Axon collateralization in primate basal ganglia and related thalamic nuclei

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Axonal collateralization in primate basal ganglia and related thalamic nuclei

Martin Parent, André Parent∗

Abstract
This paper provides an overview of the major organizational features of the basal ganglia and related thalamic centers, as delineated by the application of single-axon or single-cell labeling procedures in primates. These studies have revealed that the striatum, the external pallidum and the subthalamic nucleus harbor several types of projection neurons endowed with a highly collateralized axon that allows these neurons to interact with most components of the basal ganglia. In contrast, the internal pallidum, which is a major output structure of the basal ganglia, contains only two types of projection neurons. First, there is a minority of "limbic" pallidal neurons with a poorly branched axon that arborizes profusely within the lateral habenula, which stands out as the most densely innervated pallidal target. Second, there is a majority of pallidal "motor" neurons with a long (total axonal length up to 27 cm) and highly branched axon that provides collaterals to the ventral tiers thalamic nuclei, the brainstem pedunculopontine nucleus and the centre médian/parafascicular thalamic complex. This type of axon allows internal pallidal neurons to send efferent copies of the same information to the thalamus and brainstem and hence influence various neuronal systems scattered throughout the neuraxis. Pallidal information is conveyed to the cerebral cortex and the striatum via the thalamus, while it is projected back to different components of the basal ganglia via the numerous reentrant pathways that arise from the pedunculopontine nucleus. Virtually all neurons in the centre médian thalamic nucleus innervate massively the striatum and less prominently the primary motor cortex, which in turn projects to the striatum directly or via a collateral from long-range corticofugal pyramidal axons. The results call for a reappraisal of our current concept of the anatomical and functional organization of basal ganglia, which play a crucial role in sensorimotor integration. Our data indicate that basal ganglia and related thalamic nuclei form a widely distributed neuronal network, whose elements are endowed with a highly patterned set of axon collaterals. This morphological feature allows a complex and exquisitely precise interaction between the various basal ganglia and related thalamic nuclei. The elucidation of this finely tuned network is needed to understand the complex spatiotemporal sequence of neural events that ensures the flow of cortical information through the basal ganglia and thalamus.

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Keywords: Single-cell labeling technique; Primary motor cortex; Pallidothalamic projections; Thalamostriatal projection; Movement disorders

1. Introduction
Our knowledge of the anatomical and functional organization of the basal ganglia has greatly improved during the last two decades. This remarkable development stems from the gathering of highly valuable clinical data from patients afflicted by various forms of movement disorders and from the development of reliable animal models of neurodegenerative diseases. The bulk of information that emerged from these clinical and basic studies was crystallized into

Abbreviations: ad, anterior dorsal thalamic nucleus; AL, area lenticularis; am, anterior medial thalamic nucleus; ar, anterior ventral thalamic nucleus; bs, brachium; CB, calbindin D-28K; CD, caudate nucleus; CL, central lateral thalamic nucleus; CM, center median thalamic nucleus; CP, cerebral peduncle; CX, cerebral cortex; EP, entopeduncular nucleus; FH, Forel’s field H; GP, globus pallidus; GPr, external segment of GP (external pallidum); GPi, internal segment of GP (internal pallidum); ic, internal capsule; LF, lenticular fasciculus; LH, lateral habenular nucleus; L.O, nucleus lateralis oralis thalami; M1, primary motor cortex; MD, mediodorsal thalamic nucleus; MH, medial habenular nucleus; OT, optic tract; P.F, periaqueductal thalamic nucleus; PPN, pedunculopontine tegmental nucleus; PG, precentral gyrus; Put, putamen; RRA, retrorubral area; Rt, reticular thalamic nucleus; str, striatum; SN, substantia nigra; SNc, pars compacta of SN; SNr, pars reticulata of SN; SPr, subparafascicular thalamic nucleus; STN, subthalamic nucleus; STR, striatum; Th, thalamus; VA/VL, ventral anterior/ventral lateral thalamic nucleus; VAdc, densicellular part of VA; VAp, parvicellular part of VA, VM, ventromedial thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; Zi, zona incerta

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the form of a model of the organization of the basal ganglia that played a key role in the revival of neurosurgical therapies for Parkinson’s disease, including stereotactic lesions and deep brain stimulation (Laitinen et al., 1992; Lozano et al., 1995; Baron et al., 1996).

The basal ganglia are reciprocally linked to the cerebral cortex via a relay in the thalamus. The main axis of this cortico-basal ganglia-thalamo-cortical loop includes the following sequentially arranged elements: (1) the striatum, composed of the caudate nucleus (CD), putamen (Put) and nucleus accumbens (also termed ventral striatum); (2) the pallidum or globus pallidus, comprising an external (GPe) and an internal (GPi) segment, as well as a ventral region; (3) the substantia nigra, divided into a pars compacta (SNc) and a pars reticulata (SNr); and (4) the ventral tier thalamic nuclei (nucleus ventral anterior and ventral lateral, V A/VL), whose premotor neurons convey the information that has been processed through the basal ganglia back to the cerebral cortex (Parent and Hazrati, 1995a).

The activity of each component of the basal ganglia is modulated by numerous structures located at the margin of the main axis and which provide neurochemical inputs capable of either blocking or facilitating the flow of neural information along the basal ganglia. Among these ancillary structures are (1) the subthalamic nucleus (STN); (2) the SNr; (3) the centre médian (CM)-parafascicular (PF) thalamic complex; (4) the dorsal raphe nucleus; and (5) the pedunculopontine tegmental nucleus (PPN) (Parent, 1990).

The striatum is the major input structure of the basal ganglia. Its main afferent projections originate from entire cortical mantle, the CM/PF complex, and the SN. The glutamatergic corticostriatal projections are very massive and impose upon the striatum a specific functional organization; the sensorimotor cortex projects principally to the putamen, the associative cortex to the caudate nucleus and the limbic cortex to the nucleus accumbens. On such basis, the striatum can be subdivided into sensorimotor, associative and limbic territories. Striatal neurons located in each of these distinct functional territories play a complementary role in respect to motor behavior. Neurons in the associative territory are chiefly concerned with the anticipation and planning of movement, those in the sensorimotor territory with its execution, and the ones in the limbic territory deal more specifically with the motivational and emotional aspects of motor behavior. The GPi and the SNr represent the major output nuclei of the basal ganglia. These structures exert a tonic GABAergic inhibitory influence upon the excitatory premotor neurons located in the VA/VL thalamic nuclei and in the brainstem PPN.

The current model assumes that cortical information is conveyed through the basal ganglia in a strict parallel manner along a direct and an indirect route. The direct pathway is thought to originate from striatal neurons that contain GABA and substance P and/or dynorphin and to project monosynaptically to the GPi/SNr. The indirect pathway is believed to arise from striatal neurons that contain GABA and enkephalin and whose influence is conveyed to the GPe/SNr polysynaptically via a sequence of connections involving the GPe and the STN. This sequence comprises (1) a GABAergic inhibitory projection from the striatum to GPs; (2) an inhibitory GABAergic projection from the GPe to the STN; and (3) an excitatory glutamatergic projection from the SN to the GPs/SNr. Imbalance between the activity in the direct and indirect pathways and the resulting alterations in the GPs/SNr are thought to account for the hypokinetic and hyperkinetic features of basal ganglia disorders (Albin et al., 1989; DeLong, 1990). Despite the indisputable heuristic value of this model of the basal ganglia, recent clinical and fundamental studies have uncovered various shortcomings in this scheme of thought (Marsden and Obeso, 1994; Levy et al., 1997). Our own single-cell labeling investigations of the axonal collateralization of neurons in each major component of the primate basal ganglia have raised questions about the central tenets of the model (Parent et al., 1995; Sato et al., 2000a; Sato et al., 2000b; Parent et al., 2001). Our studies indicate that, instead of being organized in the form of a dual, parallel-processing system, the basal ganglia form a widely distributed neuronal network, whose elements are endowed with a highly patterned set of axon collaterals. The latter feature allows each basal ganglia components to interact with one another in an exquisitely precise manner. The elucidation of this finely tuned network is needed to understand the complex spatiotemporal sequence of neural events that ensures the flow of cortical information through the basal ganglia.

The present paper provides a brief review of the results of our single-cell labeling studies in primates (Parent et al., 1995; Sato et al., 2000a; Sato et al., 2000b; Parent et al., 2001). It also reports new findings on the microcircuitry of primate basal ganglia, with a special emphasis on the key position of the thalamus in this complex neuronal network, which plays a crucial role in the control of psychomotor behavior. This type of information is essential to understand how cortical information is conveyed and integrated as it courses throughout the basal ganglia and thalamus before being relayed back to the cerebral cortex.

2. Methods and techniques

2.1. Preparation of the animals

The experiments reported here were undertaken in adult cynomolgus monkeys (Macaca fascicularis) of both sexes. All surgical and animal care procedures adhered to the guidelines for the use and care of experimental animals of the Canadian Council of Animal Care. The Animal Care Committee of Laval University also approved our experimental protocol. The animals were first anesthetized with ketamin (75 mg/kg) plus xylazine (5 mg/kg) and their head placed...
in a specifically designed stereotoxic apparatus. After trepanation, a radiopaque solution (Omnipaque or iohexol, 0.8 ml of a 65% solution, Nicomed Imaging, Brandford, Ontario, Canada) was injected through a microsyringe into the right lateral ventricle. A few minutes after the injection, lateral and frontal X-ray pictures of the ventricular system were taken to precisely localize the baseline formed by the anterior and posterior commissures in each animal (Percheron, 1975).

2.2 Injection procedure

One to 3 weeks after ventriculography, the animals were anesthetized as above and placed in the same stereotoxic apparatus. They were then maintained under propofol (10 mg/ml, i.v.) anesthesia while microiontophoretic injections of either biotin dextran amine (BDA, Molecular probes, Eugene) or biocytin (N-biotynyl-L-lysine, Sigma, St. Louis, MO) were being made in different portions of each major components of the basal ganglia and associated thalamic nuclei. The various targets included the striatum, the GPe, the GPi, the STN, the CM/Pf thalamic complex and the primary motor cortex (M1). Most injections were made bilaterally. The target was aimed at by using the stereotaxic coordinates of the atlas of Szabo and Cowan (1984), as modified by the data collected from ventriculography. Microiontophoretic labeling was carried out with glass micropipettes (tip diameter 2–3 μm) filled with a solution of potassium acetate (0.5 M) plus 2% BDA or biocytin. These electrodes had impedance ranging between 10 and 15 MΩ and were used to monitor the extracellular activity of the neuronal populations encountered during the penetration of the micropipette. Once in the chosen target, the micropipette was connected to a high compliance iontophoresis system 6.0, Adobe, San Jose, CA). Most axons were entirely reconstructed along either the sagittal or frontal planes with a freezing microtome. The sections were collected serially in phosphate buffer saline (PBS, 0.1 M, pH 7.4) and processed for the visualization of BDA according to the avidin-biotin-peroxidase method (ABC, Vector Labs, Burlingame, CA). In brief, The sections were incubated overnight at 4 °C in a solution containing ABC-diluted 1:100 in PBS (0.1 M, pH 7.4), plus 1% normal rabbit serum and 1% Triton X-100. They were then rinsed twice in PBS and once in Tris buffer. The bound peroxidase was revealed by incubating the sections in a solution containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.3% nickel-ammonium sulfate, 0.009% cobalt chloride and 0.008% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6, for 10–15 min at room temperature. The reaction was terminated by a rinse in Tris buffer followed by two rinses in PBS.

One monkey was used to compare the distribution of pallidal axons with that of the calcium-binding protein calbindin D-28K (CB) at thalamic level. In such a case, the brain was cut with a freezing microtome into 50 μm thick frontal sections that were collected in cold PBS. The tracer was revealed using nickel-cobalt-intensified DAB as the chromogen (as described above) and the sections were then processed for the visualization of CB immunoreactivity, as described in detail elsewhere (Parent et al., 2001). The CB antibody was a mouse monoclonal antibody highly specific for this cytoplasmic protein (Sigma, St. Louis, MO, dilution 1:2500), and the bound peroxidase was revealed by using DAB as the chromogen.

To help identifying nuclei and structures that harbored labeled neurons and axons, most sections were counterstained for cytochrome oxidase, according to the histochemical protocol of Wong-Riley (1979). The counterstaining was performed before BDA revelation, and nickel-cobalt-intensified DAB (dark blue reaction) and un intensified DAB (diffuse brown precipitate) were used to reveal BDA and cytochrome oxidase, respectively.

2.3 Material analysis

All sections were mounted on gelatin-coated slides, dehydrated in graded alcohol, cleared in toluene, and covered with Permount. They were examined under a Nikon light microscope equipped with a camera lucida. Details of single BDA- or biocytin-labeled neurons were drawn at 200× and 400× magnifications. The terminal fields and cell body of the labeled neurons were mapped at lower magnifications to determine their topographic location according to the atlas of Szabo and Cowan (1984). The atlas of the macaque thalamus by Olszewski (1952) and specific descriptions of thalamic motor nuclei in macaques (Asanuma et al., 1983; Ilinsky and Kulius-Ilinsky, 1987; Percheron et al., 1996; Jones et al., 2001) were also used for the detailed mapping of the pallidothalamic projections. The photomicrographs were digitally captured (AGFA Studiocam, Woburn, MA) and handled with the Adobe Photoshop software (version 6.0, Adobe, San Jose, CA). Most axons were entirely reconstructed along either the sagittal or frontal planes with the help of a camera lucida. Furthermore, a computerized image-analysis system (NeuroLucida, MicroBrightField Inc., Colchester, VT) was used to reconstruct in three dimensions the entire axonal trajectory and terminal arborization of three typical GPi neurons. This computerized system was also employed to gather quantitative estimates of the number of terminal boutons and total axonal length of the same three neurons.
3. Results

3.1. The primary motor cortex

The corticostriatal projections arising from the digit area of the primary motor cortex (M1) in cynomolgus monkeys were studied after labeling small pools of neurons with BDA. The microiontophoretic injections, centered upon layer V, were made under electrophysiological guidance and the labeled axons were reconstructed from serial frontal sections with a camera lucida and a computerized image analysis system. Preliminary results from this investigation indicate that the primate striatum receives both direct and indirect projection from M1. These two types of axons occurred in about equal number and arborized mainly in the same dorsolateral sector of the putamen, which corresponds to the sensorimotor striatal territory. They formed small fascicles that coursed sinusously within the corona radiata and reached the striatum principally through its dorsolateral aspect (Fig. 1A). Axons of the first type projected directly and only to the striatum. They remained uniformly thin and unbranched throughout their trajectory to the striatum. Axons of the second type were in fact thin collaterals emitted within the corona radiata by thick, long-range fibers that descended toward the brainstem (Fig. 1C). At striatal level, axons of both types branched moderately but occupied vast striatal territories. All axonal branches were endowed with rather equally spaced varicosities of very small size. The most distal axonal segments also displayed typical pedunculated varicosities (Fig. 1D). These findings reveal that, in contrast to current beliefs, the motor corticostriatal system is not formed only by axons dedicated solely to the striatum. Cortical motor information can also be conveyed to the striatum by corticofugal axons en route to the brainstem and/or spinal cord.

3.2. The striatum

In monkeys that received injections of biocytin in the striatum, a few small neuronal pools comprising about 5–10 labeled cells per pool were visualized in each striatum. Although, the dendritic arborization of individually labeled neurons within these pools was not always distinct (Fig. 2B), each axon emerging from the pools of labeled neurons could be visualized in great detail. In some animals, the labeling procedure produced Golgi-like images of the injected perikarya and their dendritic fields, together with a detailed view of their entire axonal arborization, including the recurrent local collaterals (Fig. 2C). Only axons whose entire arborization could be traced continuously throughout the various sections were analyzed in the present study. Axons whose collaterals were intermingled with collaterals of other axons were not taken into consideration so as to avoid the problems of interpretation that may arise when two or more axons or axon collaterals are closely apposed to one another. A total of nine striatofugal axons were analyzed in detail, including three axons that were entirely reconstructed with the aid of a camera lucida. Striatofugal axons were very fine and displayed numerous irregularly spaced varicosities along their course. Short bulb-like appendages were also encountered as striatofugal axons course through the globus pallidus (Fig. 2A). All striatofugal fibers penetrated the pallidum by piercing the external medullary lamina, where they gave rise to long collaterals arborizing along the inner surface of the lamina (Fig. 2A). The parent axons continued their course medially to arborize either in the GPe alone (one axon) or, more commonly, in the GPe and GPi (eight axons, Table 1). Axons that headed toward the GPi emitted numerous collaterals along the outer border of the internal...
Fig. 2. Striatofugal axons in primates. (A) Composite two-dimensional reconstruction from superimposed camera lucida drawings of serial sections showing the pattern of arborization of a singly labeled striatofugal axon in GPe, GPi and SNr, as seen on the sagittal plane. This axon arises from a cell body (arrow) located in the middle rostrocaudal third of the putamen, near the border of the GPe. It arborizes profusely in the GPi, and less abundantly in the GPe and SNr. (B) Photomicrograph of an injection site that contains 4–5 labeled neurons. Arrowheads point to the neurons that are particularly well delineated in this plane of section. (C) This photomicrograph provides a detailed view of an injected striatal neuron. The arrow points to the initial axon segment, whereas arrowheads indicate local axon collaterals. Scale bars = 1 mm (A), 50 μm (B) and 50 μm (C).

3.3. The external pallidum

The axon-tracing study of GPe neurons in cynomolgus monkeys has revealed the existence of four distinct types of neurons: (1) neurons projecting to the GPe, STN and SNr; (2) neurons projecting to the GPe and STN; (3) neurons projecting to the STN and SNr; and (4) neurons projecting to the striatum (Table 1).

The axons of the neurons of the first and second types ran medially and caudally for a short distance within the GPe before piercing the internal medullary lamina to enter the GPi. They traveled principally in a rostrocaudal direction within the GPi and gave off 1–4 collaterals along their course. The number and length of collaterals in the GPi varied from one axon to the other. Most of the long collaterals coursed parallel to the internal medullary lamina, whereas short collaterals often formed terminal arborizations near their parent axons. After leaving the GPe, the main axon of most of these neurons traversed the internal capsule and branched into several collaterals just before entering the STN. A few other axons branched directly within the GPe itself (Fig. 3A). Collaterals penetrated the STN from its anterior and/or ventral aspects and formed fine terminal fields within a rather limited sector of the nucleus. Axons of the second type terminated in the STN, whereas axons of the first type had branches that ran further caudally and entered the SNr through its rostral pole. Occasionally, axonal branches coursed between the SNr and STN to enter the SNr through its dorsal part. At the SNr level, the GPe axonal branches became very thin and their pattern of arborization was often difficult to delineate. In cases of intensely labeled axons, however, typical pericellular terminal arborizations could be visualized in the SNr.

Axons that projected to STN and SNr (third type) were the most frequent of all types of GPe axons (Table 1). They reached their targets either by coursing through the GPe and then piercing the internal capsule, or by entering immediately within the internal capsule, thus completely avoiding the GPe. In both cases the axons terminated in a rather dense and highly focused field in the STN, whereas, they arborized more loosely and more widely in the SNr.

The GPe axons that gave off collaterals to the striatum (fourth type) terminated in the caudate nucleus or the putamen. They ramified either within the GPe itself or just after entering the striatum. Typically, pallidostratal axons coursed parallel to the lateral medullary lamina and emitted 4–5 long and thin collaterals that entered the striatum perpendicularly to the lateral medullary lamina (Fig. 3B). These collaterals ramified into a few thinner collaterals that ran dorsally and laterally in the striatum. These terminal branches covered a rather wide area of the striatum, compared to the limited terminal fields of other GPe neurons in the STN.

The three-dimensional reconstruction of some of these GPe projection neurons reveals that many of them exhibit a profuse intranuclear axon collateral system and that their dendritic and axonal arborizations are aligned along the same
Table 1
Axonal branching patterns of the major basal ganglia components and related cortical/thalamic regions in primates

<table>
<thead>
<tr>
<th>Structure, cell type and percentage</th>
<th>Axonal branching sites and degree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CX</td>
</tr>
<tr>
<td>CX (n = 15)</td>
<td>−</td>
</tr>
<tr>
<td>Type 1 (53)</td>
<td>−</td>
</tr>
<tr>
<td>Type 2 (47)</td>
<td>−</td>
</tr>
<tr>
<td>STR (n = 9)</td>
<td>−</td>
</tr>
<tr>
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<td>−</td>
</tr>
<tr>
<td>Type 2 (11)</td>
<td>−</td>
</tr>
<tr>
<td>Type 3 (78)</td>
<td>−</td>
</tr>
<tr>
<td>GPe (n = 76)</td>
<td>−</td>
</tr>
<tr>
<td>Type 1 (13)</td>
<td>−</td>
</tr>
<tr>
<td>Type 2 (18)</td>
<td>−</td>
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<tr>
<td>Type 3 (33)</td>
<td>−</td>
</tr>
<tr>
<td>Type 4 (16)</td>
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</tr>
<tr>
<td>STN (n = 75)</td>
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<tr>
<td>Type 1 (21)</td>
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</tr>
<tr>
<td>Type 2 (3)</td>
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<tr>
<td>Type 3 (40)</td>
<td>−</td>
</tr>
<tr>
<td>Type 4 (11)</td>
<td>−</td>
</tr>
<tr>
<td>Type 5 (17)</td>
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</tr>
<tr>
<td>GPi (n = 52)</td>
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</tr>
<tr>
<td>Type 1a (38)</td>
<td>−</td>
</tr>
<tr>
<td>Type 1b (42)</td>
<td>−</td>
</tr>
<tr>
<td>Type 2a (14)</td>
<td>−</td>
</tr>
<tr>
<td>Type 2b (6)</td>
<td>−</td>
</tr>
<tr>
<td>CM (n = 7)</td>
<td>+</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*a The intranuclear axonal branching is not taken into consideration.

*b Degree of axonal branching: (−) none; (+) weak; (+++) moderate; (++++) high.

rostrocaudal plane. Therefore, these neurons appear to form multiple elongated neuronal modules that are closely packed along the mediolateral plane. Labeled GPe axons displayed large varicosities often closely apposed to cell bodies and proximal dendrites in the STN, GPe and SNr, but not in the striatum.

The majority of GPe axons provided collaterals to the STN, but none of them projected only to the STN. More than half of these axons branched to the STN and SNr, while others branched in smaller proportions to the STN and GPe, and to the STN, SNr and G Pi (Table 1). These findings reveal that, besides the STN, single GPe neurons also target the SNr and the GPi via a highly collateralized axonal system.

3.4. The subthalamic nucleus

On the basis of their axonal targets, five distinct types of projection neurons have been disclosed in the primate STN: (1) neurons projecting to the SNr, GPI, and GPe, (2) neurons targeting the SNr and GPe, (3) neurons projecting to the GPe and GPe, (4) neurons targeting the GPe only, and (5) neurons sending axons toward the striatum, but whose terminal arborization could not be visualized (Table 1).

Axons of the first and second types bifurcated into a rostral and a caudal branch within the nucleus, or just after leaving the nucleus (Fig. 4A and B). The caudal branch ran caudomedially to enter the dorsal aspect of the substantia nigra. It coursed caudally through the SNr and gave off several thin collaterals to the SNc, ventrally (Fig. 4A). These axon collaterals exhibited varicosities at the SNr level, but in some cases they became too thin and too faintly labeled to be followed throughout the rostrocaudal extent of the substantia nigra (Fig. 4B). The rostral branch traveled either dorsally along the ventral border of the thalamus or ventrally along the lateral border of the subthalamic nucleus. Irrespective of their ascending trajectory, all rostrally-coursing axons pierced the internal capsule and reached the pallidum through its caudal aspect (Fig. 4A and B).

Axons of the first type gave off a few collaterals in the GPi and branched more abundantly in the GPe (Fig. 4A and B). The number of branches in each target varied from one neuron to the other, but in most cases the terminal arborization was more profuse in the GPe than in the GPi. Axons of type 2 had a caudal branch that coursed along most of the rostrocaudal extent of the SNr, but whose terminal arborization could not be visualized in detail because of the faint labeling of this most caudal branch. The rostral branch...
Fig. 3. Axonal branching of GPe neurons in primates. (A) Camera lucida reconstruction of a single BDA-labeled GPe neuron (type 2) that projects to GPi and STN, as seen on the sagittal plane. Note the dense but small and highly focused innervation of the STN. Arrow indicates the location of the cell body. (B) Camera lucida reconstruction of a type 4 GPe neuron that provides collaterals to the striatum, as seen on the sagittal plane. The arrow indicates the location of the cell body. Scale bars = 500 μm.

Axons of the third and fourth types had only a rostral branch that targeted the pallidal complex. This rostral branch coursed either ventrally along the lateral border of the STN or dorsally along the ventral border of the thalamus. Axons of the third type gave off several collaterals in the GPi before entering the GPe. However, the length and number of these collaterals vary from one axon to another. Some had several long collaterals displaying numerous terminal arborizations, while others had a few fine collaterals with only a small number of focal terminal arborizations. Axons of the fourth type traversed the GPi without emitting any collateral and arborized rather poorly within the GPe.

In contrast to the axons of the four previous types, the axons of the fifth type did not penetrate directly the ventral or caudal poles of the pallidal complex, but ran for a long distance within the internal capsule heading toward the medial border of the GPe, but did not emit any collateral at this level. These axons could be followed up to the border of the putamen, where the staining became too faint to allow the visualization of their terminal arborization.

All the terminal branches of STN axons were very fine and exhibited numerous small (<1 μm) varicosities indicative of boutons-en-passant and boutons terminaux. They were rarely seen in close contact with a cell body in any of the STN target sites. The STN axons displayed a pattern of terminal arborization that was strikingly similar in all target structures.

3.5. The internal pallidum

Based on their target sites and axonal branching patterns, the GPi in cynomolgus monkeys was found to be composed of essentially two types of neurons. The type 1 neurons will be referred to as "motor neurons" because their axons project profusely to premotor neurons located in the ventral tier thalamic nuclei and PPN. The motor neurons can be
further subdivided into two subtypes: (1) type 1a neurons, whose axon projects only to ventral tier nuclei and PPN, and (2) type 1b neurons, whose axon arborizes more profusely than type 1a and, in addition to collaterals to the ventral tier nuclei and PPN, also innervates the CM/Pf thalamic complex (Table 1). The type 2 neurons will be termed here "limbic neurons" because their axon arborizes principally within the lateral habenular nucleus (LH), a major relay in the limbic system circuitry. The limbic neurons can be further subdivided into two subtypes: (1) type 2a neurons, whose axon projects only, but very densely, to LH, and (2) type 2b neurons, whose axon innervates profusely the LH, but also provides collaterals to the anterior thalamic nuclei (Table 1).

Labeled GPi neurons had a spindle-shaped, ovoid or triangular cell body emitting 2–5 long and poorly ramified primary dendrites (Fig. 5A). Some axons of motor or limbic neurons were seen to emit one or two thin collaterals that arborized poorly within, and sometimes beyond, the somatodendritic domain of their parent neuron in the GPi. These local collaterals exhibited varicosities of various sizes reminiscent of boutons-en-passant or boutons terminaux. Besides local collaterals, the main axon of motor neurons branched either within the GPi or just after leaving this structure. In contrast, the axons of limbic neurons remained unbranched, at least until they reached the thalamus. All limbic axons exited the GPi through the lenticular fasciculus (LF, Forel’s field H2), whereas motor axons emerged either through the ansa lenticularis (AL) or the LF, irrespective of the position of their parent cell body in the GPi.

Fig. 5. (A) Photomicrograph of two Golgi-like BDA-labeled neurons in the primate GPi. (B–D) Photomicrographs of the terminal arborization of GPi axons in the VAdc thalamic nucleus (B), the PPN (C), and the Pf (D). (E) Photomicrograph showing terminal varicosities confined to the CB-rich territory of the VAdc thalamic nucleus. The dashed line traces the limit between a CB-rich sector containing a cluster of labeled varicose axons (arrow) and a CB-poor sector of the VAdc devoid of such fibers. (F) Higher magnification of pallidothalamic varicosities in close contact with CB-positive thalamic cell bodies. Scale bars = 100 µm (A, B, D, E), 50 µm (C) and 25 µm (F).
Typically, axons of type 1 neurons emitted few short and varicose collaterals in the rostral part of Forel’s field H (FH), or prerubral field, and then bifurcated more caudally in the same area. One to three main branches ascended directly to the ventral tier thalamic nuclei via the thalamic fasciculus (Forel’s field H1), while another descended without further branching toward the PPN (Fig. 6A). The main ascending branches passed through the ventromedial thalamic nucleus (VM), which corresponds to the medial part of the ventral lateral nucleus of Olszewski (1952) (Ilinsky and Kultas-Ilinsky, 1987), without emitting any collaterals or terminals at this level. It remained unbranched until it reached the densicellular part of the VA nucleus (VAdc), which corresponds closely to the oral part of the ventral lateral nucleus of Olszewski, 1952 (Ilinsky and Kultas-Ilinsky, 1987). There it broke out into several short terminal collaterals (Fig. 6D). The main branches continued more dorsally and caudally to arborize within the parvicellular part of the VA nucleus of Olszewski (1952) (VApc) (Fig. 6E). Thus, these branches spread throughout a large portion of the pallidal territory of the ventral tier nuclei, an area that is globally referred to as the nucleus lateralis oralis (LO) by Percheron and colleagues (Percheron et al., 1996).

Irrespective of their number, the initial branches that ascended to the thalamus subdivided successively into 10–15 long and thin branches that covered most of the pallidal thalamic territory. As they approached the end of their trajectory, these long branches broke out into numerous shorter terminal collaterals that formed typical glomerule-like clusters composed of a multitude of very thin, highly varicose and closely intermingled axon collaterals (Fig. 5B). Some terminal branches endowed with few varicosities also ended in the vicinity of the glomerule-like clusters, which had a mean rostrocaudal extent of about 100 μm. The number of these terminal clusters ranged from 3 to 10 per axon and, occasionally, collaterals from two distinct axons were seen to contribute to the formation of a single glomerule-like cluster. At ventral thalamic levels, the varicosities were confined to the CB-rich territory (Fig. 5E and F).

The fiber that reach the PPN arborized in the form of several short and varicose collaterals uniformly scattered along the dorsal and ventral aspects of the decussation of the superior cerebellar peduncle, within the so-called subnucleus diffusus (or pars dissipata) of the PPN (Olszewski and Baxter, 1954). A few isolated varicose collaterals, some with large size varicosities in close contact with cell bodies, were also seen in the subnucleus compactus (or pars compacta) of the PPN, more laterally. The terminal field in the PPN was markedly different from the one at thalamic level. It consisted of a small number of varicose terminal branches that emitted a few collaterals oriented at right angles from the main axonal branch (Fig. 5C). These short terminal collaterals displayed numerous varicosities, including some at their very end portions. Most of these varicosities were larger than those at thalamic level. Numerous terminal varicosities were also noted at the level of FH and the zona incerta (Zi). These terminals were part of short collaterals provided en-passage by the main axons that coursed through this area before heading to the thalamus or the brainstem.

Axons of type 1b neurons arborized more profusely than those of type 1a neurons and, in addition to collaterals to the ventral tier thalamic nuclei and PPN, also innervated the CM/Pf intralaminar complex. The axon illustrated in Fig. 6 gave rise to axonal branch (in blue) that continued its causal course for some distance before it bifurcated and gave rise to two collaterals that both reached the CM/Pf from its causal part. Another collateral (in red) divided itself into two branches within the FH. One of these two branches emitted three collaterals that arborized in the FH and Zi before it ascended dorsally and caudally to reach the CM/Pf complex, where it arborized profusely. The other branch descended toward the retrorubral area (RRA), but remained poorly ramified at this level.

The axon collaterals did not form dense plexuses in the CM/Pf as they did in the VAdc/VApc subnuclei. Instead,
the fibers became thin and varicose well before terminating and formed typical loops oriented along the longest extent of the CM/Pf complex (Fig. 5D). They emitted several short and varicose collaterals that ran at right angle to the main axonal trunk and these thin varicose terminal fibers were often seen in close contact with unlabeled cell bodies in the CM/Pf complex. Most of these fibers arborized within the medial part of the CM and the lateral part of the Pf, which correspond respectively to the pars media and the lateral sector of pars parafascicularis of the central complex, as defined by Percheron and collaborators (Fénelon et al., 1994; Percheron et al., 1996). The VA subnuclei innervated by this single neuron were found to contain only 237 axonal varicosities in comparison to 57 varicosities for the PPN and 540 for the entire CM/Pf, which appeared as the most densely innervated target of this particular type 1b axon. This 1b axon had a total axonal length of 27.22 cm.

The three-dimensional reconstruction of this neuron revealed a striking feature that appeared to be shared by all motor (type 1) neurons, which was that the widest extent of their axonal arborization occurred along the sagittal plane. It was along that plane that the entire axonal arborization was, by far, the most spread out (Fig. 6A). In contrast, the axonal arborization of the same neurons had a narrower and more compacted appearance when viewed along the frontal or horizontal planes. The axonal arborization of the type 1b neurons was thus 4.6 times more spread out along the sagittal plane than along the frontal or horizontal planes.

The axons of limbic type neurons (type 2) exited through the LF, traversed obliquely the lateral hypothalamic area by coursing along the inferior thalamic peduncle, ascended along the rostral pole of the thalamus to enter directly within the stria medullaris (Fig. 7A). From there the fiber followed a rather straight course until it approached the habenula. After a small caudal detour, the labeled axon entered directly into the habenula and immediately branched into three major collaterals that arborized within most of the LH, leaving the medial habenular nucleus completely devoid of labeled axonal varicosities. Of the three main collaterals that penetrated the LH, the first one remained rather poorly branched, the second one arborized abundantly, and the third one became extremely thin and could not be followed entirely (Fig. 7B). The pattern of arborization within the LH consisted of a
axon of these neurons revealed a similar pattern of distribution around the small, round and closely packed cell bodies in the LH. This particular axon was found to yield 647 terminal varicosities in the LH, but this number is obviously an underestimation because it was not possible to count the axon terminals of one of the three major collaterals that innervated the LH because of its faint labeling (Fig. 7B).

Although, the high density of innervation has complicated the tracing of individual axonal branches in the LH, many type 2 axons emitted one major axonal branch that crossed the midline within the interanterbular commissure and innervated the contralateral LH (Fig. 7C, inset). In cases of unilateral GPi injections, the pattern of pallidal innervation of the contralateral LH was found to be largely similar to that seen ipsilaterally, except that the density of innervation was significantly less contralaterally than ipsilaterally.

Instead of climbing immediately within the stria medullaris, other type 2a axons ascended along the reticular thalamic nucleus (Rt) and entered within the striata terminales that arched along the medullar border of the caudate nucleus near its junction with the thalamus. Further caudally, these labeled fibers progressively detached themselves from the stria terminales to course medially within the stratum zonale until they joined the stria medullaris to reach the LH.

The trajectory and branching pattern of type 2b neurons were similar to those described above for the type 2a axons, except that they provided 1–3 major collaterals to the anterior thalamic nuclei on their way to the LH. These collaterals arborized principally in the anterior median and anterior ventral thalamic nuclei. Overall, the pallidal innervation of the anterior nuclei was much less dense than that of the LH. The pattern of terminal arborization at the level of the anterior nuclei consisted essentially of a few short, thin and varicose axon collaterals that appeared to make contact en-passant with the cell bodies of the anterior thalamic nuclei.

Despite the fact that pallidal projection neurons of type 1 (motor) and 2 (limbic) were not distinguishable from one another on the basis of their somatodendritic morphology, they were found to occupy a very distinct sector of the internal pallidum. Motor neurons abounded preferentially within the large central portion of the GPi, whereas limbic neurons were largely confined to the periphery of the nucleus. Furthermore, the most profusely arborized motor neurons (type 1b neurons) tended to be more abundant ventrally in the caudalmost portion of the GPi.

3.6. The centre médian thalamic nucleus

Microiontophoretic injections of BDA combined with electrophysiological recording in the primate CM nucleus led to the Golgi-like labeling of seven large perikarya from which emerged several long, poorly branched and sparsely spined dendrites (Fig. 8C). Entire reconstructions of the axon of these neurons revealed a similar pattern of distribution. Each axon coursed through the lateral thalamic nuclei, emitted a few collaterals in the Rt, and bifurcated within the internal capsule (Fig. 8A). One branch ascended via the corona radiata to the motor cortex, where it arborized moderately in layers V and VI, and much less profusely in layer I. The other branch ran through the pallidum and entered the dorsolateral sector of the putamen (sensorimotor territory), where it broke out into 3–5 smaller collaterals (Fig. 8A). These thin branches divided into numerous shorter collaterals that displayed pedunculated varicosities organized as dense, cluster-like terminal fields that formed oblique bands restricted to specific domains or the dorsolateral putamen. In contrast to previous results obtained with double-retrograde labeling techniques, our data revealed that the motor cortex and the sensorimotor striatal territory are innervated by the same CM axons in primates and that the CM axonal arborization is much more profuse in the striatum than in the cortex. The CM axons analyzed here did not innervated extrastriatal basal ganglia components, a finding that suggests a possible difference between rodents and primates in regard to the organization of the thalamostriatal projection.

4. Discussion

4.1. The striatum

One of the central tenets of the current basal ganglia model is the segregation of the striatal output pathways. Early retrograde cell labeling investigations have supported the idea of distinct striatofugal projections arising from separate neuronal populations in the striatum (Féger and Crossman, 1984; Parent et al., 1984; Parent et al., 1989; Selemon and Goldman-Rakic, 1990; Flaherty and Graybiel, 1993). However, the results of the single-cell labeling studies described above (see also Parent et al., 1995) have revealed an abundance of striatal projection neurons with highly collateralized axons that provide branches to two or three of the striatal recipient structures. Such a high degree of axonal collateralization allows striatal neurons to send effferent copies of the same information to virtually all striatal targets, a notion that is incompatible with the current dual model of basal ganglia.

The concept of a highly collateralized striatofugal system has been confirmed and further extended in one of our previous study undertaken in the rat with the juxtapacellar labeling procedure (Wu et al., 2000). This investigation has revealed four major features of the organization of the striatofugal system in rodents. First, striatofugal system originates from medium-sized spiny neurons that project only to the globus pallidus (GP, the rodent homologue of primate GPi), to both GP and SNr, or to GP:entopeduncular nucleus (EP, the rodent homologue of primate GPd) and SNr. Second, the striatofugal system displays a high degree of axonal collateralization; about two-thirds of its axons arborize into...
two or three striatal target structures. Third, virtually all striatofugal axons send collaterals to the GP and none project exclusively to EP and/or SNr. Fourth, the three types of striatal projection neurons share similar somatodendritic morphology and have no preferential distribution in the striatum (Wu et al., 2000).

All together, these findings indicate that the striatofugal system can no longer be considered as a projection system functioning on a simple dual (direct/indirect) mode. This system now stands out as a complex and widely distributed neuronal network, whose elements exhibit a markedly branched axon collateral system by which they can influence in a multifarious manner the output nuclei of the basal ganglia.

4.2. The external pallidum

Neurons in virtually all components of the primate basal ganglia appear to be endowed with a highly collateralized axon. This is the case for the GPe where four types of neurons with a highly branched axon were found to project to various components of the basal ganglia, including its two major output structures, the GPs and the SNrs. None of the GPe neurons were found to project solely to the STN, as depicted in the current model of the basal ganglia. These results reveal that the GPe cannot be considered a simple relay nucleus in the indirect pathway. Instead, this structure appears as a major integrative nucleus that can affect virtually all components of the basal ganglia (Parent and Hazrati, 1995b). Through their widespread and highly collateralized axons, individual GPe neurons are able to provide information to the STN, as well as to directly modulate, or even block, the flow of corticostriatal information at basal ganglia output levels. This is the case of GPe neurons of type 1 whose axon projects to the STN and, at the same time, provides multiple collaterals to the GPs and the SNrs. The terminal segments of these axon collaterals exhibited very large terminal boutons that closely surrounded the cell bodies in the GPs and SNrs. Hence, while conveying neuronal information to the STN, these GPe neurons are able to exert a profound GABAergic inhibition upon neurons of the GPs and SNrs.

4.3. The subthalamic nucleus

Likewise, five different subtypes of highly branched neurons were recognized in the STN of cynomolgus monkeys. The most abundant of these STN neurons were those projecting to the GPs and GPe, followed by STN neurons projecting to the SNrs, GPs and GPe, those projecting toward the striatum, those targeting the GPe only, and finally STN neurons projecting to the SNrs and GPe (Table 1). These findings are largely in agreement with earlier data derived from antidromic invasions, intracellular cell labeling, double-retrograde fluorescent and Golgi studies in rats (DENUA et al., 1978; VANDER KOY and HATTORI, 1980; HAMMOND and YELNIK, 1983; AFSHARPOUR, 1985; KITA and KITAI, 1987; GRANATA and KITAI, 1989). These investigations have emphasized the highly branched nature of the axons of STN neurons, a finding that led to the concept that virtually all STN neurons have a similar somatodendritic morphology and an axon that branches to the pallidum and the substantia nigra. This concept has become one of the cornerstones of the current model of the organization of the basal ganglia.

The results of our single-axon-tracing study in monkeys, however, call for a re-evaluation of the present conception of the anatomical organization of the STN. Indeed, STN neurons with axons branching to both GPs and SNrs have been visualized in cynomolgus monkeys, but all of them were found to send collateral to the GPe as well. Furthermore, these type 1 neurons represent less than one-fourth of the total number of labeled STN neurons. These results indicate that STN neurons with a rostral axonal branch only (types 3–5) are about three times more numerous than STN neurons with rostral and caudal axonal branches (types 1 and 2). The group of rostrally projecting STN neurons is itself heterogeneous. It comprises neurons projecting to both GPe and GPs (type 3), which represent nearly half of all STN labeled neurons, as well as neurons whose axon arborizes within the GPe only (type 4) or courses toward the striatum (type 5) (Table 1).

These results reveal that the STN is not a monolithic entity; instead, this nucleus harbors several subtypes of projection neurons, each endowed with a highly patterned set of collaterals. This organizational feature allows STN neurons to exert a multifarious effect not only on the GPe, with which the STN is reciprocally connected, but also on the two major output structures of the basal ganglia (SNrs and GPs).

4.4. The internal pallidum

In contrast to the other basal ganglia components, the GPi appears to be composed of only two types of projection neurons, (1) the limbic neurons projecting specifically and very densely to the lateral habenular nucleus, and (2) the motor neurons arborizing profusely within the ventral tier of thalamic nuclei. Although, limbic neurons appear to represent only 10% of the total neuronal populations in the GPs (PARENT et al., 1999, 2001), their presence indicates that the basal ganglia may be able to influence neural mechanisms other than the ones strictly related to somatic musculature and movement. This aspect has been largely neglected in our recent attempts to redefine the functional organization of the basal ganglia.

Another important organizational feature of the pallidofugal system relates to the fact that the pallidothalamic projection is part of a much wider neuronal network. Virtually all single pallidothalamic fibers that we have traced were collaterals of single-axons that also innervated the PPN (type 1 neurons) and, in about 50% of the cases, the CM/VP complex as well (type 1b neurons). This form of organization implies that single pallidal neurons can send efferent copies of the
in view of the fact that this part of the GPi is a good target territory (Fénelon et al., 1990). This finding is interesting region crossed by the fibers of the putamen sensorimotor abundant in the caudal and ventral sector of the GPi, a colleagues to refer to the CM/Pf complex) are particularly keys, which has revealed that pallidal neurons projecting results of a previous retrograde cell labeling study in mon-

in the nucleus. The latter finding is congruent with the
dominant in the caudalventral portion of the GPi than elsewhere (Parent and De Bellefeuille, 1982; Parent et al., 1999).

The projection from GPi to CM in primates was first reported by Nauta and Mehler (1966) in their pioneering anterograde degeneration study of the basal ganglia. The existence of that projection was later confirmed by numer-

ous anterograde tract-tracing and retrograde cell labeling studies in monkeys (see Sadikot et al., 1992). A similar projection arising from rodent and feline EP was also doc-

umented (Nauta, 1974; Carter and Fibiger, 1978; Hendry et al., 1979; Larsen and McBride, 1979). Here, we demon-

strate that the pallidointeraminar projection arises from collaterals of single-axons that project also to the ventral tier thalamic nuclei and PPN. This finding is in agreement with the original suggestion made by Nauta and Mehler (1966) that the CM/PI pallidal innervation derives prin-
cipally from collaterals of fibers that arborize within the ventral tier thalamic nuclei, as confirmed by studies undert-
taken with double-retrograde fluorescent method (Parent and De Bellefeuille, 1983) and single-axon-tracing tech-
nique (Arecchi-Bouchhioua et al., 1997).

The pallidofugal axons that provide collaterals to the CM/PI (motor type 1b) are by far the most widely arborized axons and their cell body of origin are slightly more abun-
dant in the caudalventral portion of the GPi than elsewhere in the nucleus. The latter finding is congruent with the results of a previous retrograde cell labeling study in mon-
keys, which has revealed that pallidal neurons projecting to the “central complex” (the term used by Percheron and colleagues to refer to the CM/PI complex) are particularly abundant in the caudal and ventral sector of the GPi, a region crossed by the fibers of the putamen sensorimotor territory (Fénélon et al., 1998). This finding is interesting in view of the fact that this part of the GPi is a good target for placing radiofrequency lesions (Vitek et al., 1998) or electrical stimulators (Krack et al., 1998) in the hope to allevi-
ate hypostatic and hyperkinetic movement disorders. The pallidointeraminar fibers traced here terminated massively in the CM, but also provided a significant input to the lateral part of the Pf via long and varicose collaterals that form loops between the two structures (Fig. 5D).

The pallidodemental projection differs in many respects from the pallidothalamic and pallidointeraminar projec-
tions. In most cases, it was composed of a single fiber that emerged rather close from the cell body and remained poorly branched and uniformly thick throughout its long trajectory toward the PPN. It is only when it reached the PPN that the fiber broke out into numerous collaterals that arborized within both the pars diffusa and compacta of the PPN. The fact that the number of varicosities is about 5–10 times less numerous in the PPN than in thalamus does not indicate that the pallidodemental branch is functionally less impor-
tant than the pallidothalamic branch. First, this difference in the number of terminals might reflect, at least in part, the enormous size variation that exists between the small PPN and the voluminous lateral thalamic mass. Second, the pal-
idodemental terminal field is much more focused than the widespread thalamic innervation and it comprises terminal collaterals with large varicosities that closely surround PPN neurons. Third, the fiber that descends to the PPN remains largely unbranched, whereas the pallidothalamic innervation derives from multiple, long and thin collaterals that branch frequently. Since nerve conduction velocity is directly propor-
tional to the axon diameter and inversely proportional to the degree of axonal branching, it may be presumed that PPN neurons receive GPi information well before thalamic neurons. This spatiotemporal mode of organization of single pallidofugal axons is important because the PPN is known to project back to many basal ganglia structures, principally the SNC, the STN and the GPi (Lavoie and Parent, 1994a). This feedback projection, which is excitatory and medi-
ated through acetylcholine and glutamate, or both (Steriade et al., 1988; Di Loreto et al., 1992; Lavoie and Parent, 1994b; Charara et al., 1996), could play an important role in the functional organization of the basal ganglia.

4.5. The primary motor cortex and the centre médian nucleus

Neurons with highly branched axons have also been disclosed in two of the major sources of afferents to the striatum, namely the M1 areas of the cerebral cortex and the CM nucleus of the thalamus. As for the cerebral cortex, the corticostratal projection has long been regarded as distinct from other corticofugal fiber systems (see Jones, 1985). However, some of our previous single-axon-tracing studies in rodents have revealed that virtually all corticostriatal ax-
ons are collaterals of long-range corticofugal neurons that project toward the brainstem and spinal cord (Lévesque et al., 1996), as predicted a long time ago by Ramón y Cajal (1911) on the basis of Golgi-stained materials. Yet, despite this demonstration in rodents, the belief in the existence of a corticofugal projection strictly dedicated to the striatum has persisted. For example, this view has been advocated rec-
ently to explain the electrophysiological behavior of corti-
costratal neurons during motor activity in primates (Turner and DeLong, 2000). The results of our single-axon-tracing study in cynomolgus monkeys indicate that the organiza-
tion of the corticostratal projection in primates might differ from that in rodents. As in rodents, layer V neurons that project to the striatum via collateral of their long-range axon have been detected in cynomolgus monkeys, but M1
neurons that project directly to the striatum have also been documented. The small sample of labeled cortical neurons gathered up to now in the primate M1 area does not allow us to speculate on the relative number of these two types of corticostriatal neurons, which both arborize in the sensorimotor territory of the striatum, or on their laminar localization in primary motor cortex. Nevertheless, the existence of these two types of corticostriatal neurons, which are likely to possess distinct physiological properties, indicates that the primate motor cortex has both a direct and an indirect access to the striatum and can thus influence the input stage of the basal ganglia in a highly complex and subtle manner.

The CM has long been known to be a major source of striatal afferents (see review by Parent, 1986). Our own experiments with bulk injections of anterograde tracers in squirrel monkeys have revealed that the CM nucleus projects specifically to the sensorimotor striatal territory, whereas the PI and the subparafascicular (SPF) nuclei innervate the associative and limbic striatal territories, respectively (Sadikot et al., 1992). Hence, the CM/PI/SPF nuclear complex appears to innervate massively and in a highly ordered fashion the entire rostrocaudal and mediolateral extents of the striatum in primates. The close link that exists between the CM nucleus and the basal ganglia is further attested by the recent demonstration that CM neurons degenerate in Parkinson's disease (Henderson et al., 2000). However, in addition to its close relationship with the striatum, the CM nucleus also belongs to the so-called posterior intralaminar thalamic nuclei, which are known to exert a state-dependent influence over vast cortical areas through highly collateralized projections (Jones, 1998, 2001; Steriade, 2000).

The fact that the posterior intralaminar nuclei project to the cortex as well as to the striatum raises the question of knowing whether these two projections arise from the same or different neurons in the CM. Our previous attempts to answer this fundamental question has yielded two opposite answers. A retrograde double-labeling experiment in squirrel monkeys has indicated that neurons in the caudal intralaminar nuclei projecting to the cortex are distinct from those projecting to the striatum (Sadikot et al., 1992). At CM levels, neurons projecting to the cortex were mostly confined to the lateral aspect of the nucleus, while those projecting to the striatum were more numerous and centrally located (Sadikot et al., 1992). In contrast, a more recent axon-tracing study in rats has revealed that virtually all neurons in the PI nucleus project to both striatum and cerebral cortex (Deschênes et al., 1996). We believe that this discrepancy is essentially due to a methodological difference and does not reflect a fundamental variation between rodents and primates in the organization of the posterior intralaminar nuclei. The single-axon-tracing technique provides direct evidence of axonal collateralization, whereas the retrograde double-labeling procedure yields data that cannot be considered definitive. One of the major drawbacks of the retrograde double-labeling technique is the false single-labeled neurons that may be encountered if the two injections sites are not in perfect register, that is, if the two tracers do not reach in equal amount the two terminal fields of the same axon. This limitation is particularly acute when the two terminal fields, whose exact topographic location can hardly be known in advance, are of unequal expanse and density, as it seems to be the case for the striatal and cortical terminal fields of CM axons. The data of the present single-axon-tracing study reveal that, irrespective of their location in the nucleus, most CM neurons are endowed with an axon that has a branch that arborizes densely in the rather restricted somatosensory territory of the striatum, and another branch that innervates much more diffusely a vast area of the motor cortex. It must mentioned, however, that the number of CM labeled neurons is at present too small to eliminate the possibility that some CM neurons might project only to the striatum or only to the cortex. This study is currently being pursued with the aim of sampling virtually the entire CM nucleus, as well as the PI nucleus, to produce a picture as detailed as possible of the organization of the CM/PI projection system in primates.

In any event, that existence of CM neurons with a bifurcating axon that innervates both the striatum and the cortex calls for a re-evaluation of the so-called Nauta-Mehler loop, which is one of the numerous, closed, ancillary loops that characterize the microcircuitry of the basal ganglia. This three-synaptic striato-thalamo-striatal loop, first described by Walde Nauta and William Mehler in their pioneering paper published in 1966, involves: (1) the striatopallidal projection, which arises from neurons located in the sensorimotor striatal territory that project to the core of the GPi; (2) the pallidothalamic projection, which originates from neurons in the core of the GPi that project to the CM/PI thalamic complex; and (3) the thalamostriatal projection, which stems from CM neurons that project back to the sensorimotor striatal territory. The fact that most CM neurons that innervate the sensorimotor striatal territory also project to the cerebral cortex indicates that the Nauta-Mehler loop is an open rather than a closed ancillary loop. The CM neurons endowed with an axon that branches to both striatum and cortex can no longer be considered as simple feedback elements. Their branched axon allows them to influence neurons in the sensorimotor striatal territory directly, as predicted in the Nauta-Mehler scheme, but also indirectly via a relay in the motor cortex, which projects back to the sensorimotor striatal territory. The open nature of the loop is reinforced by that fact about half of the GPi neurons that project to the CM also project to the ventral thalamus, as well as to the PPN brainstem nucleus. The latter nucleus is known to project back in a rather massive manner to the GPi. It may thus act as a reentrant pathway by modifying directly the firing pattern of the pallidal neurons that project to the CM nucleus. Likewise, the GPi neurons that project to both the CM and the ventral thalamus are able to influence striatal neurons both directly, as illustrated in the Nauta-Mehler loop, but also indirectly by influencing the corticostriatal neurons that receive input from the motor thalamic nuclei. Altogether,
these findings indicate that the Nauta-Mehler loop should no longer considered as a closed system.

5. Concluding remarks

The single-cell labeling studies reviewed above reveal that axonal collateralization is one of the major organizational features of the basal ganglia and, to a lesser extent, the thalamus. These investigations have shown that each major component of the primate basal ganglia, together with the basal ganglia-related thalamic centers, is composed of various types of neurons with distinct axonal arborization patterns. This morphological approach has helped to uncover a rather unexpected picture of the organization of the basal ganglia that has little in common with the current dual, parallel-processing type of model. Our data indicate that the basal ganglia are organized as a widely distributed neural network, whose individual elements are endowed with a highly patterned set of collaterals. The latter morphological feature allows each basal ganglia component to interact with one another, as well as with some major thalamic centers. The elucidation of this complex microcircuity is a prerequisite for understanding how cortical information flows through the basal ganglia and the thalamus, particularly in respect to the spatiotemporal sequence of neural events that underlies both normal and abnormal motor behavior.

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