Thalamic innervation of striatal and subthalamic neurons projecting to the rat entopeduncular nucleus

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Abstract

The present study analyses the anatomical arrangement of the projections linking the Wistar rat parafascicular thalamic nucleus (PF) and basal ganglia structures, such as the striatum and the subthalamic nucleus (STN), by using neuroanatomical tract-tracing techniques. Both the thalamostriatal and the striato-entopeduncular projections were topographically organized, and several areas of overlap between identified circuits were noticed, sustaining the existence of up to three separated channels within the Nauta–Mehler loop. Thalamic afferents arising from dorsolateral PF territories are in register with striatofugal neurons located in dorsolateral striatal areas, which in turn project to dorsolateral regions of the entopeduncular nucleus (ENT). Medial ENT regions are innervated by striatal neurons located within medial striatal territories, these neurons being the target for thalamic afferents coming from medial PF areas. Finally, afferents from neurons located in ventrolateral PF areas approached striatal neurons in ventral and lateral striatal territories, which in turn project towards ventral and lateral ENT regions. Efferent STN neurons projecting to ENT were found to be the apparent postsynaptic target for thalamo-subthalamic axons. The thalamo-subthalamic projection was also topographically organized. Medial, central and lateral STN territories are innervated by thalamic neurons located within medial, ventrolateral and dorsolateral PF areas, respectively. Thus, each individual PF subregion projects in a segregated fashion to specific parts of the striato-entopeduncular and subthalamo-entopeduncular systems. These circuits enabled the caudal intralaminar nuclei to modulate basal ganglia output.

Introduction

Thalamostriatal projections rank among one of the most prominent sources of afferents to the striatum. The main sources of thalamic inputs to the striatum are the caudal intralaminar nuclei, namely the centromedian-parafascicular thalamic complex in primates (CM-PF) (Parent *et al.*, 1983; Nakano *et al.*, 1990; Sadikot *et al.*, 1990, 1992a,b; Fenelon *et al.*, 1991; Sidibé & Smith, 1996) or the parafascicular nucleus in rodents (PF) (Féger *et al.*, 1994; Deschênes *et al.*, 1995, 1996; Rudkin & Sadikot, 1999; Gonzalo *et al.*, 2002; Vercelli *et al.*, 2003). Striatal neurons receiving thalamic afferents project to neurons located in the entopeduncular nucleus (ENT), which in turn innervate the caudal intralaminar nuclei by virtue of the Nauta–Mehler loop (Nauta & Mehler, 1966). Thalamic afferents to STN have been reported in rats, cats and primates (Sugimoto & Hattori, 1983; Sugimoto *et al.*, 1983; Royce & Mourey, 1985; Sadikot *et al.*, 1992a; Marini *et al.*, 1999). In rats, PF is known to contain neurons projecting to the striatum as well as neurons projecting to STN (Féger *et al.*, 1994). Furthermore, a subpopulation of PF neurons projects simultaneously to the striatum and to STN, via axon collaterals (Deschênes *et al.*, 1996). The thalamo-subthalamic projections arising from PF are known to be excitatory and contain

glutamate as neurotransmitter (Mouroux & Féger, 1993; Mouroux *et al.*, 1995).

The original pathophysiological model of the basal ganglia implicitly conceived the control of the output nuclei (substantia nigra pars reticulata and ENT or globus pallidus pars interna in primates) as a balance between neuronal activity in the direct and the indirect pathways, leading to decreased firing rate (i.e. movement facilitation) and increased firing rate (i.e. movement inhibition) (Crossman, 1987; Albin *et al.*, 1989; DeLong, 1990). Accordingly, the motor circuit is understood as part of a series of separate loops linking successively the cortex, the striatum, basal ganglia output nuclei, the thalamus and back to the cortex (Alexander *et al.*, 1986; Alexander & Crutcher, 1990). This view was further elaborated (Marsden & Obeso, 1994; Mink, 1996) and has prevailed in many respects until the present time (Mink, 2003). However, recent advances in physiological and anatomical techniques have revealed a more sophisticated organization (reviewed in Bolam *et al.*, 2000; Bar-Gad & Bergman, 2001). Thus, the indirect circuit is actually understood as a neuronal network of its own, directly linked with the cortex, where the STN and globus pallidus pars externa (GPe) and interna (GPi) are reciprocally and tightly interconnected to modulate basal ganglia output (Shink *et al.*, 1996; Joel & Weiner, 1997; Nambu, 2004). In addition, the role of internal or transversal pathways, i.e. circuits connecting the thalamus with the striatum and STN or the GPe-STN-GPi/SNr loop, had not received a great deal of attention until recently (Bolam *et al.*, 2000;

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Obeso *et al.*, 2000; Bevan *et al.*, 2002) but these pathways may serve as an important feed-back function to guarantee stability of basal ganglia neuronal output activity (Obeso *et al.*, 2000; Rodríguez *et al.*, 2003). In this regard, ibotenate-induced unilateral lesion of PF in healthy rats resulted in notorious changes in the metabolic activity of several basal ganglia-related nuclei such as the striatum, GP, ENT, SNr and STN (Bacci *et al.*, 2002). Furthermore, a pathophysiological role for the CM-PF in Parkinson's disease has been suggested (Gonzalo *et al.*, 2002). Thus, in dopamine-depleted rats, the metabolic activity of PF neurons projecting to STN is increased by up to 62% compared to sham-lesioned animals (Orieux *et al.*, 2000). The increased metabolic activity of the output nuclei was reduced to control levels after a thalamic lesion with ibotenic acid in PF (Bacci *et al.*, 2004). Finally, it was recently shown that CM-PF is an important site of nondopaminergic degeneration in Parkinson's disease (Henderson *et al.*, 2000a,b).

We believe the intralaminar nuclei of the thalamus may play a modulatory function of the basal ganglia, particularly with respect to motor control. If so, they should be anatomically organized in a topographically distinct way, capable of simultaneously influencing neuronal activity in both the indirect and direct circuits. The present report investigates the anatomical circuits connecting the caudal intralaminar nuclei with the striatum and STN. Emphasis was placed on the analysis of the topography of thalamostriatal and thalamo-subthalamic projections, together with the identification of several subpopulations of projection neurons presumptively approached by thalamic afferents within targeted structures.

Materials and methods

A total number of 54 female Wistar rats with a body weight ranging from 210 to 260 g were used in this study. Animals were deeply anaesthetized with an intramuscularly injected mixture of four parts of Ketaset® (1% of a solution of ketamine) and three parts of Rompun® (2% of a solution of xylazine). Rats were handled according to the European Council Directive 86/609/EEC as well as in accordance with the Society for Neuroscience Policy on the Use of Animals in Neuroscience Research. Conducted experiments received the approval of the Ethical Committee for Animal Testing of the University of Navarra (ref. 037/2000).

The rats were placed in a stereotaxic frame (David Kopf frame for small animals) and all tracers were injected in a single surgical session. Stereotaxic coordinates were taken from the atlas of Paxinos & Watson (1998). The anterograde tracer biotinylated dextran amine (BDA, Molecular Probes Europe, Leiden, The Netherlands) was iontophoretically delivered in the left parafascicular nucleus (PF, stereotaxic coordinates ranging from bregma -3.8 to -4.4 mm, lateral between 0.6 and 1.6 mm from midline, and 5–6 mm ventral to the pia) as a 10% solution in 0.01 M phosphate buffer (PB), pH 7.25, through a glass micropipette (inner diameter ranging from 25 to 40 μ m) using a positive-pulsed direct current (5 μ A, 7 s on/off). Injection time took approximately 3–5 min and once completed, the micropipette was left in place for 10 min before withdrawal. During removal of the micropipette, the current was reversed in order to minimize tracer uptake through the injection track. Next, 0.5 μ L of a 2% solution in PB of the retrograde tracer cholera toxin subunit β (CTB, List Biological Laboratories, Campbell, CA) were pressure injected through a Hamilton syringe in the ipsilateral substantia nigra reticulata (SNr, stereotaxic coordinates ranging from bregma -5.6 to -6.04 mm, lateral between 1.2 and 2.3 mm from midline, and 7.2–8.2 mm ventral to the pia). Finally, the retrograde tracer Fluoro-Gold (FG, Fluorochrome Inc., Englewood, CA) was iontophoretically delivered in the ipsilateral entopeduncular nucleus (ENT, stereotaxic coordinates ranging

from bregma -2.3 to -2.6 mm, lateral between 2 and 3.1 mm from midline, and 7–7.8 mm ventral to the pia) as a 2% solution in 0.1 M cacodylate buffer, pH 7.3, using the same parameters described for BDA injections.

Animals were anaesthetized with an overdose of 10% chloral hydrate in distilled water 7 days post-injection, and perfused transcardially. The perfusates consisted of a saline Ringer solution followed immediately by 500 mL of a cold fixative solution containing 4% paraformaldehyde, 0.1% glutaraldehyde and 0.2% of saturated picric acid in 0.125 M PB, pH 7.4. After perfusion, the skull was opened and the brain removed and stored in a cryoprotective solution (Rosene *et al.*, 1986) containing 20% glycerin and 2% dimethylsulphoxide in 0.125 M PB, pH 7.4. Frozen coronal sections (40 μ m-thick) were obtained in a sliding microtome and collected in 0.125 M PB, pH 7.4. Material from triple labelling experiments was divided into series of adjacent sections, which were subjected to: (i) Nissl stain, (ii) sequential BDA, CTB and FG immunohistochemistry and (iii) ACh-E stain.

Histology

A quick description of the conducted methodology is given below. More detailed information dealing with histological processing can be obtained elsewhere (Köbber *et al.*, 2000; Lanciego *et al.*, 2000).

The BDA protocol was conducted firstly, by using an ABC solution (ABC kit standard PK-4000, Vector Laboratories, Burlingame, CA) for 90 min at room temperature. Sections were then reacted for 5–10 min in nickel-enhanced diaminobenzidine (DAB-Ni; DAB from Sigma Chemical Co., St. Louis, MO), resulting in a black precipitate. Sections were then incubated in a cocktail solution of primary antisera containing 1 : 2000 goat anti-CTB (List Biological) and 1 : 2000 rabbit anti-FG (Chemicon, Temecula, CA) for 60 h at 4 °C. Next, incubation was continued with a cocktail solution of bridge antisera containing 1 : 50 donkey anti-goat (Nordic Immunological Laboratories, Tilburg, The Netherlands) and 1 : 50 swine anti-rabbit (Dako, Copenhagen, Denmark) for 2 h at room temperature. Sections were then incubated in a peroxidase-anti-peroxidase (PAP) complex raised in goat (1 : 600 goat-PAP, 90 min at room temperature, Nordic) and CTB-containing neurons were finally visualized in brown using a regular DAB solution. Sections were further incubated in a PAP complex raised in rabbit (1 : 600 rabbit-PAP, 90 min at room temperature, Dako) and neurons displaying retrogradely transported FG were finally stained by using Vector® VIP substrate (V-VIP).

Sections were mounted on glass slides using a 2% solution of gelatin (Merck, Darmstadt, Germany) in 0.05 M Tris/HCl pH 7.6, dried at room temperature, quickly dehydrated in toluene and coverslipped with Entellan® (Merck). All antisera used in this procedure were diluted in 0.05 M TBS, pH 8.0, with 0.5% Triton X-100 (Sigma, TBS-Tx). The incubations in the primary antisera were implemented with 2% of bovine serum albumin (BSA, Merck). Extensive washing with 0.05 M TBS-Tx pH 8.0 was carried out throughout the procedure. Several rinsing steps were conducted prior to and after the reactions with the different chromogen solutions.

Data analysis

The accurate localization of injection sites as well as structures displaying tracer was achieved by using a Panasonic digital camera (DMC) for plotting purposes. Briefly, 8–9 rostrocaudal levels of the rat striatum (taken from the series devoted to triple labelling with BDA, CTB and FG) were scanned directly from the microscope (Zeiss Axioscop) by using the DMC camera. During the procedure, ten snapshots covering the area comprising the striatum (final magnification of 100 $\times$, resolution 300 ppi, RGB) were taken. Then, the snapshots were photo-mounted in a single digital file by using Adobe

FIG. 1. Schematic representation of the localization and extent of the injections sites. Top, Fluoro-Gold injection sites into the entopeduncular nucleus; middle, injections of biotinylated dextran amine within the parafascicular nucleus; bottom, deposits of cholera toxin β subunit in the substantia nigra reticulata.

FIG. 2. Schematic representation of the structures displaying tracer at different rostrocaudal levels of the striatum. Striatal neurons projecting to the entopeduncular nucleus or to the substantia nigra reticulata are labelled in purple or in brown, respectively. Each dot represents one single labelled neuron. A grey shaded area represents terminal fields of thalamostriatal afferents. Thalamic afferents coming from medial areas in PF are distributed through medial territories of the striatum (case no. 066, left), while the dorsolateral striatal territories are innervated by thalamic afferents coming from dorsolateral PF areas (case no. 067, right). A similar organization was observed when dealing with the distribution striato-entopeduncular projecting neurons: medial areas of the entopeduncular nucleus are innervated by neurons located in periventricular territories of the striatum, while the striatal neurons targeting dorsolateral areas of the entopeduncular nucleus are mainly located in dorsolateral areas of the striatum.

Photoshop 6.0 software, exported to a vectorial drawing software (Deneba Canvas 5.0) and labelled as layer 1. Subsequently, the structures displaying tracer and the gross striatal boundaries were plotted in a separate layer (named as layer 2). Next, a similar procedure was conducted for scanning both the adjacent Nissl-stained and ACh-E-stained sections (up to ten snapshots, final magnification of 100 $\times$, resolution of 72 ppi, B & W). These digital files were also exported to the former Canvas file (layers 3 and 5) and drawn for cytoarchitectural (layer 4) or chemoarchitectural (layer 6) references. Combining these layers, it is possible to delineate the striatal boundaries accurately. Finally, layers 1, 3 and 5 were deleted and the remaining layers were flattened onto a single, final layer, comprising the plotted structures as well as the striatal delineation. The precise localization of the injection sites was also achieved by using this procedure.

Results

Technical considerations

Results reported here were based on the analysis of nine cases in which the injection sites were confined to their respective target areas, without noticeable tracer leakage along the injection tract. Only those cases in which the three target areas (PF, ENT and SNr) were accurately approached ($n=9$) were considered valid for this study. A schematic representation of the injection sites is shown in Fig. 1.

In all reported cases, restricted tracer deposits were confined within the nuclear boundaries. The total extent of ENT was covered with the

FG-related injection sites. Regarding the PF thalamic nuclei, three extra animals receiving a single BDA injection in medial PF territories were added to our study in order to ensure a complete mapping of the whole extension of the PF nucleus. Finally, the retrograde tracer CTB was placed in medial parts of SNr in all cases comprised in the present study, and therefore data related to striatonigral projections only reflect the distribution of striatal neurons projecting to medial territories of SNr.

Distribution of striatofugal neurons giving rise to the direct pathway

Striatal neurons projecting to ENT were distributed throughout the entire rostrocaudal extent of the striatum. The localization of striatal retrogradely labelled neurons varied as a function of the FG deposit within ENT territories. According to projection patterns, three distinctive ENT territories (medial, ventral and laterodorsal) were identified. The delivery of FG into medial territories of ENT (cases 064 and 066) resulted in the appearance of retrogradely labelled neurons throughout the pre- and post-commissural striatum, with a marked preference for medial striatal areas, close to the lateral ventricle (Fig. 2A). In contrast, after tracer injections targeting laterodorsal areas of ENT (cases 014, 059, 067 and 112), a considerable amount of retrogradely labelled neurons were noticed in dorsolateral regions of the pre- and post-commissural striatum, with a preferred distribution for lateral areas of the post-commissural striatum and dorsal territories when considering the pre-commissural striatum (Fig. 2B). Finally,

FIG. 3. Photomicrographs of coronal sections through the rat striatum illustrating the arrival of thalamostriatal afferents and the striatal neuronal subpopulations apparently targeted by these afferents. (A and B) Relationships between thalamostriatal afferents and striatal neurons projecting to either the entopeduncular nucleus or to the substantia nigra reticulata. Terminal fields of thalamic afferents displayed a patchy distribution within the dorsal striatum, these patches often containing neurons projecting to the entopeduncular nucleus (purple-labelled) as well as neurons projecting to the substantia nigra reticulata (brown-labelled). Scale bar, 120 μm (A); 30 μm (B).

striatal neurons located in ventral regions of the pre- and post-commissural striatum displayed a tendency to project to ventral areas of ENT (cases 063 and 092, not illustrated). Thus, different striatal regions were distinctly connected with corresponding similar areas of ENT. Neurons located within medial regions of the striatum mainly project to medial areas of ENT, neurons located laterodorsally displayed a marked tendency for projecting to the laterodorsal ENT and neurons distributed through central and ventral striatal territories innervate ventral areas of the ENT. Figure 4A illustrates the overall organization of striato-entopeduncular projections.

As mentioned earlier an accurate topography of the striatonigral projection could not be further disclosed because our injections were restricted to medial areas of SNr. Overall, the largest numbers of labelled neurons were preferentially found in medial territories of the pre- and post-commissural striatum, with a slight tendency for a more lateral and caudal localization when the injection sites were placed more laterally within SNr.

Topography of thalamostriatal projections arising from the parafascicular nucleus

As a result of injections of the anterograde tracer BDA into the thalamic PF nucleus, a strong labelling was seen in the striatum. Thalamic afferents displayed a patchy distribution within the striatum (Fig. 3A and B). These results indicate that thalamostriatal projections arising from PF are distributed throughout the entire rostrocaudal, mediolateral and dorsoventral territories of the striatum.

Obvious differences in the striatal distribution of thalamic afferents were found related to the different injection sites within several PF regions. According to the projection patterns, up to four PF areas (medial, ventral lateral, dorsolateral and rostral) were identified. The delivery of BDA into medial PF areas (cases 064 and 066) resulted in strong thalamostriatal projections spreading over pre- and post-commissural striatal regions, with a marked preference for a periventricular localization in medial areas of the striatum (Fig. 2A). Neurons located

FIG. 4. Topographical distribution of striato-entopeduncular projections (A), thalamostriatal projections (B) and thalamo-subthalamic projections (C). When considering thalamostriatal projections together with the distribution of striato-entopeduncular projecting-cells, one might easily appreciate that both pathways are tightly interconnected from each other, therefore supporting the existence of separate channels within the Nauta–Mehler loop. Regarding the thalamo-subthalamic projection, limbic-related areas of PF are represented in medial STN territories, while axons coming from sensorimotor-related areas of PF displayed a tendency for a more lateral localization in STN.

FIG. 5. Thalamo-subthalamic projections. (A and B) Photomicrographs taken from coronal sections through the subthalamic nucleus showing the distribution of labelled elements. (A) Low-power magnification photomicrograph showing retrogradely labelled neurons projecting to the entopeduncular nucleus, these neurons being located in the subthalamic nucleus as well as in the zona incerta. The terminal fields of thalamo-subthalamic axons are stained in brown, this labelling being restricted to the subthalamic nucleus, without entering in the zona incerta. Case no. 112. Scale bar, 700 μm . (B) Inset taken from A at higher magnification, showing a dense plexus of thalamic axons spreading through almost the entire extent of the subthalamic nucleus. These thin axons contain numerous varicosities. Retrogradely labelled subthalamic neurons projecting to the entopeduncular nucleus are often comprised within the terminal fields of thalamo-subthalamic afferents. Scale bar, 175 μm . (C and D) Photomicrographs taken from sagittal sections through the subthalamic nucleus. (C) Photomicrograph showing that the entire extension of the subthalamic nucleus is covered by labelled neurons projecting to the entopeduncular nucleus. It might also be appreciated a rostrocaudally orientated band of thalamic axons covering the rostral half of the subthalamic nucleus. Scale bar, 350 μm . (D) Inset taken from C at higher magnification in order to better illustrate that subthalamic neurons projecting to the entopeduncular nucleus are clearly in register with afferents coming from the parafascicular nucleus. Scale bar, 175 μm .

in the ventral lateral areas of PF (cases 059, 063, 092 and 112) project to ventral and lateral territories of both the pre- and post-commissural striatum, slightly moving dorsally in the pre-commissural striatum. The largest amount of striatal extension covered by thalamostriatal afferents was observed after tracer deposits within the dorsolateral areas of PF (cases 014 and 067). Projections coming from these PF areas spread over dorsal and lateral territories of the pre- and post-commissural striatum according to a patchy distribution (Figs 2B, and 3A and B), also reaching dorsal periventricular regions as well as central territories in the pre-commissural striatum. Finally, PF neurons located in the rostral pole of the nucleus send their axons to the entire rostrocaudal axis of the striatum, with a slight preponderance of the post-commissural striatum. Thalamostriatal axons arising from rostral areas of PF extended over lateral and ventral territories of the post-commissural striatum, moving to central territories in rostral levels of the striatum, always maintaining a preferred ventral localization. Overall, projections coming from medial, dorsolateral and ventrolateral PF areas remained largely segregated from each other within the striatum, while the pattern of projection seen after rostral PF injections must be seen as a mixture of all the other three distinctive patterns, and therefore a true topographical arrangement for the thalamostriatal projections could only be clearly disclosed when dealing with PF

levels located at a distance from bregma ranging between -4.0 and -4.4 mm. Figure 4B illustrates the overall organization of PF projections to the striatum.

Relationships between thalamostriatal afferents and striatofugal neurons projecting through the direct pathway

Neurons located in medial regions of PF project preferentially to medial territories of the pre- and post-commissural striatum, these regions containing striatofugal neurons, which in turn project to medial areas of ENT. Thalamic projections coming from neurons located dorsolaterally within PF are distributed within dorsal and lateral striatal areas, these territories being the ones that contain striatal neurons projecting to dorsolateral regions of ENT. Finally, ventral areas within ENT are innervated by afferents coming from projection neurons located in central and ventrolateral striatal regions, these regions in turn receiving afferents arising from thalamic neurons located in rostral and ventrolateral PF territories (Fig. 4A and B).

Relationships between thalamic afferents to STN and STN neurons projecting to ENT

As expected, all animals receiving a FG injection in ENT displayed an enormous amount of retrogradely labelled neurons in STN. Labelled

neurons were distributed throughout the entire extent of STN. The deposits of the retrograde tracer CTB into the SNr also labelled a large number of neurons in the STN. However, precise analysis of this projection was quite limited by the reduced efficacy of CTB for retrograde labelling when compared to FG and the high density of FG labelled neurons in the STN.

An increased complexity was noticed when analysing the thalamo-subthalamic projection. All PF regions (rostral, medial, dorsolateral and ventral lateral) supply afferents to STN, these projections being organized topographically according to a mediolateral axis. Thalamo-subthalamic afferents reaching lateral STN areas were appreciated after BDA injections located in laterodorsal territories of PF (cases 014, 059 and 067), these projections were more robust when dealing with injections placed on the more caudal levels of PF (cases 059 and 067). The remaining PF areas also participate on the thalamo-subthalamic projections, although to a lesser extent. Central STN territories were mainly innervated by thalamic neurons located in ventral lateral PF regions. Finally, thalamic axons arising from medial and rostral PF regions reach medial STN territories. Figure 4C illustrates the overall organization of thalamo-subthalamic projections.

The parent neurons of the subthalamo-entopeduncular projection were often found to be in register with PF afferents reaching the STN nucleus (Fig. 5), therefore suggesting that STN efferent neurons projecting to ENT are the presumptive postsynaptic target for these thalamic axons arising from PF.

Discussion

We report in detail the topography of the thalamostriatal and thalamo-subthalamic projections, together with the identification of several distinct neuronal subpopulations within targeted structures by means of retrograde tract-tracing techniques and immunocytochemical procedures. This study expands previous analysis on the relationship between the thalamus and the basal ganglia and provides the anatomical basis for an important functional role of the thalamic caudal intralaminar nuclei as a modulator of neuronal activity in the BG.

First of all, it is pertinent to discuss some methodological limitations of the study here. The visualization of the labelled structures has been conducted by brightfield microscopy. This method enabled us to assess the presence of overlapping areas between labelled structures, although the question of whether one individual axon could lead to real synaptic contacts with a particular neuronal profile cannot be definitively resolved without conducting further analysis under either the electron microscope or the confocal microscope. A second limitation concerns the intrinsic nature of retrograde tracing techniques. Even with the use of sensitive retrograde tracers such as Fluoro-Gold, the retrogradely transported tracer is restricted to the neuronal soma and to the initial portions of the thickest main dendrites. The possible existence of synaptic contacts between thalamic afferents and dendritic shafts or spines located far away from the neuronal soma cannot be disclosed unless performing intracellular labelling techniques (with Lucifer Yellow, for example). The delivery of small extracellular deposits of BDA into restricted regions of PF indicated that thalamic afferents reaching both the striatum and STN arise from the same PF area, although the question of whether or not one individual PF neuron might project simultaneously to the striatum and STN, via axon collaterals, cannot be answered with the methodology here applied. Another primary concern is the possibility of Fluoro-Gold (FG) uptake by striatal fibers passing through the ENT towards more distal targets. Certainly, the ENT in rodents is a particularly fibre-rich area and some discrepancy remains regarding the FG uptake by fibers of passage

FIG. 6. Flow chart summarizing position of transversal circuits arising from the caudal intralaminar nuclei within basal ganglia circuitry. (A) Classic model for the cortico-basal ganglia-thalamo-cortical motor loop, showing the direct and the indirect circuits. (B). The transversal position of the circuits under scrutiny within the classic basal ganglia model. The thalamostriatal-striatopallidal-pallidothalamic loop (known as the Nauta-Mehler loop) is illustrated in purple, while a second loop comprising thalamo-subthalamic-subthalamopallidal-pallidothalamic projections is shown in green. Taken together, the anatomical arrangement of these circuits supports the idea that PF neurons might exert a powerful influence on basal ganglia output.

(Schmued & Fallon, 1986; Pieribone & Aston-Jones, 1988; Dado *et al.*, 1990; Divac & Mogensen, 1990). However, there is general agreement to consider minimal the amount of FG uptake by damaged fibers traversing the injection site. This uptake is indeed kept to a minimum by means of the iontophoretic delivery of the dye (Pieribone & Aston-Jones, 1988; Schmued & Heimer, 1990). Furthermore, possible FG uptake by fibers of passage going to SNr will result in the appearance of striatal double-labelled neurons, containing both transported FG and CTB. After extensive screening of our material, conducted under brightfield illumination, no double-labelled neurons were found throughout any striatal area. The latter technical point is very relevant here as we know that the combination of two colourimetric precipitates (DAB and V-VIP) allowed an accurate evaluation of colocalizing antigens within one single neuronal profile (Lanciego & Giménez-Amaya, 1999). Overall, we consider that none of these limitations invalidate our results that disclosed firstly a very accurate topographical organization of the thalamostriatal and thalamo-subthalamic projections and secondly, neuronal subpopulations located within the terminal fields.

Separate channels within the Nauta–Mehler loop

The apparent nonspecific nature of the intralaminar nuclei can no longer be maintained when considering the thalamostriatal projections (Groenewegen & Berendse, 1994). Several studies carried out in rats, cats and primates have demonstrated the evidence of topographical patterns of projections to the striatum (Sadikot *et al.*, 1992a,b; Groenewegen & Berendse, 1994; Smith *et al.*, 1994; Giménez-Amaya *et al.*, 1995; Sidibé & Smith, 1996; Groenewegen *et al.*, 1999; Mengual *et al.*, 1999; Vercelli *et al.*, 2003). The primate post-commissural putamen, a region related to sensorimotor processing, is innervated by CM neurons (the primate analogue to lateral areas of PF in rodents). However, the associative- and limbic-related areas represented by the pre-commissural putamen, the caudate nucleus and the accumbens receive axons arising from neurons located in PF (an analogue to medial territories of the rodent PF; see Sadikot *et al.*, 1992a,b; Giménez-Amaya *et al.*, 1995). Studies carried out in monkeys showed that CM neurons make synaptic contacts preferentially with sensorimotor-related neurons of the putamen, which in turn project to the internal division of the globus pallidum (GPi; Sidibé and Smith, 1996). Furthermore, GPi neurons send their axons to CM neurons (Sidibé *et al.*, 1997). Projections from the output BG nuclei in primates to the caudal intralaminar nuclei are organized in segregated circuits. Four separated channels were recently described by Sidibé *et al.* (2002), as follows: (i) a sensorimotor-related circuit, comprising ventrolateral regions of GPi, medial two-thirds of CM and the post-commissural putamen; (ii) a limbic-related circuit, containing the rostromedial pole of GPi, rostral two thirds of PF and the accumbens nucleus, and (iii) two associative circuits, one connecting SNr, PF and the caudate nucleus, the other comprising the dorsal third of GPi, dorsolateral areas of PF and the post-commissural putamen. Rodent data presented here support the existence of a similar organization when considering the relationships between thalamostriatal projections and striato-entopeduncular projecting neurons: neurons located in dorsolateral territories of PF project to dorsolateral striatal areas, which in turn project to dorsolateral territories of ENT. A similar organization was established for thalamostriatal afferents coming from medial PF areas and reaching striatofugal neurons located in medial striatal territories, these neurons in turn projecting towards medial portions of ENT. The same happens when considering ventral ENT territories receiving afferents from neurons located within central and ventral striatal areas, the latter innervated by thalamic neurons located in ventrolateral regions of PF.

Thalamo-subthalamic projections and subthalamo-entopeduncular projecting neurons

The existence of direct projections from CM-PF reaching the STN has been reported in rats, cats and primates (Sugimoto & Hattori, 1983; Sugimoto *et al.*, 1983; Royce & Mourey, 1985; Sadikot *et al.*, 1992a; Marini *et al.*, 1999), although an accurate topography as well as the identity of postsynaptic targeted neurons remains to date unknown. A previous study performed by reconstruction of individual labelled axons demonstrated that at least a subpopulation of PF neurons is able to project simultaneously, via axon collaterals, to both the striatum and STN (Deschênes *et al.*, 1996). Our data conclusively shows that PF neurons, beside their massive projection to the striatum, also send strong and topographically organized projections to STN. This is in keeping with prior anatomical and physiological studies showing a discrete topographical and functional organization of the STN according to its afferent/efferent connectivity. In both humans and monkeys, the dorsolateral region of STN mainly contains movement-related neurons (Wichmann *et al.*, 1994; Rodríguez-Oroz *et al.*, 2001; Theodosopoulos *et al.*, 2003) that projects to the lateral (motor) region of either the external and internal globus pallidum and receives afferents from the cortical motor areas (Nambu *et al.*, 1996; Nambu *et al.*, 1997). On the other hand, the medio-ventral region is mainly connected with the antero-medial (associative and limbic) regions of the globus pallidum and receives cortical afferents from the prefrontal cortex (Nambu *et al.*, 2000).

The caudal intralaminar nuclei and their putative influence on basal ganglia output: functional implications

Data reported here about the architecture of thalamostriatal and thalamo-subthalamic pathways support the idea that PF neurons might exert a dual control on basal ganglia output nuclei (Fig. 6). On one side, the activation of PF neurons should excite striatal GABAergic neurons projecting to ENT (the direct pathway) leading to thalamic disinhibition. Thus, the Nauta–Mehler loop should be considered a facilitatory or feed-forward loop. On the other side, discharges of PF neurons should increase STN excitatory drive onto ENT neurons and therefore cause increased inhibitory output to the thalamus. As thalamic afferents to STN induce the activation of the last step of the indirect pathway, it should be considered as an inhibitory feedback loop. Both the thalamo-striato-entopeduncular and the thalamo-subthalamo-entopeduncular pathways are disynaptic and presumably have similar conduction velocities. Accordingly, these two loops appear properly designed to contribute to the characteristic dual facilitatory/inhibitory effect of the thalamocortical projection, which has been best characterized for the motor loop (DeLong, 1990; Mink & Thach, 1993). Besides the functional role potentially played by thalamo-striato-entopeduncular and thalamo-subthalamo-entopeduncular loops, one should keep in mind that PF neurons can also gain direct access to ENT and SNr, as described by Marini *et al.* (1999). Furthermore, the existence of direct projections linking the caudal intralaminar nuclei and the lateral globus pallidus was reported by Kincaid *et al.* (1991). Only functional (i.e. neurophysiological and behavioural) studies could allow a more accurate estimation of the relative importance of these different projections.

The basal ganglia play a crucial role in motor control by setting the appropriate balance between facilitation of the desired action and suppression of unwanted muscular activity. The caudal intralaminar nuclei are intimately linked with the motor cortex by powerful reciprocal connections (Jones & Leavitt, 1974; Herkenham, 1980; Berendse & Groenewegen, 1990, 1991). Altogether, the caudal intralaminar nuclei appear to be capable of modulating basal ganglia activity according to the cortical command. The precise role of these

nuclei within the hierarchy of events associated with movement control such as movement initiation or termination, self-induced and externally triggered movements, rapid ballistic vs. slow ramp movements, etc., has to be ascertained by physiological studies. It is worth noting that striatal interneurons are also reached by thalamostriatal afferents (Lapper & Bolam, 1992; Bennet & Bolam, 1994; Rudkin & Sadikot, 1999; Sidibé & Smith, 1999). Striatal interneurons are mainly inhibitory and sustain powerful mechanisms synchronizing the firing of striatofugal neurons (Kawaguchi *et al.*, 1995). We envisage the activation of striatal interneurons by thalamic afferents in parallel with the activation of striatofugal neurons, possibly providing another mechanism to select and focus the transmission of corticostriatal signals in the direct pathway.

The exquisite design of the thalamo-basal ganglia connections is likely to have pathophysiological implications. Lesion or derangement of the caudal intralaminar nuclei could distort movement selection, leading to involuntary movements such as dystonia or tics. On the other hand, increased activity of the caudal intralaminar nuclei should probably be associated with overdriving of the indirect pathway by virtue of predominant excitatory tone of the STN projections onto ENT neurons (or GPi in primates), causing a reduction of movement, that is parkinsonism. Indeed, unilateral 6-OHDA lesion of the nigrostriatal pathway in rats has been associated with hyperactivity of PF neurons projecting to STN (Orieux *et al.*, 2000) and, intriguingly, significant cell loss has been found in the CM-PF complex in brains of patients suffering from Parkinson's disease (Henderson *et al.*, 2000a,b). The precise significance of these findings requires further experimental studies.

In conclusion, we found that neurons from the same localized subregions of the caudal intralaminar nuclei project in a well-organized fashion to specific zones of the striatum and STN. This depicts the existence of two transversal thalamo-ganglionic loops very likely to play an important role in setting the balance between facilitation and inhibition of the basal ganglia output to the thalamocortical projection. We have discussed the possible functional implications of this anatomical organization in terms of the motor loop and the control of movement but in fact, a similar arrangement is present for the associative and limbic loops, suggesting an even wider putative functional role for the caudal intralaminar nuclei.

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Abbreviations

BDA, biotinylated dextran amine; CM-PF, centromedian-parafascicular thalamic complex; CTB, cholera toxin subunit β ; ENT, entopeduncular nucleus; Gpe, globus pallidum pars externa; Gpi, globus pallidum pars interna; FG, Fluoro-Gold; PAP, peroxidase-anti-peroxidase; PB, phosphate buffer; PF, parafascicular thalamic nucleus; SNr, substantia nigra reticulata; STN, subthalamic nucleus.

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