Intralaminar Thalamic Nuclei Lesions: Widespread Impact on Dopamine Denervation-Mediated Cellular Defects in the Rat Basal Ganglia

JEAN-JACQUES BACCI, MSc, PHILIPPE KACHIDIAN, PhD, LYDIA KERKERIAN-LE GOFF, PhD, AND PASCAL SALIN, PhD

Abstract. Intralaminar thalamic nuclei represent a major site of non-dopaminergic degeneration in Parkinson disease, but the impact of this degeneration on the pathophysiological functioning of basal ganglia remains unknown. To address this issue, we compared the effects of 6-hydroxydopamine-induced lesions of nigral dopamine neurons alone or combined with ibotenate-induced lesions of intralaminar thalamic neurons on markers of neuronal metabolic activity in the rat basal ganglia using in situ hybridization histochemistry. Thalamic lesions prevented most of the dopamine denervation-induced changes (i.e. the increases in mRNA levels of enkephalin and GAD67 in the striatum, of GAD67 in the globus pallidus and entopeduncular nucleus, and of cytochrome oxidase subunit-I in the subthalamic nucleus), but did not affect the downregulation of striatal substance P and upregulation of GAD67 in the substantia nigra pars reticulata. We also provide immunohistochemical evidence that thalamic lesions markedly decreased striatal expression of the vesicular glutamate transporter vGluT2, confirming the association of this transporter with the thalamic projections to the basal ganglia. Altogether, these data reveal a major antagonistic influence of thalamic and dopaminergic afferents onto the basal ganglia and suggest that degeneration of thalamic neurons in Parkinson disease may represent an important factor counteracting expression of the defects associated with the dopamine denervation.

Key Words: 6-hydroxydopamine lesion; Basal ganglia; Cytochrome oxidase; Glutamate decarboxylase; In situ hybridization; Intralaminar thalamic nuclei; Parkinson disease.

INTRODUCTION

Parkinson disease is characterized primarily by the massive loss of dopaminergic neurons in the substantia nigra pars compacta. However, it has been long recognized that this disease is also associated with neurodegeneration in non-dopaminergic sites, including the pedunculopontine nuclei, locus coeruleus (1, 2), and raphe nuclei (3). Recently, Henderson et al (4, 5) reported an important neuronal degeneration (40%–55% cell loss) in the caudal intralaminar thalamic nuclei, i.e. the centromedian-parafascicular complex (CM/Pf), which is suggested to represent an early event in the disease probably paralleling the degeneration of dopamine neurons.

After cortical input, CM/Pf neurons represent the major source of excitatory extrinsic afferents to the basal ganglia complex (6–8), using glutamate as a neurotransmitter (9, 10). These thalamic nuclei innervate not only the striatum (11, 12) but also the globus pallidus (GP) (12–14), the subthalamic nucleus (STN) (15, 16), and the basal ganglia output structures, i.e. the substantia nigra pars reticulata (SNr) and the entopeduncular nucleus (EP) (17). Given the quantitative importance of the projections from CM/Pf to the striatum (18), thalamic influence on the basal ganglia network has long been thought to be mediated mainly through regulation of striatal outflow. However, the electrophysiological studies by Mouroux et al (7, 8) have more recently emphasized the importance of the CM/Pf as a complex acting in parallel on individual structures of the basal ganglia network. Accordingly, we provided evidence that lesions of intralaminar thalamic nuclei in rats induce a pattern of changes in neurotransmitter-related gene expression in the basal ganglia structures that better fit with removal of direct excitatory thalamic input onto these structures than with changes in intrinsic basal ganglia loops (19). Based on these data, the degeneration of CM/Pf neurons can be expected to have major insights onto the pathological functioning of basal ganglia in Parkinson disease.

The aim of this study was to investigate the impact of thalamic degeneration on the dopamine denervation-mediated cellular defects in the basal ganglia. To address this issue, we compared the effects of 6-hydroxydopamine-induced lesions of nigral dopamine neurons alone or in combination with ibotenate-induced lesions of intralaminar thalamic neurons on neurotransmitter-related gene expression in the rat basal ganglia by means of in situ hybridization histochemistry. The majority of neurons in the striatum, GP, EP, and SNr are GABAergic. The levels of mRNAs encoding the 67-kDa isoform of glutamate decarboxylase (GAD67), the GABA synthesizing enzyme, have been analyzed as an index of GABAergic activity in these structures. In the striatum, the responsiveness of the 2 efferent neuronal populations has been further distinguished by measuring gene expression of preproenkephalin A (Enk), a marker of the striatal neurons innervating primarily the GP, and preprotachykinin (SP), a marker of the neurons at the origin of the direct
pathway innervating the SNr and EP. In parallel, the metabolic activity of STN neurons was assessed using cytochrome oxidase subunit I (Col) mRNA expression. Finally, as PF neurons have been shown to express high levels of transcripts encoding the vesicular glutamate transporter vGluT2 (20), expression of the transporter protein was analyzed in the striatum by immunohistochemistry to assess the extent of thalamic denervation and examine the possible impact of dopamine deafferentation on thalamostriatal projections.

MATERIALS AND METHODS

Surgery and Tissue Preparation

All experiments were carried out on female Wistar rats (Iffa Credo, L’arbresle, France), weighing 180 to 200 g at the time of surgery. They were housed in groups of 3 at constant temperature (21°C ± 1°C) with a fixed 12-hour light/dark cycle and had free access to food and water. The experimental protocols involving animals and their care strictly conformed to the guidelines of the French Agriculture and Forestry Ministry (Decree 87–848).

Fourteen animals under chloral hydrate anesthesia (250 mg/kg i.p.) received a unilateral stereotaxic injection of 8 μg of 6-hydroxydopamine (6-OHDA; Sigma, St. Quentin-Fallavier, France) dissolved in 4 μl of NaCl 0.9% plus 0.1% ascorbic acid into the left substantia nigra pars compacta. The stereotaxic coordinates of the injection site were anterior (A): 2.2 mm; lateral (L): 2.0 mm; dorsoventral (DV): 3.3 mm; with the incisor bar at +5 mm according to the stereotaxic atlas of De Groot (21). The animals were then separated into 2 groups: one without any subsequent surgery (n = 5) and the other submitted to a unilateral injection of ibotenic acid (6 nmol/0.6 μl; TO-CRIS, Bristol, UK) into the left thalamus (n = 9) at the interaural coordinates: (A): 4.6 mm; (L): 1.0 mm; (DV): 4.0 mm, with the incisor bar at 2.4 mm below the interaural plane with respect to the stereotaxic atlas of Paxinos and Watson (22). The 2 injections were done in the same surgical session at the rate of 0.1 μl/min using a motor-driven injector. Two animals with the combined lesions died within the first week after surgery and were excluded from the study.

Anesthetized unoperated rats, 6-OHDA-lesioned animals, and combined 6-OHDA/thalamic-lesioned animals were killed by decapitation after a 2-week-postlesion survival time. The brains were removed quickly, frozen in dry ice, and kept at -80°C until cryostat sectioning. Based on the stereotaxic atlas of Paxinos and Watson (22), coronal 10-μm-thick tissue sections were cut at -20°C at rostral striatal level (between interaural coordinates A: 9.2 to 10.2 mm), and at the level of the GP (A: 8.2 to 7.2 mm), EP (A: 6.7 to 5.7 mm), STN (A: 5.4 to 4.8 mm), SNr (A: 4.2 to 3.2 mm), and intralaminar thalamic nuclei (A: 7.6 to 6.2 mm). Sections were then thaw-mounted onto SuperFrost plus glass slides (Fisher Scientific, Elancourt, France) and stored at -80°C until specific treatment.

Animal Selection Based on Histological Staining and 3H-mazindol Binding Experiments

The location and extent of the ibotenate-induced lesions were examined at thalamic level under light microscope (Nikon) on brain cresyl violet-stained sections. For each animal, the lesion size was determined and drawn on schematic coronal section diagrams. Only animals with thalamic lesions centered on the parafascicular nucleus were selected for the following experiments.

The extent of the 6-OHDA-induced dopamine denervation was examined at striatal level by autoradiographic labeling of dopamine uptake sites using [3]H-mazindol as a ligand (25). In brief, after being air-dried and rinsed, striatal sections were incubated for 40 min at 4°C with 15 nM of [3]H-mazindol (NEN Life Science Products, Boston, MA; specific activity: 17 Ci/m mole) in 50 mM TRIS buffer containing 300 mM NaCl, 5 mM KCl added with 0.3 μM of desipramine to avoid binding on noradrenalin transporters. After 2 rinses in the incubation buffer and 1 rinse in cold distilled water, sections were rapidly dehydrated and exposed for 2 weeks to [3]H-sensitive Hyperfilm (Amersham Pharmacia, Orsay, France). Quantitative analysis was done by optical density measurement in the striatum from digitized autoradiographic images using the BIOCOM image analysis system with Densitrag software (BIOCIM, Les Ulis, France), as reported previously (26). Six sections at 150-μm intervals between interaural coordinates (A): 9.2 to 10.2 mm were analyzed per individual, with analysis being restricted to the dorsal striatum (corresponding to the putamen), i.e. excluding the more ventral part of the structure and nucleus accumbens. Only animals showing a total loss of [3]H mazindol binding (>90%) in the striatum ipsilateral to the dopaminergic lesions were included in the study.

On the basis of the above-described criteria, 2 animals with incorrect location of the thalamic lesions (lateral and anterior lesions affecting the ventral thalamic nuclear group) were excluded from the combined lesion group, and 1 animal with partial dopamine denervation was excluded from the dopamine lesion group. The following experiments were then conducted on 5 control animals, 4 rats with the 6-OHDA-induced dopamine lesions alone and 5 rats with the combined dopaminergic and thalamic lesions.

Immunohistochemistry of vGluT2

The denervation resulting from the thalamic lesions were further examined by immunolabeling of the vesicular glutamate transporter vGluT2, previously referred to as differentiation-associated Na+-dependent inorganic cotransporter, DNPI (23). The protein was detected by a radio-immunohistochemical method using [125]I-radiolabeled secondary antibodies, as previously described (24). In brief, sections were fixed with 4% paraformaldehyde for 15 min. After preincubation in 0.1 M PBS solution containing 3% BSA, 1% goat normal serum, and 2 mM NaCl for 1 h, sections were incubated overnight in a rabbit anti-DNPI antiserum diluted 1/10,000, then with [125]I-labeled goat anti-rabbit IgG (0.25 μCi/ml, Amersham Pharmacia Biotech) for 2 h. Characteristics and specificity of the antibodies have been extensively studied by Herzog et al (24). The antibodies were kindly provided by Dr. S. El Mestikawy. Sections were apposed to X-ray films (Kodak Biomax MS from Eastman Kodak, Rochester, NY) for 5 days.

In Situ Hybridization Histochemistry

Probes were 43 to 48 mer synthetic oligonucleotides selected on the basis of the published sequence of preproenkephalin A

(27), preprotachykinin (28), GAD67 (29), and of CoI (30). Probes were 3’-end-labeled by terminal deoxynucleotide transferase (Roche, Meylan, France) with 32S-dATP (NEN Life Science Products; 1,300 Ci/mmol) using a DNA-tailing kit (NEN Life Science Products). The radiolabeled probes were then extracted on Nensorb purification column (Roche).

All solutions used for in situ hybridization were treated with diethyl pyrocarbonate and autoclaved to avoid RNase degradation. Slide-mounted sections were postfixed for 5 min in 3% paraformaldehyde, incubated in prehybridization buffer containing 2× standard saline citrate (SSC) and 1× Denhardt’s solution, acetylated for 10 min with 0.25% acetic anhydride in 0.1 M triethanolamine, and treated for 30 min in 0.1 M Tris-glycine before being dehydrated in ethanol and air-dried. Each section was covered with 35 μl of hybridization solution (4× SSC with 50% formamide, 10% dextran sulfate, 1× Denhardt’s solution, 0.25 mg/ml E. coli tRNA, and 0.5 mg/ml sheared salmon sperm DNA) containing the radiolabeled probe (radioactivity level about 500,000 cpm per section) and incubated overnight at 40°C in humid chambers. Sections were then rapidly rinsed in ice-cold 2× SSC, then treated successively for 10 and 40 min with 1× SSC at room temperature, 1× SSC at 42°C, and with 0.1× SSC at 42°C. Sections were then dehydrated in ethanol and air-dried.

Striatal sections were apposed to Kodak Bio-Max MR-1 film and exposure time was adjusted to avoid film saturation (6 to 8 days for neuropeptides and GAD67 mRNA detection). Sections at GP, EP, SNr, and STN levels were dipped in photographic liquid emulsion (LM1 from Amersham) and exposed at 4°C for 6 h for CoI mRNA labeling and about 20 days for GADs and neuropeptides. The X-ray films and the dipped slides were developed with Kodak D-19 for 3 min at 20°C, rinsed, and fixed. Sections were then counterstained with toluidine-blue and cover-slipped with Depex mounting medium. Brain sections from control and experimental groups of animals were run together in the same experimental session and exposed side by side on the same autoradiographic film.

**Data Analysis**

Levels of autoradiographic labeling were quantified by a computerized imaging system (BIOCOM, Les Ulis, France). Analysis at striatal level was performed from autoradiographic films and was restricted to the dorsal part of the structure. Grey levels were converted to relative optical density by using standard internal curves. The background signal was determined for each section by scanning the corpus callosum and subtracted from values obtained in the striatum on the same section. The mean optical density was determined from 3 sections of each animal. The data from all the animals per condition were then averaged and expressed as percent ± SEM of the corresponding control values.

For cellular analysis, GAD67 mRNA levels in the GP, EP, SNr, and CoI mRNA levels in STN were determined by the quantification of the number of silver grains in neurons randomly picked in each side of sections. The tissue section was observed under dark-field epi-illumination with an immersion ×20 objective of a Nikon Optiphot microscope connected to a COHU camera and the digitized images were transferred to the screen of a video monitor with a resulting magnification of ×1,000. Using a computer-assisted analysis system (Visioscan software from BIOCOM), a standard curve was done by measuring the optical density of the black background of the slide and by measuring the mean optical density of a defined number of silver grains, with each measure being conducted several times at different places of the section. The optical density was then measured over each neuronal cell body and converted into a number of silver grains per cell with respect to the standard curve. In the present experiments, background labeling was extremely low, so that the corresponding value was not subtracted. For GAD67, background labeling was estimated at a distance from labeled cells in the structures of interest. For CoI mRNA, background labeling was determined from an adjacent area devoid of neurons (cerebral peduncle). Indeed, because this marker is encoded by the mitochondrial genome, labeling is concentrated but not restricted to cell bodies and extends to processes so that measurements at a distance from labeled perikarya overestimate background. A random sample of 40 neurons (identified first under bright-field illumination) per section and per brain side was measured in 3 sections from each animal and the individual mean number of silver grains per cell was determined. The data from all the animals per condition were then averaged and expressed as the mean of the mean number of silver grains per cell. Experimental data are presented as percent ± SEM of the corresponding controls.

Statistical analysis of data was performed using a one-way ANOVA followed by Scheffé’s test for multiple group comparison. A significance of p < 0.05 was required for rejection of the null hypothesis.

**RESULTS**

**Thalamic Lesions**

In the selected animals, the ibotenate-induced thalamic lesions were centered on the Pf as illustrated in Figure 1. The lesion site was characterized by a total loss of neurons accompanied by marked gliosis that provided a dark aspect. The lesions typically extended to the lateral part of the centromedian thalamic nucleus, the major part of centrolateral and paracentral thalamic nuclei, and only a part of the mediodorsal and posterior thalamic nuclear groups, as previously reported (19). For all the animals, the lesions did not affect the ventral thalamic nuclear group.

**Nigrostriatal Dopamine Denervation**

Selected animals showed an extensive dopamine denervation in the striatum ipsilateral to the 6-OHDA injection, as assessed by the total loss in 3H-mazindol binding as compared to control labeling (Fig. 2). The mean decrease was of 96.98% ± 1.09% in the group with the dopamine lesion alone and of 93.59% ± 1.59% in the group with combined 6-OHDA and thalamic lesions. No significant difference in the amplitude of the depletion between the 2 experimental groups was noticed. No significant change was found in the side contralateral to the lesions.
Neuropeptides and GAD67 mRNA Expression in the Striatum

The effects of unilateral dopamine lesion alone or combined with thalamic lesions on striatal Enk, SP, and GAD67 mRNA expression were analyzed at structural scale using macroautoradiography. The results are illustrated in Figure 3.

The dopamine lesion alone induced an ipsilateral increase in Enk and GAD67 mRNA expression (+52.7% ± 7.7% and +45.2% ± 5.0%, respectively) and a significant decrease in SP mRNA expression (~−45.1% ± 3.3%). In the combined dopamine and thalamic lesion condition, Enk and GAD67 mRNA levels in the ipsilateral striatum were significantly decreased compared to values obtained in animals with the dopamine lesion alone and were no more different from control values. In contrast, levels of SP mRNA did not differ significantly from values obtained in animals with the dopamine lesion alone and remained significantly decreased (~−32.7% ± 3.5%) compared to control values.

GAD67 and Col mRNA Expression in Other Basal Ganglia Structures

Analyses were performed at cellular scale using microautoradiography. In the GP (Fig. 4), 6-OHDA-induced lesions of dopamine neurons resulted in a significant increase in GAD67 levels in the side ipsilateral to the lesion (+62.8% ± 10.6%). This increase was not observed in the animals with combined dopamine and thalamic lesions; the values obtained being no more different from control and significantly decreased compared to animals with the dopamine lesion.

In the STN (Fig. 4), the dopamine lesion alone induced a significant increase in Col mRNA expression in the side
Fig. 2. Digitized autoradiographic images (A) and quantitative analysis (B) of 3H mazindol binding on frontal section of the striatum illustrating the dopamine depletion in the animals with the 6-OHDA lesions alone or combined with the thalamic lesions. The data presented in the graphs are the means ± SEM of the optical density values determined from the n animals per condition and are expressed as percentages of control. I: ipsilateral side; C: contralateral side; *lesion side. **p < 0.01 compared with control values using Scheffe’s test. Scale bar: 1 mm.

ipsilateral to the lesion (+24.2% ± 4.3%). A slight but non-significant increase was measured in the contralateral side (+16.9% ± 5.1%). In animals with the combined dopamine and thalamic lesions, no change in CoI mRNA expression was measured compared to control animals but values were significantly decreased compared to values from animals with only dopamine lesions.

In the EP (Fig. 5), 6-OHDA lesion induced an ipsilateral increase in GAD67 mRNA expression (+69.9% ± 6.5%). In animals with combined 6-OHDA/thalamic lesions, GAD67 mRNA levels were bilaterally decreased not only compared to values obtained in animals with the dopamine lesion alone but also compared to control values (−26.1% ± 3.6% ipsilateral and −34.7% ± 2.7% contralateral).

In the SNr (Fig. 5), GAD67 mRNA expression showed significant increases after 6-OHDA-induced lesion (+20.5% ± 3.4%). In the combined 6-OHDA/thalamic lesion condition, GAD67 mRNA levels remained significantly increased in the side ipsilateral to the lesions (+14.5% ± 2.3%) compared with controls.

Striatal Expression of vGluT2 (Fig. 6)

The striatum showed intense immunohistochemical signal for vGluT2. The distribution of labeling appeared as relatively uniform, except the most medial zone bordering the ventricles that showed stronger labeling. After 6-OHDA-induced lesion, striatal vGluT2 immunolabeling was not significantly modified, although a slight increase (+24%) was measured. In the combined 6-OHDA/thalamic lesion condition, vGluT2 immunolabeling was markedly decreased in the side ipsilateral to the lesions (−67.5% ± 8.98%), without significant change in the contralateral side, as compared with control values. In pilot experiments we verified that thalamic lesions alone induced a similar reduction at the same survival time (−57% ± 3.36%, data not shown).

DISCUSSION

The findings of this study together with our previous data on the effects of thalamic lesion alone (19, 31), summarized in the Table, reveal that thalamic and dopamine inputs have mostly antagonistic influence on neurotransmitter-related gene expression in the basal ganglia structures. In the combined lesion condition, the only significant cellular changes in the markers examined in the basal ganglia consist of a decrease in SP in the so-called “direct pathway” connecting the striatum to the basal ganglia output structures and opposite changes in GAD67 in those output structures (increase in SNr and decrease in EP). Therefore, thalamic lesioning restricts the expression of dopamine lesion-mediated cellular defects in basal ganglia. These experimental data provide new insights into the interacting circuits affected in PD by suggesting that the degeneration of CM/Pf observed in this disease may partially counteract the consequences of dopamine neuron loss. In addition, our study provides evidence that vGluT2 expression in the striatum is substantially associated with the thalamostriatal projections.

Ultrastructural studies have shown that vGluT2 in the striatum mostly localizes to synaptic vesicle clusters in axonal terminals forming asymmetrical synaptic contacts,
Fig. 3. Digitized autoradiographic images of frontal sections at rostral striatal level (A) and quantitative analysis (B) showing the effects of unilateral 6-OHDA lesions alone or combined with ibotenate-induced thalamic lesions on GAD67, enkephalin, and substance P mRNA expression. Data are expressed as percent ± SEM of the mean control optical density. I: ipsilateral side; C: contralateral side; * lesion side. **p < 0.01 compared to control values and $$$ p < 0.01 compared to 6-OHDA values by Scheffé’s test. Scale bar: 2 mm.

a feature characteristic of excitatory amino acid-containing striatal projections (24). The striatum receives 2 major glutamatergic projections originating from the cerebral cortex and thalamus, regions that exhibit vGluT2 mRNA at different levels: very high in the thalamic nuclei and low to moderate in the cerebral cortex (20, 23, 24). Here we provide the first evidence that striatal vGluT2 expression is primarily associated with intralaminar thalamic afferents by showing that unilateral lesion of these nuclei reduces vGluT2 immunolabeling in the ipsilateral striatum by more than 50%. This study also shows that lesions of nigral dopamine neurons do not significantly affect the expression of vGluT2 in the striatum. Therefore, this protein is unlikely to be associated with nigrostriatal projections despite the data in the literature suggesting a glutamatergic component in this pathway (32–34) and showing high level of DNPI mRNA expression in the substantia nigra in human tissue (35). In addition, preliminary data indicate that striatal vGluT2 immunolabeling is not modified by cortical lesions (not
Fig. 4. Effects of unilateral 6-OHDA-induced lesions alone or combined with ibotenate-induced thalamic lesions on the expression of GAD67 mRNAs in the globus pallidus and CoI in the subthalamic nucleus. A: Photomicrographs taken under dark-field epi-illumination of emulsion-coated sections showing the effects of 6-OHDA and/or thalamic lesion on the expression of GAD67 mRNAs in the GP and CoI in the STN ipsilateral to the lesion. Note that compared to GAD67, CoI mRNA labeling is more diffuse because this marker, which is encoded by the mitochondrial genome, is expressed in both perikarya and cell processes. Scale bar: 50 μm. B: Histograms representing the mean of silver grain number per labeled neuron on the side ipsilateral
shown). Striatal vGluT2 expression can be then considered as a reliable marker of the integrity of thalamostriatal projections, which may be useful to assess the extent of thalamic denervation in patients with PD. On the other hand, whether or not expression of vGluT2 can represent a dynamic marker of activity remains to be determined, so that the lack of effect of the dopamine lesions on striatal vGluT2 expression cannot be interpreted in terms of absence of change in the activity of the thalamostriatal pathway.

The other effects of the 6-OHDA-induced lesions of nigral dopamine neurons reported here, that is, the decrease in gene expression of SP in the striatum, the increase in striatal mRNA expression of Enk and increase in GAD67 mRNA levels in the striatum, GP, EP, and SNr, as well as the increase in CoI mRNA in the STN, are consistent with most of the previous data in the literature (26, 36–39).

We previously assessed the effects of intralaminar thalamic nuclei lesion alone and reported segregated changes in neurotransmitter related gene expression in the basal ganglia (19). Here, we examined the impact of this lesion on expression of the dopamine denervation-mediated changes in the basal ganglia. As illustrated in the Table, the effects of the combined thalamic/dopamine lesions on the markers examined in all the basal ganglia structures in the lesion side correspond more or less to an addition of the effects of each lesion considered separately. Interestingly, 2 types of responses are evidenced. In the one case, the thalamic lesion has no effect alone and does not affect the dopamine denervation-induced changes, suggesting that these changes are independent of thalamic input. This concerns SP mRNA expression in the striatum and GAD67 mRNA in the SNr. It could be that the neurons expressing these markers do not undergo direct thalamic control. It could also be that the control exerted by thalamic afferents onto these neurons is not tonic. The latter hypothesis is favored by the anatomical studies showing that SNr receives direct thalamic inputs from CM/Pf (17), and that striatal neurons at the origin of the “direct pathway” connecting the striatum to the basal ganglia output structures (which are known to express SP) are preferential targets of thalamic afferents in primates (40). In the other case, the thalamic lesion has opposite effects of those of the dopamine lesion and prevents the dopamine lesion-mediated changes. This concerns mRNA expression of Enk in the striatum and of GAD67 mRNA levels in the striatum and pallidal complex, as well as CoI mRNA expression in the STN. These data reveal a global antagonistic influence of the thalamic and nigral dopamine inputs on the neuronal systems forming the “indirect pathway” connecting the striatum to basal ganglia output structures, i.e. the striatopallidal and pallido-subthalamic GABA neurons, and the subthalamic neurons projecting to the basal ganglia output structures, SNr, and EP.

The striatum is the main recipient of nigral dopaminergic and of thalamic inputs. However, all basal ganglia structures also receive thalamic afferents and undergo local dopamine control. At odds with the classical schemes of basal ganglia functioning, several lines of evidence now indicate that the effects of dopamine denervation in the GP and STN cannot be viewed simply as the consequences of the striatal modifications through the so-called “indirect pathway” (41, 42). For instance, whereas striatopallidal and pallido-subthalamic neurons are inhibitory, dopamine lesions induce parallel increases in gene expression of markers of the activity of striatopallidal, GP, and STN neurons (42). In recent re-evaluation of these schemes, the effects of dopamine lesions at the GP level are rather viewed as a consequence of abnormal activation of STN, which is itself viewed as a result of direct removal of dopamine influence and/or reactive increase in excitatory afferents of the STN. Similar interpretations, in terms of local changes in individual basal ganglia structures rather than striatal-driven cascade of changes, can be drawn regarding the effects of thalamic lesions, which decreases markers of neuronal activity in the 3 links of the indirect pathway and prevents the dopamine lesion-mediated increases in these structures. The fact that normalization of the effects of dopamine lesioning by combined thalamic lesioning apparently corresponds to a summation of opposite effects of each lesion does not necessarily imply that the changes induced by dopamine denervation are independent on thalamic input, but suggests that, if involved, thalamic input may be overactive. In this connection, evidence has been provided for increased metabolic activity of identified parafascicular neurons projecting to STN (43), so that removal of thalamic intermediate can directly account for the prevention of the dopamine lesion-mediated changes in STN reported here. It cannot be inferred from data obtained at STN level that dopamine lesion-induced changes in the other neuronal links of the “indirect pathway” are also directly dependent on thalamic input, since evidence has been provided that projections from parafascicular nucleus to STN and striatum arise mostly from separate neuronal populations (44), and since electrophysiological recordings have shown no stable change in the activity of parafascicular neurons after dopamine lesioning (45).

(I) and contralateral (C) to the lesion, expressed as percent ± SEM of the corresponding controls. **p < 0.01 compared to control values and *p < 0.01 compared to 6-OHDA values by Scheffé’s test.
Fig. 5. Effects of unilateral 6-OHDA-induced lesions alone or combined with ibotenate-induced thalamic lesions on the expression of GAD67 mRNAs in the entopeduncular nucleus and substantia nigra pars reticulata. A: Photomicrographs taken under dark-field epi-illumination of emulsion-coated sections showing the effects of 6-OHDA and/or thalamic lesions on the expression of GAD67 mRNAs in the EP and SNr ipsilateral to the lesion. Scale bar: 50 μm. B: Histograms representing the mean of silver grain number per labeled neuron on the side ipsilateral (I) and contralateral (C) to the lesion, expressed as percent ± SEM of the corresponding controls. **p < 0.01 compared to control values and $$p < 0.01 compared to 6-OHDA values using Scheffé’s test.
Fig. 6. Digitized autoradiographic images of frontal sections at rostral striatal level (A) and quantitative analysis (B) showing the effects of unilateral 6-OHDA lesions alone or combined with ibotenate-induced thalamic lesions on vGluT2 immunolabeling. Data are expressed as percent ± SEM of the mean control optical density. I: ipsilateral side; C: contralateral side; *lesion side. **p < 0.01 compared to control values using Scheffé’s test. Scale bar: 2 mm.

The present data show that lesions of intralaminar thalamic nuclei counteracts the cellular defects induced by dopamine denervation in the EP and the structures involved in the “indirect pathway” without interfering with the reactivity of the “direct pathway” and SNr. These experimental data may provide major insights into the pathophysiological functioning of basal ganglia in PD given the recent finding that intralaminar thalamic nuclei represent a major non-dopaminergic site of degeneration in this disease (4, 5). It can be inferred that CM/Pf degeneration in PD, if affecting the neurons projecting to all the basal ganglia structures, leads to a predominance of the dysfunction of the direct pathway and SNr in the expression of PD symptoms. This could explain discrepancies between neuropathological data and experimental data obtained after selective lesions of dopamine neurons regarding markers of striatopallidal and GP neuron activity (46–48). However, this view is difficult to reconcile with the abundant literature stressing the central involvement of STN abnormal activation in the pathophysiology of PD, as exemplified by the therapeutic benefits provided by the surgical treatments targeting this structure (49–51). As thalamic projections from intralaminar thalamic nuclei to the striatum and STN originate mainly in segregated neuronal populations (44), a possible conciliating hypothesis is that thalamic degeneration in PD, which affects only part of the neurons in CM/Pf (30%–40%), may concern primarily output neurons forming the collateralized ascending projections to the GP, striatum, and cortex while relatively sparing descending pathways to the STN and output structures of the basal ganglia. On the other hand, whereas the loss of dopamine neurons is recognized to be progressive, that of intralaminar thalamic neurons has been reported to be an early phenomenon, independent of disease onset or stage and severity of symptoms (4). Therefore, an alternative hypothesis is that thalamic degeneration in PD may delay rather than prevent the cellular and functional defects produced by dopamine loss.

TABLE

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Summary of data obtained after separate or combined unilateral 6-OHDA and thalamic lesions on mRNA expression of neuropeptides in the striatum, of GAD67 in the striatum, globus pallidus (GP), entopeduncular nucleus (EP), substantia nigra pars reticulata (SNr), and of CoI in the subthalamic nucleus (STN) on the lesion side. The effects of thalamic lesioning alone mentioned are those previously reported (19, 31).

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