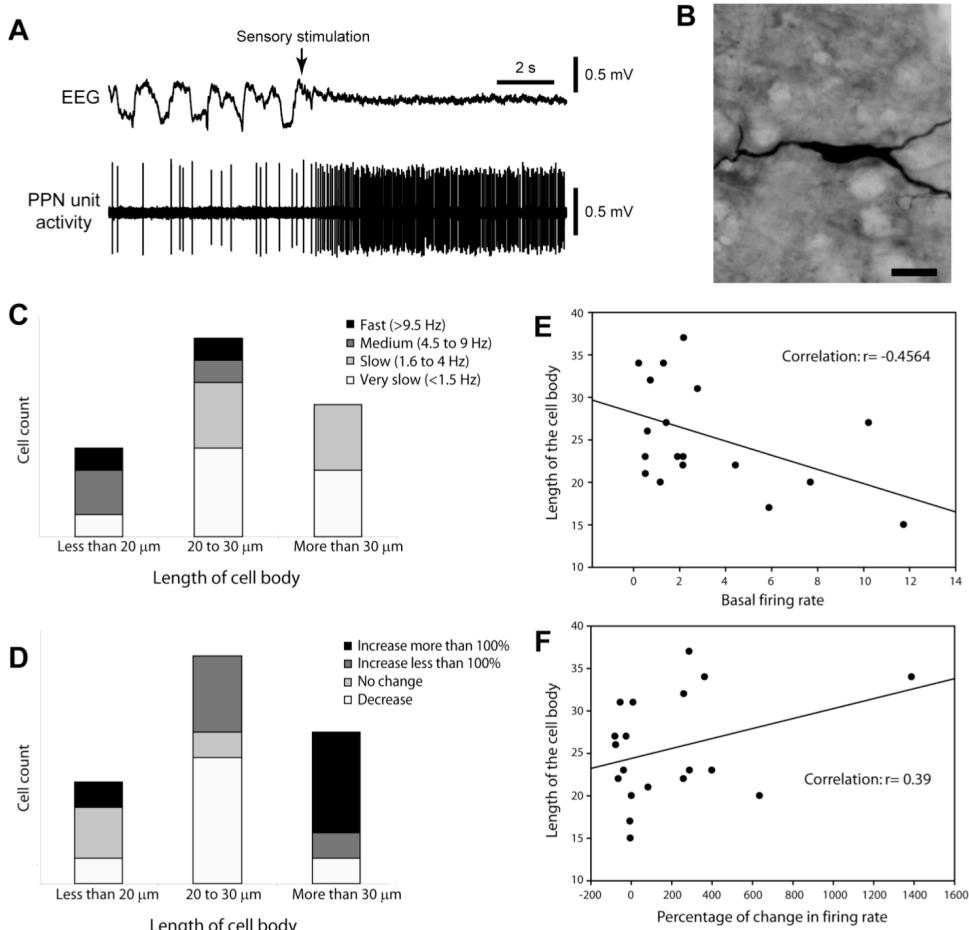


Figure 1. Correlation between physiology and morphology of single PPN neurons recorded and labelled by the juxtacellular method. Extracellular single-unit recordings are used to evaluate the basal firing rate and the change in the firing rate during and after the sensory stimulation; the EEG changes from a slow-wave activity to an activated state characterized by smaller-amplitude, faster-frequency rhythmic activity (A). The recorded cells are then labelled with neurobiotin and their morphology analyzed (B), to correlate the length of the cell body in the longest axis (μm) with the basal firing rate (C, E) and the response to the sensory stimulation (D, F). Scale bar = 20 μm .

4. PHYSIOLOGICAL CHARACTERISTICS OF SINGLE CELLS, AND CORRELATION WITH FUNCTION

The *reticular activating system* (RAS; Moruzzi and Magoun, 1949) is located in the brainstem and projects diffusely to the anterior brain. The tonic activity of neurons of the RAS maintains the waking state and is reinforced by sensory inputs. The PPN contributes to this system by its cholinergic projection, and possible glutamatergic projection, to the intralaminar thalamic nuclei. The PPN is in a position to modulate inputs coming from the spinal cord, and then modify the activity of the forebrain through the generation of fast rhythmic activity (see Figure 1A), producing a global activation of the brain. Steriade and colleagues (1991) have shown the involvement of the mesopontine cholinergic nuclei (which include the PPN) in triggering and maintaining the activation of thalamocortical projections, thereby facilitating the activation of the cortex. Stimulation of the PPN is followed by cortical activation, with a 10-20 msec delay, and typically produces a long-lasting depolarization of thalamocortical cells that is mediated by muscarinic acetylcholine receptors. This depolarization is associated with prolonged gamma oscillations in the EEG (Steriade et al., 1991), that are indicative of the activated or aroused brain state. PPN stimulation is able to produce a decrease in slower rhythms (0-8 Hz) in the cortex, due to the reduction or suppression of the long-lasting hyperpolarizations of thalamic and cortical cells, as well as to increase the power of fast rhythms (24-33 Hz) (Steriade et al., 1993). This typical cortical activation is obtained following either the electrical stimulation of PPN by pulse trains, or chemical stimulation of PPN by administration of cholinergic or glutamatergic agonists, and is blocked by GABA agonists. In agreement with these observations, changes in the activity of neurons in the PPN are associated with the cortical activation that is observed in particular brain states like wakefulness and REM sleep. Moreover, in addition to the disruption of the oscillations of the thalamocortical projections during slow-wave activity, the PPN is also involved in the generation and maintenance of ponto-geniculo-occipital (PGO) waves. PGO waves are phasic field potentials, of 150 µV of amplitude and 100 msec of duration in rats, which originate in the pons and are transferred to the lateral geniculate body and the occipital cortex immediately before the onset of REM sleep. They have been implicated in high-level brain functions, such as sensorimotor integration and learning and memory. PPN neurons contribute to PGO waves by firing in a bursting pattern during the transition from slow-wave activity to the activated state of REM sleep (for a review see Datta, 1997).

In addition to the PPN's role in arousal and the generation of specific thalamic and cortical rhythms as part of the RAS, it is also involved in locomotion (for other PPN functions, see Alderson and Winn's chapter in this book), and indeed forms part of the *mesencephalic locomotor region* (MLR). Stimulation of the MLR produces locomotion, but with a typical delay (2s) between the stimulation and the movement (stepping). This has led to the suggestion that locomotion is gradually recruited rather than immediately induced (Garcia-Rill, 1991). Garcia-Rill and colleagues (for a review see Garcia-Rill et al., 2004) have shown that rapid stimulation of the PPN produces a startle response, but if the stimulation is ramped, it will produce stepping movements. In fact, the effect of this stimulation is closely dependent on the frequency of the stimuli: the PPN, as well as other mesopontine sites, requires a train of stimuli delivered at 40-60 Hz before locomotion develops, in comparison to other locomotor regions, which require lower frequencies. Similarly, the effect of PPN stimulation on its targets (i.e., pontine reticular neurons) also

varies according to the frequency of stimulation (from no response to a prolonged response), even when the duration of the stimulus remained constant (Garcia-Rill et al., 2001). These variable responses may relate to variations in the co-release of neurotransmitters from PPN terminals. In addition to firing rate, firing pattern is associated with different behaviours; thus, tonic firing is related to initiation or termination of locomotion, whereas burst firing is related to the stepping frequency (Garcia-Rill and Skinner, 1988). Furthermore, there may be a topography of physiological characteristics within the PPN; the proportion of bursting cells seems to increase in the dorsal part of the PPN, whereas tonic neurons predominate in the ventral part. The behavioural response to the stimulation of PPN neurons also seems to vary from a locomotor response to a change in the muscle tone, depending on whether the electrode is within the PPN or surrounding its dorsal or ventral borders (Takakusaki et al., 1997).

The fact that the same PPN neuron types exhibit different firing patterns in relation to different tasks (Kobayashi and Isa, 2002) presents a target for future PPN research. Thus, it is possible that a single PPN neuron fires in a bursting pattern in relation to a particular behaviour (e.g., stepping frequency in locomotion, PGO waves), and then a phasic pattern in response to a sensory stimulus. The different firing patterns probably depend on the activation or inhibition conditions, that is, the combination of neurotransmitters released and the combination of receptors expressed in each neuron. A single PPN neuron could thus be involved in a wide variety of functions, its role being dependent on the inputs activated and its activity history at any particular point in time. In addition, the differences in the physiological features of single cells related to different functions, together with the high degree of neurotransmitter co-expression, raises the possibility that PPN cells could differentially release neurotransmitters depending on their firing pattern.

5. RECIPROCAL CONNECTIONS WITH THE BASAL GANGLIA

Anterograde and retrograde studies conducted over the last two decades have demonstrated that a large number of nuclei receive projections from PPN neurons. Almost every functional system has been reported to receive PPN inputs. Targets of the PPN include nuclei in the basal forebrain, such as the lateral hypothalamus and amygdala, the tectum (superior colliculus), zona incerta, all basal ganglia nuclei, and areas located in the brainstem and spinal cord. Not only does the PPN send out widespread projections, it also receives a varied and extensive input from numerous nuclei, and on many occasions, the projections are reciprocal (for comprehensive reviews, refer to Pahapill and Lozano, 2000; Usunoff et al., 2003).

Accordingly, the axonal projections of these cells are long and far-reaching; some axons are collateralized and contact multiple target nuclei, although to what degree still remains unknown. It is generally considered that the majority of PPN neurons have either ascending or descending projections, although some studies (including our own unpublished work) have shown that the axons of PPN neurons collateralise and extend in both directions (Semba et al., 1990). The long, ascending projection axons tend to travel by one of two avenues (Shute and Lewis, 1967): dorsally towards the thalamus, or ventrally towards the basal ganglia and rostral forebrain, with many other targets receiving collaterals along the way. In addition to these ascending projection tracts, there are also descending projections. Targets of descending axons include nuclei of the

brainstem reticular formation, such as the pontis oralis and caudalis (PnO and PnC, respectively; Grofova and Keane, 1991), and even the spinal cord. The varied structures served by these numerous pathways are crucially involved in the control of movement and sensorimotor coordination (basal ganglia), sleep-wake mechanisms and arousal (thalamus), and locomotion and autonomic functions (brainstem and spinal cord). With this in mind, it is not surprising that the PPN has been implicated in such a large repertoire of functions. In particular, the importance of the PPN in diverse functions is exemplified by the complex, reciprocal connections that it forms with the basal ganglia (Figure 2; Mena-Segovia et al., 2004a). In light of the cellular heterogeneity of the PPN, and the difficulty in delineating the boundaries of the PPN, it is perhaps more useful to define the PPN, and its possible sub-territories, on the basis of connectivity.

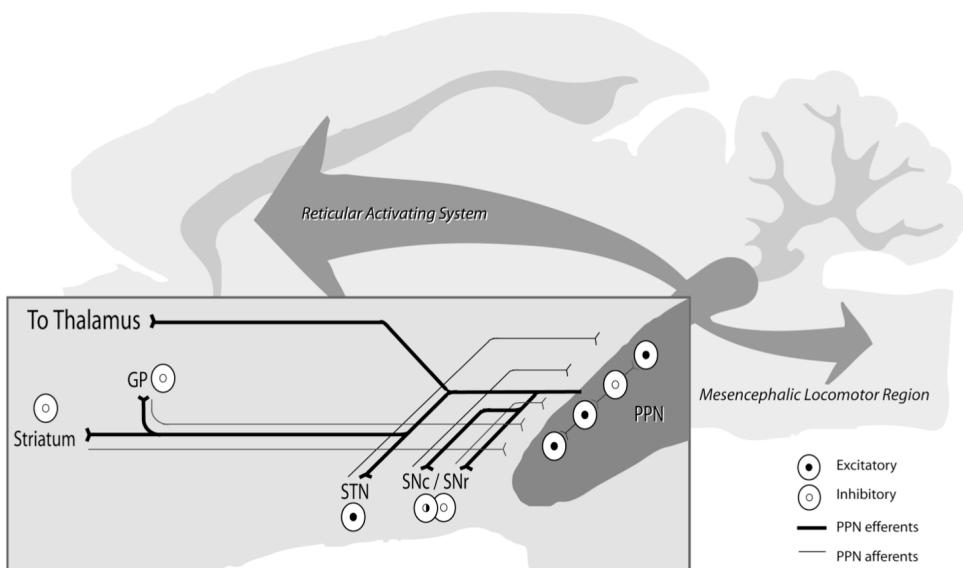


Figure 2. Schematic representation of the main PPN connections. Besides being part of two major behavioral systems, the reticular activating system and the mesencephalic locomotor region, the pedunculopontine nucleus (PPN) is highly interconnected with the basal ganglia. All of the nuclei of the basal ganglia project to, and receive inputs from the PPN. The SNC is represented as a modulatory (excitation/inhibition) connection. SNr, substantia nigra pars reticulata; SNC, substantia nigra pars compacta; STN, subthalamic nucleus; GP, globus pallidus.

5.1. Connections from the PPN to the Basal Ganglia

In light of the increasing evidence of the importance of non-cholinergic neurons in the PPN, several studies have reported that glutamatergic and GABAergic afferents from the PPN innervate different regions in the basal ganglia. The PPN projects to the SNr and the internal segment of the globus pallidus (GPi), as well as to the substantia nigra pars compacta (SNC; Lavoie and Parent, 1994a; Spann and Grofova, 1989; Spann and Grofova, 1991). The neurons giving rise to these projections have been shown to contain glutamate, in addition to expressing ChAT (Lavoie and Parent, 1994a). Lavoie and Parent (1994c) further showed that most PPN neurons projecting to the substantia nigra in the squirrel

monkey are located in the more medial, non-cholinergic area, and that a small proportion are located within the cholinergic cell population; in addition, neurons located in the core of the PPN provide the greatest innervation of the basal ganglia. Similarly, cholinergic terminals in the STN and entopeduncular nucleus (EP, rat equivalent of GPi), which are presumed to be derived from the PPN, contain glutamate (Clarke et al., 1997). At least a component of the mesopontine projection is GABAergic (Bevan and Bolam, 1995). Anterograde tracing, combined with immunolabelling for GABA or glutamate, in primates (Charara et al., 1996), suggests that the PPN has a varied and extensive influence over neurons of the SNc and ventral tegmental area (VTA). Furthermore, dopamine neurons in both regions have been shown to receive input from cholinergic terminals presumed to be derived from the PPN (Bolam et al., 1991; Garzon et al., 1999). We have also shown the existence of glutamatergic and GABAergic projections from the PPN to the VTA at the electron microscopic level using the post-embedding method (Figure 3). Our results showed that 13 of 14 (93%) anterogradely-labelled PPN terminals in the VTA were glutamate immunopositive and completely devoid of GABA immunolabelling (Figure 3A and 3B). In contrast, when the anterograde tracer injection was placed in the area medial to the cholinergic core of the PPN (referred to by some authors as PPN pars dissipata), 5 of 8 (63%) terminals were GABA-immunopositive (Figure 3C and 3D).

5.2. Connections from the Basal Ganglia to the PPN

The basal ganglia output nuclei, SNr and GPi or EP, seem to be the major afferents to the PPN derived from the basal ganglia, providing a prominent GABAergic input to the PPN (Figure 2). Tracing studies combined with immunocytochemistry suggest that nigral GABAergic afferents form synaptic contact mainly with non-cholinergic neurons, some of which have been identified as glutamatergic, and to a lesser extent, the cholinergic neurons (Grofova and Zhou, 1998). These findings are supported by electrophysiological evidence demonstrating an inhibitory effect of the nigral projection, mediated by GABA-A receptors, on the activity of non-cholinergic and cholinergic neurons in the PPN (Saitoh et al., 2003). It is interesting to note that, following injections of tracers both in the SNr in rats and in the GPi of primates, retrogradely labelled neurons in the PPN are sometimes seen to receive input from anterogradely labelled nigral axons, indicating that the interconnections are, at least in part, reciprocal (Grofova and Zhou 1998; Shink et al. 1997). In addition to the output nuclei of the basal ganglia, the STN also provides a prominent, but in this case glutamatergic, innervation of the PPN (Hammond et al., 1983; Steininger et al., 1992).

It should be noted, however, that by virtue of the PPN innervation by the output nuclei, every division of the basal ganglia is in a position, at least indirectly, to influence the activity of neurons in the PPN. Indeed recent functional studies are in support of this (Mena-Segovia et al., 2004b). Although differential inputs and outputs of the PPN in relation to the basal ganglia have been identified, it remains to be established how well the topographical organisation of the cortico-basal ganglia-thalamocortical circuits is maintained at the level of the PPN, or whether the PPN is a major site for the integration of information derived from different functional territories of the basal ganglia (Shink et al., 1997).

5.3. Regulation of the PPN by the Basal Ganglia

The influence of the basal ganglia upon the PPN depends of course on the activity of the direct and indirect pathways. As mentioned above, the major inputs from the basal ganglia are the glutamatergic projection from the STN and the GABAergic projections

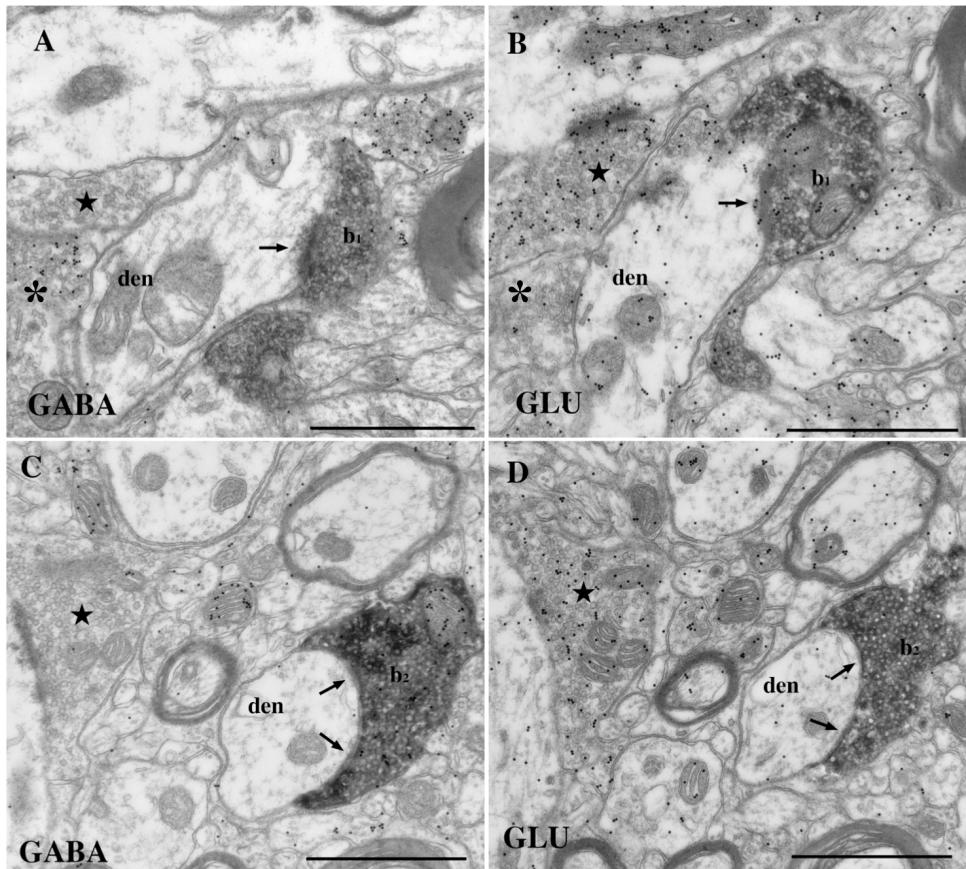


Figure 3. Pairs of electron micrographs of terminals in the VTA that were anterogradely labelled following injections of BDA in the PPN (A, B) and medial to the core of cholinergic neurons in the PPN (C, D). One of each pair of serial sections was immunolabelled to reveal GABA (A, C; GABA) and the other to reveal glutamate (B, D; GLU) using the post-embedding immunogold method. (A, B) The BDA-labelled bouton **b₁**, is forming an asymmetric synapse (arrow) and is associated with a low level of GABA immunolabelling and a high level of glutamate immunolabelling. Note that adjacent to the BDA-labelled terminal, another unlabelled terminal (star) makes asymmetric synaptic contact with a different dendrite (den) and also has low levels of GABA and high levels of glutamate immunolabelling, while the bouton labelled with an asterisk has high levels of GABA and low levels of glutamate immunolabelling. (C, D) The BDA-labelled bouton **b₂**, is in symmetrical synaptic contact (arrows) with a dendrite and is associated with a high level of GABA immunolabelling and a low level of glutamate immunolabelling. Note that adjacent to the BDA-labelled terminal, another unlabelled terminal (star), makes asymmetric synaptic contact with a different dendrite and has low levels of GABA and high levels of glutamate immunolabelling. Scale bars = 1 μm. (Data from Ross, Bevan, et al., unpublished.)

from the SNr and GPi/EP. Both pathways and neurotransmitters have been shown to produce significant effects on PPN function: in Parkinson's disease or its models, in which the basal ganglia output is overactive, there are marked changes in PPN neuron activity. In these conditions the PPN is driven by an increased inhibitory input from the basal ganglia output nuclei and an increased excitatory input from the STN, which is reflected in decreased or increased firing of PPN neurons (for a review see Mena-Segovia et al., 2004a; Pahapill and Lozano, 2000), although the types of cells affected in these ways are unknown. The increased activity of afferents from SNr and STN have also been confirmed in a microdialysis study showing increased extracellular levels of both GABA and glutamate in the PPN following a 6-hydroxydopamine lesion of the SNC (Blanco-Lezcano et al., 2005). These changes have been proposed to underlie the akinesia in Parkinson's disease, which improved following the blockade of GABA-A receptors (Nandi et al., 2002).

6. CONCLUSIONS

The dense interconnections of the PPN and basal ganglia enable the two structures to maintain a close functional relationship that has significant bearing on a wide range of behaviours, including sleep/arousal, locomotion, and posture. Our synthesis of the literature suggests that it is no longer sufficient to view PPN neurons as playing roles in only one of two distinct effector systems, namely the RAS and MLR, but that individual PPN neurons are ideally suited to play central roles in *both* systems. The impact of this hypothesis on our understanding of basal ganglia function is difficult to predict at this time. What is certain, however, is that an appreciation of the functional integration of the PPN and basal ganglia is critical for a better understanding of the roles played by each circuit in behaviour. The cellular heterogeneity present in the PPN indicates that the development of a realistic scheme for functional integration is a major challenge for the future. In meeting this challenge, it is imperative that *correlative* analyses of the physiology, neurochemistry and morphology of the non-cholinergic neurons, as well as the cholinergic neurons, of the PPN are undertaken. Furthermore, it is important that we consider the different functional aspects of the PPN at the level of single *identified* neurons. Using this and other approaches, it will be possible to dissect the influences of the basal ganglia and PPN as they work together as a single 'system', rather than two separate entities.

7. ACKNOWLEDGEMENTS

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