Loss of Parvalbumin-Positive Neurons From the Globus Pallidus in Animal Models of Parkinson Disease

Diana Fernández-Suárez, Marta Celorio, Jose L. Lanciego, MD, PhD, Rafael Franco, PhD, and María S. Aymerich, PhD

Abstract
The external segment of the globus pallidus (GPe) in humans and the equivalent structure in rodents, the globus pallidus (GP), influence signal processing in the basal ganglia under normal and pathological conditions. Parvalbumin (PV) immunoreactivity defines 2 main neuronal subpopulations in the GP/GPe: PV-immunopositive cells that project mainly to the subthalamic nucleus and the internal segment of the GP and PV-negative cells that mainly project to the striatum. We evaluated the number of neurons in the GP/GPe in animal models of Parkinson disease. In rats, dopaminergic denervation with 6-hydroxydopamine (6-OHDA) provoked a significant decrease in the number of GP neurons (12% ± 4%, p < 0.05), which specifically affected the PV+ subpopulation. A similar trend was observed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned rats. Taken together, these findings provide evidence for nondopaminergic neuronal cell loss in the basal ganglia of 6-OHDA-lesioned rats and suggest that a similar loss may occur in the MPTP monkey. These data suggest that in patients with Parkinson disease, the loss of GABAergic neurons projecting to the subthalamic nucleus may contribute to the hyperactivity of this nucleus despite the absence of gross alterations in GAD mRNA expression.

Key Words: 6-OHDA rat, Basal ganglia, External segment, Globus pallidus, MPTP, Neurodegeneration, Parkinson disease, Parvalbumin, Primate.

INTRODUCTION
The external segment of the globus pallidus (GPe) is regarded as a relay nucleus in the indirect pathway of the basal ganglia circuitry, connecting the striatum with the subthalamic nucleus (STN) in humans and nonhuman primates. Motor control is achieved via the direct and indirect pathways by the flow of information between the input nucleus, the striatum and the output structures, the internal segment of the globus pallidus (Gpi), and the substantia nigra pars reticulata (1, 2). In Parkinson disease (PD), hypoactivity of the GPe may contribute to the hyperactivity of the output nuclei, giving rise to akinesia (1–3). However, far from being a simple relay nucleus, the GPe is critically positioned to influence the activity of all basal ganglia structures (4–8). Accordingly, the basal ganglia may be viewed as a network of regulatory loops, with the GPe positioned centrally to regulate the activity of afferent and efferent nuclei (9–11).

The human GPe and the equivalent structure in rodents, the globus pallidus (GP), are composed of networks of inhibitory GABAergic projection neurons (12). The inhibitory activity of GABAergic nuclei can be assessed by in situ hybridization (ISH) to quantify the mRNA expression of the 2 isoforms of glutamic acid decarboxylase (GAD), GAD65 and GAD67, enzymes that mediate the synthesis of GABA. In cerebellar Purkinje cells, GAD67 mRNA levels increase in parallel with the spike activity (13). By contrast, in the GP/GPe, an increase in GAD67 mRNA expression observed in the GP/GPe of parkinsonian animals (14–17) does not correlate with increased neuronal activity (18–23).

Distinct neuronal subpopulations have been described in the GP/GPe based on their projection targets and their expression of the Ca2+-binding protein parvalbumin (PV). In the GP, one third of the pallidal neurons project to the striatum (24) and do not express PV (25–27), whereas the remaining neurons project to the STN, the entopeduncular nucleus (Gpi in primates), and the substantia nigra pars reticulata (6, 24, 28) and they are PV immunopositive (PV positive) (25–27). The electrophysiological properties of the 2 GP neuronal subpopulations have been extensively characterized in the 6-hydroxydopamine (6-OHDA) rat model (27). In monkeys, 30% of GPe neurons project to the striatum (29), whereas the remainder project to the STN and the Gpi, substantia nigra pars reticulata, or both (7).

To explain the hyperactivity of the STN, the classical model predicts hypoactivity of the GP/GPe, assuming that all neurons project to the STN. However, the proposed role of the GP/GPe in basal ganglia circuits is under revision because new connections between the GP/GPe and other nuclei are now described. The main electrophysiological feature of the GP/GPe in parkinsonian conditions is a decrease in the firing rate (18, 22, 30, 31), which contrasts with the enhanced expression of biochemical markers of GABAergic activity in
animal models of PD (14–17). Neuronal loss has been described in the GPe of individuals with Tourette syndrome (32) and in patients with progressive supranuclear palsy affecting specifically the PV-positive pallidal neuronal subpopulation (33). Because the hyperactivity of the STN may be caused by a specific decrease in the cell number and/or the GABAergic activity of the PV-positive pallidal neurons projecting mainly to the STN, we investigated whether dopaminergic cell loss affected the number and the activity of GP/GPe neurons. Accordingly, we used Nissl staining and immunofluorescence assays to quantify the number of PV-positive and PV-negative after dopamine depletion in 2 animal models of PD, 6-OHDA–treated rats and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–treated monkeys.

MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats (220–240 g) were obtained from Harlan (Barcelona, Spain) and housed in standard animal facilities. Adult male cynomolgus monkeys (Macaca fascicularis; body weight, 3–4.5 kg) were from R.C. Hartelust BV (Tilburg, Netherlands). The animals were maintained in a temperature- and humidity-controlled environment on a 12/12-hour light/dark cycle with ad libitum access to food and water. All the procedures involving animals were performed in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals. The experimental design was approved by the Ethical Committee for Animal Testing of the University of Navarra (ref. 060/07 and 019/2008) and the Department of Health of the Government of Navarra (ref. NA-UNAV-04-08).

Experimental Parkinsonism
Rats were deeply anesthetized by intraperitoneal injection of a mixture of ketamine (75 mg/kg, Imalgene 500; Merial, Barcelona, Spain) and xylazine (10 mg/kg, Rompun; Bayer, Barcelona, Spain) and then placed in a stereotoxic frame (David Kopf, Hertfordshire, UK) with a syringe (Hamilton, Bonaduz, Switzerland) attached. Rats received 2 injections of 6-OHDA (Sigma, St. Louis, MO) into the medial forebrain bundle (12 μg in 4 μL of saline containing 0.1% ascorbic acid; Sigma) using the following coordinates relative to the bregma (34): AP, −3.6 mm; ML, 1.8 mm; DV, 8.2 mm; and AP, −3.6 mm; ML, 1.8 mm; DV, 8.6 mm. Five weeks after 6-OHDA injection, rotational behavior was induced in rats by intraperitoneal injection of amphetamine (5 mg/kg in 0.9% saline solution; Sigma) and monitored using a computerized rotometer (Panlab, Barcelona, Spain). The number of full (360 degrees) left and right body turns was recorded during a period of 90 minutes. The net rotational asymmetry score was calculated by subtracting contralateral from ipsilateral full-body turns. Animals were killed 48 hours after amphetamine administration.

In monkeys, the neurotoxin MPTP (Sigma) was administered once a week (0.3 mg/kg as a 0.2% solution in saline) intravenously until a stable parkinsonian syndrome was observed. The severity of the MPTP-induced parkinsonian syndrome was evaluated using Kurlan Scale (35), which rates parkinsonian motor symptoms on a cumulative scale, with the highest score reflecting maximum severity (29). Monkeys reached a stable score of more than 21 points that was maintained throughout an MPTP washout period of 2 months (with an estimated variation of ± 2 points).

Tissue Processing
Rats were anesthetized with an overdose of 10% chloral hydrate (Panreac, Barcelona, Spain) in distilled water and transcardially perfused with a fixative solution containing 4% paraformaldehyde (Merck, Darmstadt, Germany) in 0.125 mol/L PBS (pH 7.4). The same fixative solution was used for monkeys, with the addition of 0.4% glutaraldehyde. The brain of each animal was removed and cryoprotected for 48 hours in a solution containing 20% glycerin (Panreac) and 2% dimethylsulfoxide (Sigma) in PBS. All solutions used for perfusion and cryoprotection were treated with 0.1% diethlypyrocarbonate (Sigma) and autoclaved. Frozen coronal microtome sections (40 μm thick) were collected in cryoprotective solution and stored at −80°C.

Nissl Staining
Sections were mounted on glass slides using 0.2% solution of gelatin (Merck) in 0.05 mol/L Trizma base (pH 7.6; Sigma) and dried at room temperature (RT) overnight. After 30 minutes of incubation with a mixture of chloroform (Panreac) and ethanol (Panreac), sections were sequentially hydrated with 100%, 90%, 70%, and 50% alcohol and rinsed in distilled water for 5 minutes. Nissl staining was performed by incubating the sections for 5 minutes in a solution containing 8.7 mmol/L thionine (Sigma), 30 mmol/L NaOH, and 1.2% ethanol. The sections were then washed with distilled water, dehydrated sequentially in increasing concentrations of alcohol for 2 minutes, lightened with acetic alcohol (Panreac), cleared in xylene for 6 minutes, and coverslipped with DPX (BDH Chemicals, Poole, UK).

Immunohistochemistry
Free-floating sections were washed with PBS eliminate, and endogenous peroxidase activity was inactivated by incubation for 30 minutes with 0.03% H2O2 in methanol (Sigma). After washing 3 times with PBS, the tissue was incubated for 40 minutes with blocking solution (4% normal goat serum, 0.05% Triton X-100 [Sigma] and 4% bovine serum albumin [Merck] in PBS) and exposed overnight at RT to the primary antibodies diluted in blocking solution. Rabbit anti-tyrosine hydroxylase ([TH] 1:1000; Chemicon, Temecula, CA) was detected by incubating sections in biotinylated goat anti-rabbit (1:1000; Jackson ImmunoResearch Laboratories, West Grove, PA) in blocking solution for 2 hours at RT. The biotinylated antibody was detected with peroxidase-conjugated avidin (1:5000; Sigma) for 90 minutes at RT, which was visualized with 0.05% diaminobenzidine (Sigma) in 0.03% H2O2/Trizma-HCl (pH 7.6). The sections were mounted on glass slides in a 0.2% solution of gelatin in 0.05 mol/L Tris (pH 7.6; Sigma), dried, and dehydrated in toluene (Panreac) for 12 minutes.
before coverslipping with DPX. For fluorescent immunohistochemistry, mouse anti-NeuN (1:1000; Millipore, Temecula, CA) and rabbit anti-PV (1:1000; Abcam, Cambridge, UK) antibody binding were detected with Alexa-568 donkey antiserum (1:1000; Invitrogen), respectively, diluted in blocking solution and incubated with the sections for 2 hours at RT. After several washes with PBS, the sections were mounted, dehydrated, and coverslipped as described above.

**Fluorescent In Situ Hybridization**

Sense and antisense riboprobes for rat GAD65 and GAD67 were prepared from the GAD65 and GAD67 cDNAs generously provided by Drs. A.J. Tobin and N.J.K. Tillakaratne (36). Riboprobe synthesis and hybridization were performed as previously described (37). Specificity of the riboprobes was tested by colorimetric ISH to confirm labeling using the antisense probe and its absence using the sense probe.

**Triple Labeling**

For the detection of GAD mRNAs in combination with double immunohistochemistry after performing ISH, the tissue was washed extensively and immunohistochemistry was performed as described above.

**Image Analysis**

The optical density of striatal TH-immunoreactive fibers was measured in each animal at 6 different rostrocaudal levels, from +2.16 mm to −0.36 mm relative to the bregma (34). Images of coronal sections were taken under the same conditions using a digital camera (DXM1200F) coupled to a Nikon Eclipse microscope (Barcelona, Spain). The digitized images were analyzed using Metamorph image analysis software (Molecular Devices, Sunnyvale, CA). The number of TH-immunoreactive neurons in the substantia nigra pars compacta was assessed in 3 consecutive sections from the medial terminal nucleus of the accessory optic tract, −5.28 mm relative to the bregma (34), and all immunoreactive cells lateral to the medial terminal nucleus of the accessory optic tract were counted. The GP was analyzed in tissue sections taken from 5 different rostrocaudal levels, from −0.6 mm to −1.2 mm relative to the bregma (34). Three images from each section and hemisphere were obtained in the dorsoventral axis on an LSM 510 confocal microscope (Zeiss, Madrid, Spain) using a 40× oil objective, and a total of 15 images per slide were analyzed. A projection of each stack was analyzed using Metamorph software. The total number of NeuN-positive, PV-positive, and PV-negative cells was determined, and the average fluorescence intensity of the hybridized probe was quantified in each cell. The monkey GPe was analyzed in 1 coronal section, obtaining images along the dorsoventral and mediolateral axes.

**Stereological Counting**

The number of Nissl-stained neurons present in the GP/GPe was determined by unbiased design-based stereology. Stereological counting in 6-OHDA rats (5 animals per group) was performed in 5 coronal GP sections (40 μm thick) taken at uniform intervals (400 μm) that covered the entire rostrocaudal extent of the nucleus. In MPTP monkeys (2 controls and 3 MPTP-treated animals), we analyzed 7 such coronal GPe sections covering a rostrocaudal extension ranging from −2 mm to −5 mm caudal to the anterior commissure (38). The reference volume of the GP/GPe was calculated from images obtained with the 2× objective using a point count array according to Cavalieri principles (39). The cross-sectional area of the nucleus was measured, and the reference volume (Vr) for the entire GP/GPe was estimated using the following equation:

\[ Vr = \frac{Ta}{ap} \sum_{i=1}^{n} Pi \]

where T is the section thickness, ap is the area of each point, and Pi is the number of points falling within the GP/GPe. All stereological counting was performed using an Olympus Bx61 microscope equipped with a camera (model DP71; Olympus, Hicksville, NY) and a stage connected to an XYZ stepper (Hoersholm, Denmark). The GP/GPe region was outlined at low magnification (4×) to estimate the area. The number of labeled neurons was calculated at 100× magnification under oil immersion using randomized meander sampling and the optical dissector methods. The optical dissector height was 12 μm, and the sampling area and counting frame were 3% and 3,025.6 μm² for rats and 5% and 4,538 μm² for monkeys, respectively. Unbiased counting was performed by an investigator blind to the treatment, and the total number of Nissl-positive neurons (N) was calculated using the following formula:

\[ N = \sum Q \frac{1}{h asf ssf} \]

where \( \sum Q \) is the total number of particles counted, \( t \) is the mean section thickness, \( h \) is the height of the optical dissector, \( asf \) is the area sampling fraction, and \( ssf \) is the section sampling fraction. Neuronal density (D) was determined using the following formula:

\[ D = N / Vr \]

**Statistical Analysis**

The data in the graphs are presented as the mean ± SEM. All data were analyzed using SPSS 15.0 software. A mixed-model analysis of variance (ANOVA) was used to detect statistical differences in cell counts and cell density between the 2 hemispheres (intrasubject factor) in sham- and 6-OHDA–lesioned animals (intersubject factor), followed by a post hoc corrected Student t-test. To analyze GAD mRNA expression, 3-way ANOVA was used to identify differences between the 3 factors: group, hemisphere, and cell type.

**RESULTS**

**Neuronal Subpopulations in 6-OHDA–Lesioned Rats**

The PV-positive and PV-negative subpopulations of pallidal neurons were studied in 6-OHDA–lesioned rats. The
extent of the lesion was first assessed 5 weeks after the injection of 6-OHDA by quantifying amphetamine-induced rotational behavior ipsilateral to the lesion side (40). Sham-lesioned animals exhibited no rotational behavior, whereas lesioned animals that rotated more than 6 turns per minute toward the lesioned side were analyzed further (Fig. 1A). Anti-TH immunohistochemistry revealed nigrostriatal cell loss at the level of the striatum and substantia nigra pars compacta (Fig. 1B), and densitometric analysis of TH-positive fibers in the striatum (Fig. 1C) and the number of TH-positive cells in the substantia nigra pars compacta (Fig. 1D) revealed almost complete loss of dopaminergic neurons.

Changes in pallidal neuron subpopulations in the absence of dopaminergic input to the basal ganglia were analyzed by NeuN and PV immunohistochemistry. This was combined with ISH to assess GAD65 (Fig. 2A) and GAD67 (Fig. 2B) mRNA expression in PV-positive and PV-negative cells. Cell numbers in the GP were determined by counting NeuN-positive cells (Fig. 3A). Mixed-model ANOVA revealed significant differences when both hemisphere and group were taken into account (F1,9 = 7.3, p = 0.025). Post hoc tests demonstrated a significant decrease (13% ± 4%, p < 0.05) in the number of NeuN-positive cells in the GP ipsilateral to the 6-OHDA injection site versus the contralateral GP, in which the neuronal number was similar to that observed in both hemispheres of sham-lesioned rats. To determine whether this decrease was specific to a given subtype of pallidal neurons, double labeled NeuN-positive/PV-positive cells were counted (Fig. 3B). In the GP contralateral to the 6-OHDA lesion in parkinsonian animals, the number of PV-positive cells was similar to that found in sham-lesioned animals. By contrast, there were significantly fewer PV-positive cells in the ipsilateral GP of 6-OHDA-lesioned animals versus the same hemisphere in sham-lesioned animals. Mixed-model ANOVA analysis of the total cell number in both subpopulations revealed significant differences (F1,9 = 11.2, p < 0.01) when both hemisphere and group were taken into account. Furthermore, post hoc tests confirmed the significant decrease (21% ± 5%) in the NeuN-positive/PV-positive subpopulation versus both the contralateral hemisphere of the same animal and the sham-lesioned rats. Unbiased stereological counting of Nissl-stained neurons in the GP (Fig. 3C, D) confirmed a significant decrease (12% ± 4%, p < 0.05) in the numbers of pallidal neurons in the hemisphere ipsilateral to the 6-OHDA lesion.

To investigate the potential changes in PV-positive and PV-negative neuronal activity in the rat GP, we analyzed GAD65 and GAD67 mRNA expression (Fig. 4). The 6-OHDA lesion did not induce significant changes in GAD65 mRNA levels in any pallidal subpopulation, PV positive or PV negative (Fig. 4A), although GAD65 mRNA expression was greater in PV-negative versus PV-positive cells (3-way ANOVA, F1,44 = 19.1, p < 0.001). By contrast, the expression of GAD67 mRNA was similar in the 2 neuronal subtypes, and it was unaffected by 6-OHDA lesion (Fig. 4B). Taken together, these results indicate that, in parkinsonian conditions, a specific loss of PV-positive cells occurs in the GP that is not accompanied by gross alterations in GAD mRNA expression in either neuronal subpopulation.
To determine whether the number of pallidal neurons also decreased in the primate model of PD, we analyzed the cell density in the GPe of MPTP-treated monkeys. Nissl-stained sections covering the rostrocaudal extension of the GPe were analyzed (Fig. 5A) and the total number of neurons in 2 controls and 3 MPTP-treated animals was quantified blindly by stereology. In agreement with the results obtained in the 6-OHDA-lesioned rats, there were lower numbers

**Neuronal Subpopulations in MPTP-Treated Monkeys**

To determine whether the number of pallidal neurons also decreased in the primate model of PD, we analyzed the cell density in the GPe of MPTP-treated monkeys. Nissl-stained sections covering the rostrocaudal extension of the GPe were analyzed (Fig. 5A) and the total number of neurons in 2 controls and 3 MPTP-treated animals was quantified blindly by stereology. In agreement with the results obtained in the 6-OHDA-lesioned rats, there were lower numbers...
(29%) of Nissl-stained neurons in the GPe of the MPTP-treated monkeys. Subsequently, double immunostaining for NeuN and PV was performed in samples from each animal in equivalent coronal sections taken 3.5 mm caudal to the anterior commissure (Fig. 5C) (38). Data corresponding to several fields from every section (20 images per section) were processed blindly (Fig. 5D). Despite the limited availability of primates that limited statistical analysis, there was a clear trend toward a lower proportion of PV-positive cells in samples from MPTP-treated monkeys. Taken together, these
findings indicate a loss of pallidal neurons in the 6-OHDA rat model that likely also occurs in the MPTP monkey model, resulting in an imbalance in the proportion of PV-positive versus PV-negative cells.

DISCUSSION

Using stereology and NeuN immunostaining, we have demonstrated a significant neuronal loss in the GP of 6-OHDA-lesioned rats, specifically affecting PV-positive pallidal neurons. A similar trend was observed in the GPe of MPTP-treated primates, suggesting that this phenomenon may also occur in PD patients. To our knowledge, this is the first demonstration of nondopaminergic cell loss in the basal ganglia in animal models of PD.

Analysis of neuronal subpopulations based on PV expression revealed a specific decrease in the total number of PV-positive cells in the GP of 6-OHDA-lesioned rats. Interestingly, PV-negative cells were unaffected by the loss of the nigrostriatal pathway. A loss of immunoreactivity rather than PV-positive cells per se has been described in the zona incerta of 6-OHDA-lesioned rats (41). In the present study, the total number of cells in the GP was reduced, although the number of PV-negative neurons was unaffected. The most likely explanation for these results is a reduction in the total number of GP neurons caused by the specific loss of PV-positive cells. Because PV-positive neurons project mainly to the STN (8, 26, 27), these findings suggest that the hyperactivity of the STN in parkinsonism may be caused at least in part by the specific loss of pallidal neurons projecting to this nucleus.

It was previously reported that there were no significant differences in the total number of PV-positive neurons in the human GPe (6 PD and 5 control samples) (33). However, the GPe volumes analyzed were different from those in the present study. When considering the cell density rather than the absolute counts, there is a trend toward the decrease in the density of PV-positive cells in the GPe of PD patients that would be consistent with the findings reported here. It is likely that this trend to a decrease in PV-positive cell density in human subjects (p = 0.08, Mann-Whitney U test) and in monkeys (Fig. 5) would reach significance in an analysis of a larger sample size.

The N-methyl-D-aspartate receptor-mediated loss of NeuN-positive cells may be triggered by haloperidol treatment after administering high doses of methamphetamine, which is excitotoxic to GABAergic, but not to dopaminergic, neurons (42). Because of the reciprocal projections between the GPe and the STN, the hyperactivity of the STN in PD may induce excitotoxic damage in the pallidal subpopulation of PV-positive neurons. These excitotoxic effects may be highly localized, as suggested by the comparable overall glutamate content in 6-OHDA- and sham-lesioned rats (data not shown). To the best of our knowledge, calcification is the only evidence of postsynaptic glutamate-induced damage in the GP/GPe. In the central nervous system, calcification is a progressive and regulated process that depends on the vulnerability of the affected area to glutamate (43, 46) and may be mediated by AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and N-methyl-D-aspartate receptor activation (47, 48).
Levels of GAD65 and GAD67 mRNA expression have been studied previously to analyze changes in GABAergic activity in the parkinsonian GP/GPe. The expression of GAD65 seems not to vary in any animal model of PD (15, 17, 49), whereas increased GAD67 expression has been described in the GPe of MPTP-treated monkeys using radioactive ISH (16, 50) and immunohistochemical assays (49). Interestingly, radioactive ISH in MPTP-treated primates identified alterations in GAD67 mRNA levels exclusively at one of the 4 rostrocaudal levels analyzed in the GPe (17). In the 6-OHDA rat model (14, 15), larger increases in GAD67 mRNA were observed in the hemisphere ipsilateral to the 6-OHDA lesion than in the contralateral hemisphere or in either hemisphere in sham-lesioned animals. Radioactive ISH studies also revealed increases in GAD67 mRNA expression in the GP of 6-OHDA-lesioned rats at $-0.92$ mm relative to bregma (51). Using nonradioactive ISH, we sought to identify alterations in the GAD mRNA expression that could reconcile the classical model assumption of GP/GPe hypoactivity with the reports of unchanged GAD65 and elevated GAD67 levels (mRNA and/or protein). The analysis of 5 sections from $0.6$ to $1.2$ mm relative to bregma (34) revealed that PV-positive and PV-negative cells display similar levels of mRNA for GADs in the GP of sham and 6-OHDA rats. Thus, the GABAergic activity of
PV-negative neurons, which project mainly to the striatum and that are not affected by the dopaminergic lesion, seems to be similar to that of control animals. The specific loss (21%) of GP PV-positive neurons in 6-OHDA–lesioned rats and the similar trend observed in MPTP-treated monkeys could result in a significant alteration in the GABAergic input to the STN. On one hand, a decrease in innervation from the GP/GPe would lead to the reduced GABA release observed in the STN of MPTP-treated rhesus monkeys (22). On the other hand, in the 6-OHDA rat model, it has been shown that prototypic GABAergic neurons that innervate downstream basal ganglia nuclei like the STN are mainly PV-positive and fire antiphase to the STN neurons (27). Therefore, a reduction of PV-positive pallidal projecting neurons likely could contribute to the STN hyperactivity described in parkinsonian conditions.

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REFERENCES

5. Smith Y, Bolam JP. The output neurones and the dopaminergic neurones of the substantia nigra receive a GABA-containing input from the globus pallidus in the rat. J Comp Neurol 1990;296:47–64
13. Drenger SM, Ottnans GA. Rapid increases in cerebellar Purkinje cell glutamatic acid decarboxylase (GAD67) mRNA after lesion-induced increases in cell firing. Brain Res 1993;615:175–79
15. Soghomonian JJ, Chesselet MF. Effects of nigrostral lesions on the levels of messenger RNAs encoding two isoforms of glutamate decarboxylase in the globus pallidus and entopeduncular nucleus of the rat. Synapse 1992;11:124–33
17. Schneider JS, Wade TV. Experimental parkinsonism is associated with increased pallidal GAD gene expression and is reversed by site-directed antisense gene therapy. Mov Disord 2003;18:32–40
18. Pan HS, Walters JR. Unilateral lesion of the nigrostriatal pathway decreases the firing rate and alters the firing pattern of globus pallidus neurons in the rat. Synapse 1994;20:165–70
47. Petegnief V, Saura J, Dewar D, et al. Long-term effects of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate and 6-nitro-7-sulphamoylbenzo(1)quinoxaline-2,3-dione in the rat basal ganglia: Calcification changes in glutamate receptors and glial reactions. Neuroscience 1999;94:105–15