Glutamic Acid Decarboxylase 67 mRNA Regulation in Two Globus Pallidus Neuron Populations by Dopamine and the Subthalamic Nucleus

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The globus pallidus (GP) consists of two neuron populations, distinguished according to their immunoreactivity for parvalbumin (PV). The PV-immunoreactive (PV+) neurons project preferentially to “downstream” targets such as the subthalamic and entopeduncular nuclei, whereas neurons lacking PV (PV− neurons) project preferentially to the striatum, suggesting a role for PV− cells in feedback to striatal neurons. Although dopamine D2 antagonist administration induces immediate early gene expression preferentially in PV− GP neurons, little is known about long-term regulation of PV− versus PV+ GP neurons. Nigral 6-hydroxydopamine (6-OHDA) lesions or repeated D2-class antagonist injections have been shown to increase pallidal expression of glutamate decarboxylase (GAD₆₇ isoform) mRNA. This increase in GAD₆₇ is believed to be secondary to activation of excitatory subthalamopallidal projections. The current study examined the effects of subthalamic nucleus (STN) lesion on 6-OHDA- or repeated D2 antagonist-induced changes in GP GAD₆₇ mRNA expression in PV+ and PV− neurons. Five or 21 d after nigral 6-OHDA injections or after 3, 7, or 21 d of D2 antagonist administration, GAD₆₇ mRNA increased in both the PV− and PV+ GP neurons, but the magnitude of the increase was significantly greater in PV− neurons. By contrast, STN lesion resulted in declines in GAD₆₇ mRNA in both cell populations, with the decreases in PV+ neurons exceeding those in PV− neurons. Furthermore, STN lesion completely blocked 6-OHDA- or D2 antagonist-induced GAD₆₇ mRNA increases in PV+ cells but only partly offset the GAD₆₇ mRNA increase in PV− pallidal neurons. Thus, the PV+ and PV− neurons are influenced in qualitatively similar ways by dopamine and the STN, but these cell types exhibit contrasting degrees of regulation by the dopaminergic and STN perturbations. This pattern of results has implications for pallidal control of striatal versus downstream basal ganglia nuclei.

Key words: dopamine; GAD; globus pallidus (pallidum); Parkinson; substantia nigra; subthalamic nucleus

Introduction

Far from being a simple relay nucleus for striatal efference, the rodent globus pallidus (GP) is critically positioned to influence activity in all other basal ganglia structures (Staines et al., 1981; Smith and Bolam, 1990; Bevan et al., 1997; Sato et al., 2000). The GP is a heterogeneous structure (Kita, 1994; Rajakumar et al., 1994; Hoover and Marshall, 1999) whose neuron gene expression can be differentially regulated by pallidal afferent projections (Ruskin and Marshall, 1997; Hoover and Marshall, 2002; Billings and Marshall, 2003). The identification of distinct pallidal neuron populations has aided our understanding of this structure. In general, pallidal neurons that are retrogradely labeled from the subthalamic nucleus (STN) and the basal ganglia output nuclei contain the calcium-binding protein parvalbumin (PV), whereas pallidal neurons retrogradely labeled from the striatum more fre-

quently contain the opioid precursor preproenkephalin (PPE) mRNA (Ruskin and Marshall, 1997; Hoover and Marshall, 1999, 2002). Parvalbumin and PPE rarely colocalize in pallidal neurons (Hoover and Marshall, 1999, 2002).

Although the GP receives moderate innervation by dopamine (DA) terminals (Fallon and Moore, 1978; Lindvall and Björklund, 1979), the function of dopamine in GP remains poorly understood. One suggestion is that DA acts presynaptically on D2-class receptors, decreasing GABA release from striatopallidal terminals (Floran et al., 1997; Cooper and Stanford, 2000). However, other pallidal responses are not easily reconciled with this suggestion. Systemic or intrapallidal administration of a D2-class antagonist induces Fos, the protein product of the immediate early gene (IEG) *c-fos*, almost exclusively in GP neurons that lack PV (Ruskin and Marshall, 1997; Billings and Marshall, 2003), findings not mimicked by intrapallidal application of a GABA_A agonist.

An important unanswered question is whether these short-term changes in IEG induction predict longer-term alterations in neurotransmitter-related gene expression within the relevant cell populations. Here we investigate changes in mRNA for the 67 kDa isoform of glutamic acid decarboxylase (GAD), the key enzyme in GABA neurotransmission within all GP neurons (Oertel

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and Mugnaini, 1984). Although previous studies have indicated that GP GAD₆₇ mRNA levels increase after DA neuron degeneration or chronic dopamine receptor blockade (Kincaid et al., 1992; Soghomonian and Chesselet, 1992; Delfs et al., 1995a), no previous studies have examined this the regulation of this mRNA in specific GP cell populations.

The regulation of pallidal gene expression by DA appears also to involve the STN. Because STN lesions reverse 6-hydroxydopamine (6-OHDA) lesion-induced pallidal GAD₆₇ mRNA increase, the GAD₆₇ mRNA increase after 6-OHDA lesions may be triggered by increased subthalamopallidal activity (Delfs et al., 1995b). Therefore, 6-OHDA lesion-induced changes in GP late gene expression may result from a combination of increased subthalamopallidal glutamatergic input and decreased nigropallidal dopaminergic input, influences that could vary depending on pallidal cell type. Because previous work (Ruskin and Marshall, 1997; Hoover and Marshall, 2002; Billings and Marshall, 2003) demonstrated that PV[−]/PPE⁺ pallidostriatal neurons are selectively sensitive to dopaminergic D2 antagonism, this population of neurons provides the most likely site for DA–glutamate interactions.

This study explores the population specificity of pallidal GAD₆₇ mRNA regulation after 6-OHDA lesion or repeated dopamine antagonist administration. In light of those findings, we also examined the effects of STN lesions on these changes in GAD₆₇ mRNA in PV⁺ and PV[−] pallidal neurons.

Materials and Methods

Adult male Sprague Dawley rats ($n = 190$; Charles River Laboratories, Hollister, CA) weighing 200–275 gm at the beginning of the experiment were individually housed and kept on a 12 hr light/dark schedule. All rats were given *ad libitum* access to food and water. Experiments were performed during the light portion of the diurnal cycle. All animal procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and all appropriate measures were taken to minimize pain and discomfort in experimental animals.

Experiment 1: nigral 6-hydroxydopamine lesions

For the nigral DA cell lesions, rats ($n = 22$) weighing 200–250 gm at the time of surgery were anesthetized with Equithesin (10 mM sodium pentobarbital, 256.8 mM chloral hydrate, 86 mM MgSO₄, 10.5% propylene glycol, and 12% ethanol, administered at 4.2 mg/kg, i.p.) and placed in a Kopf stereotaxic apparatus. Eight micrograms of 6-OHDA hydrobromide (HBr) (as base; Sigma, St. Louis, MO) dissolved in 4 μ l of 0.1% ascorbic acid in 0.9% saline ($n = 16$) or the vehicle ($n = 6$) were injected into the left ventral tegmentum through a 28 gauge cannula at +2.6 mm from the interaural plane, +1.0 mm from midline, and −7.8 mm from dura. Vehicle injections (saline) were −6.8 mm from dura to prevent nonspecific damage to the substantia nigra attributable to cannula penetration. Thirty minutes before the 6-OHDA HBr or vehicle infusion, desipramine HCl (Sigma; 15 mg/kg, i.p., as base) was administered to each animal to antagonize the uptake of the 6-OHDA HBr into norepinephrine-containing cells or in sham lesion animals to serve as a control for the effects of desipramine alone. Animals in this study were perfused, as described below, 5 or 21 d after lesion (eight lesioned animals and three sham-lesioned animals at each time point). Tyrosine hydroxylase (TH) immunocytochemistry was performed on coronal sections through the substantia nigra pars compacta (SNc) from these animals to verify the extent of dopamine cell loss in 6-OHDA-lesioned animals. All animals included in the study had fewer than five TH-immunoreactive cells in each section of the SNc examined (Fig. 1D). No damage was noted in the uninjected hemisphere (Fig. 1C).

Experiment 2: repeated dopamine antagonist injections

For the repeated injection study, rats ($n = 72$) weighing 250–275 gm at the beginning of the experiment were injected with the D2-class antagonist eticlopride (Research Biochemicals, St. Louis, MO; 1 mg/kg, s.c.; $n =$

Figure 1. Photomicrographs of cresyl violet-stained sections of the STN of intact (A) and kainic acid-lesioned (B) animals and of TH-immunoreactive neurons in the SNc of intact (C) and 6-OHDA-lesioned (D) animals. In A, arrows denote the borders of the STN. Note the gliosis in B and the lack of intact STN neurons. Also note the near total lack of TH-immunoreactive neurons in D.

24), the D1-class antagonist *R*(+/-)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine maleate (SCH-23390) (Schering Corp., Bloomfield, NJ; 1 mg/kg, i.p.; $n = 24$), or saline (1 mg/kg; $n = 24$) for 3, 7, or 21 d ($n = 8$ at each time point). All animals were perfused, as described below, 24 hr after the last injection.

Experiments 3 and 4: combined subthalamic nucleus lesion and 6-OHDA or repeated D2 antagonist administration

STN lesion and 6-hydroxydopamine lesion study. For the subthalamic nucleus lesions, rats ($n = 32$) weighing 250–275 gm at the time of surgery were anesthetized with Equithesin (4.2 ml/kg, i.p.) and placed in a Kopf stereotaxic apparatus. Animals receiving STN lesions ($n = 16$) received 0.3 μ g of kainic acid dissolved in 0.1 μ l of 0.9% sterile saline into the left subthalamic nucleus at +5.1 mm from interaural, +2.6 mm from midline, and −7.4 mm ventral to dura. Sham animals ($n = 16$) received an equal amount of sterile saline at the same coordinates, except the dorsal-ventral coordinate was adjusted to −6.4 ventral to dura to avoid damage to the subthalamic nucleus attributable to cannula penetration. Animals were allowed to recover for 6 d in their home cages before receiving a 6-OHDA ($n = 16$) or sham lesion ($n = 16$) of the SNc as described above.

Of the 32 animals in this study, 8 received an STN lesion and 6-OHDA lesion (LL group); 8 received an STN lesion and a sham SNc lesion (LS group); 8 received a sham STN lesion and a 6-OHDA SNc lesion (SL group); and 8 received sham lesions in both structures (SS group). Five days after the 6-OHDA or sham SNc lesion, animals were transcardially perfused as described below.

All animals included in the LS and LL groups had complete STN lesions as verified with cresyl violet staining of sections through the entire STN (Fig. 1B). No damage was noted in the contralateral STN (Fig. 1A). All animals included in the SL and LL groups had fewer than five TH-immunoreactive cells in every SNc section examined. No damage was noted in the unlesioned hemisphere.

STN lesion and repeated injection study. For the combined STN lesion and repeated injection study, rats ($n = 64$) weighing 250–275 gm at the beginning of the experiment were given an STN lesion ($n = 32$) or a sham lesion ($n = 32$) as described above and allowed 6 d to recover after the lesion. Then animals were injected twice daily with the D2 antagonist eticlopride (Research Biochemicals; 1 mg/kg, s.c.; $n = 32$), or saline (1 ml/kg; $n = 32$) for 3 or 7 d. Therefore, there were eight groups in this study: STN lesion combined with 3 d eticlopride (L-3E) or 3 d saline (L-3V); sham STN lesion combined with 3 d eticlopride (S-3E) or 3 d saline (S-3V); STN lesion combined with 7 d eticlopride (L-7E) or 7 d

saline (L-7V); and sham STN lesion combined with 7 d eticlopride (S-7E) or 7 d saline (S-7V). All groups consisted of eight animals. All animals were perfused, as described below, 24 hr after the last injection.

Tissue processing

At their respective time points, animals from each study were transcardially perfused with ice-cold 0.1 M PBS (60 ml) followed by 4% paraformaldehyde (120 ml). After perfusion, the brains were rapidly removed and postfixed overnight in 4% paraformaldehyde, followed by cryoprotection for 24 hr in 30% sucrose in 0.1 M phosphate buffer (PB). Coronal sections (30 μ m) through the GP were cut on a sliding microtome. GP sections were taken 0.4–1.1 mm caudal to bregma (Paxinos and Watson, 1998), which includes the GP regions most relevant to defined characteristics of basal ganglia circuitry (Shammah-Lagnado et al., 1996). All sections were stored in sterile 0.1 M PB until being processed for GAD₆₇ mRNA *in situ* hybridization later the same day. Tissue was processed through free-floating *in situ* hybridization using a 2.1 kb GAD₆₇ antisense riboprobe transcribed in the presence of [³⁵S]UTP with a T7 RNA polymerase promoter. The GAD₆₇ cDNA was kindly donated by Dr. A. Tobin (University of California, Los Angeles, CA). Hybridization procedures consisted of sequentially rinsing the tissue in 0.75% glycine in 0.1 M PB, 0.1 M PB, 0.25% acetic anhydride in 0.1 M triethanolamine, 2 $\times$ SSC, a series of ascending and descending alcohols, and chloroform treatment. Sections were then immersed in hybridization buffer that contained the ³⁵S-labeled riboprobe at a density of 15,000 cpm/ μ l. Hybridization was performed overnight at 55°C. Sections were then washed through two formamide and SSC rinses followed by an RNase buffer and RNase A treatment (20 mg/ml) and SSC rinses. Immediately subsequent to the GAD₆₇ mRNA *in situ* hybridization, the tissue was processed for PV immunocytochemistry. The sections were incubated in 5% normal horse serum (NHS; Vector Laboratories, Burlingame, CA) followed by incubation in 1% NHS (Vector Laboratories) containing an anti-PV monoclonal antibody (1:1000; Sigma) for 48 hr at 4°C. Sections were then incubated with a biotinylated horse anti-mouse secondary antibody (1:200, 2 hr at room temperature; Jackson ImmunoResearch, West Grove, PA), followed by avidin-biotin-peroxidase complex (1 hr; Vector Laboratories). PV was visualized by using 6% 3,3'-diaminobenzidine (Pierce, Rockford, IL), and sections were then rinsed in 0.1 M Tris buffer and mounted on gelatin-coated slides. Two slides per animal to be used in analysis were dipped in NTB-2 emulsion (Eastman Kodak Co., Rochester, NY) for 5–8 d; one slide per animal was packed for 2 d against Raymax β film to gauge the strength of the *in situ* hybridization signal to determine how long slides should be kept in emulsion. Film and slides were developed with Kodak D-19, and slides were counterstained with hematoxylin-eosin (H&E) and coverslipped.

Tissue analysis

Fields in the GP were imaged at an optical magnification of 400 $\times$ using an Optiphot microscope (Nikon, Melville, NY) interfaced to a microcomputer imaging device image analysis system (Imaging Research, St. Catharines, Ontario, Canada) calibrated to quantify the density of silver grains (representative of ³⁵S-GAD₆₇ mRNA) and to assess their association with PV+ neurons. Because parvalbumin is not distributed equally throughout the GP (Rajakumar et al., 1994), the GP was subdivided into 12 equally sized regions at approximately -0.92 mm from bregma (Paxinos and Watson, 1998) (see Fig. 3). Two channels were linked in the image analysis system. The first channel contained a bright-field image of one of the 12 regions in the GP. This channel was linked to a second channel that contained a dark-field representation of the same bright-field image. Using a sampling tool that was slightly larger than the largest PV+ neuron in each sector, cells were selected in bright field to allow identification of whether they were PV+, and the computer performed the grain counts in the corresponding dark-field channel (grains appeared as white pixels on a black screen). All neurons in each field (both PV+ and H&E-only) were quantified. (Pixel densities associated with 95–110 GP cells were collected for each animal or hemisphere.) Background levels of grain density were determined for each animal by using the sampling tool (of a size similar to the size described above) to measure pixel densities over the corpus callosum (for a total of 30 background readings per animal).

Statistical analysis

For all experiments, statistical analyses determined whether (1) GAD₆₇ mRNA increased in pallidal neurons of treated animals (Kincaid et al., 1992; Soghomonian and Chesselet, 1992; Delfs et al., 1995a), (2) any such increase occurred in predominantly PV+ or PV– pallidal neurons, (3) the duration of dopamine antagonist treatment or the interval since 6-OHDA infusion affected any cell population specificity of changes in GAD₆₇ mRNA expression, and (4) any changes in pallidal GAD₆₇ mRNA expression differed by GP region. GAD₆₇ mRNA (pixels per cell) was measured, and numbers were collapsed into a quadrant value (see Fig. 3).

For all groups, initial repeated measures ANOVA indicated there were no within-subjects regional differences. Consequently, the values for each quadrant were summed and collapsed into one value for PV+ and another value for PV– pallidal neurons for each animal. All further statistical analyses were conducted using these values. Repeated measures ANOVAs and subsequent simple main effects analyses were used to determine differences in GAD₆₇ mRNA expression with respect to treatment condition (or hemisphere) or cell type specificity.

Because substantial differences in the absolute values of GAD₆₇ mRNA were found between the two *in situ* hybridization reactions for experiment 1 (presumably because of differences in emulsion coating resulting in higher levels of GAD₆₇ mRNA and background labeling or other unknown variations in the hybridization), an initial repeated measures ANOVA with a single within-subject factor (hemisphere) was used to establish a main effect of lesion for this study. This ANOVA included “background readings” from the corpus callosum as a covariate (ANCOVA). To analyze the differences in GAD₆₇ mRNA levels in the two pallidal neuron cell types as well as to analyze any group effects, values were normalized to “percent increase from unlesioned hemisphere” for each cell type in each GP quadrant. No regional differences were noted with these values; they were therefore collapsed into a single value for PV+ and one for PV– GP neurons.

For experiment 2, one-way ANOVAs and *post hoc* Scheffé tests were used to explore differences between time points. For experiment 4, comparisons between groups (3 and 7 d injection groups) were made using independent sample *t* tests. In all statistical comparisons, the dependent variable was GAD₆₇ mRNA expression, unless otherwise noted. All values were considered significantly different at a $p < 0.05$ level.

Results

GAD₆₇ mRNA labeling

GAD₆₇ mRNA labeling was found in punctate clusters that overlaid PV+ neurons, H&E-counterstained neurons, or both (Fig. 2; see Fig. 6). A group of silver grains was considered a cluster if >10 pixels were contained within the sampling tool area, although clusters in this study contained at least 25 pixels per cell. Callosal pixel levels were well below cellular labeling levels (data not shown). Parvalbumin-positive neurons were easily identified by distinct brown labeling of the entire soma as well as neuronal processes. Neurons labeled by H&E stain only (i.e., PV– neurons) had clear, purple nuclei; glial cells labeled with H&E had dark, very small nuclei and were not included in the quantification. Soma size was used as an additional criterion for distinguishing glia from neurons when nuclear staining was questionable. Although PV+ cells are slightly larger than PV– cells, both types are substantially larger than glial cells. All GAD₆₇ mRNA quantification of silver grains was carried out under dark-field illumination, and identification of cells was performed under bright-field illumination.

Experiment 1: nigral 6-hydroxydopamine lesions

The first experiment examined the effects of nigral 6-OHDA injections on GAD₆₇ mRNA expression in PV+ and PV– neurons 5 and 21 d postoperatively. Overall GAD₆₇ mRNA levels were found to be significantly greater in the GP of the 6-OHDA-injected compared with uninjected hemispheres [ANCOVA;

Figure 2. Photomicrograph of GAD₆₇ mRNA in PV+ (brown cells) and PV– (yellow arrowheads) GP neurons of animals in experiment 1 (*A, B*) and experiment 2 (*C–E*). *A, B*, The unlesioned hemisphere is depicted in *A*, and the lesioned hemisphere is depicted in *B*. Note the increase in GAD₆₇ mRNA in the lesioned hemisphere overall and the more pronounced increase in PV– GP neurons compared with PV+ GP neurons. *C–E*, GAD₆₇ mRNA in PV+ (brown cells) and PV– (yellow arrows) GP neurons in vehicle-treated (*C*), SCH-23390-treated (*D*), and eticlopride-treated (*E*) animals. Note the eticlopride-induced increase in GAD₆₇ mRNA overall and the more pronounced increase in GAD₆₇ mRNA in PV– compared with PV+ GP neurons. Scale bar, 50 μ m.

$F_{(1,13)} = 102.8$; $p < 0.001$; Fig. 3]. GAD₆₇ mRNA was markedly increased in both the PV– and PV+ cells, and subsequent ANOVA using the normalized (percentage) values indicated that GAD₆₇ mRNA levels were significantly higher in the PV– than PV+ cells [$F_{(1,14)} = 29.9$; $p < 0.001$; Fig. 3]. However, neither a significant effect of days after lesion (group) nor a group $\times$ cell type interaction was detected.

Experiment 2: repeated dopamine antagonist injections

In the second experiment, animals received twice daily injections of the D2-class antagonist eticlopride, the D1-class antagonist SCH-23390, or the saline vehicle for 3, 7, or 21 d. Tissue was processed for GAD₆₇ mRNA *in situ* hybridization followed by PV immunocytochemistry.

Three day treatment

In animals injected for 3 d, the drug treatment condition (eticlopride, SCH-23390, or vehicle) significantly affected pallidal GAD₆₇ mRNA expression [$F_{(1,21)} = 7038.9$; $p < 0.001$; Fig. 4]. For both cell types, GAD₆₇ mRNA levels in eticlopride-treated animals were higher than in SCH-23390-treated animals [$F_{(1,21)} = 2010.6$; $p < 0.001$ for PV+ neurons; $F_{(1,21)} = 2145.2$; $p < 0.001$ for PV– neurons] or vehicle-treated animals [$F_{(1,21)} = 1907.9$; $p < 0.001$ for PV+ neurons; $F_{(1,21)} = 2276.1$; $p < 0.001$ for PV– neurons]. Moreover, GAD₆₇ mRNA levels between SCH-23390- and vehicle-treated animals did not differ for either cell type.

ANOVA also revealed a significant effect of cell type [$F_{(1,21)} = 11.6$; $p < 0.01$] as well as a significant drug treatment $\times$ cell type interaction [$F_{(2,21)} = 4.2$; $p < 0.05$]. Animals treated with eticlopride for 3 d had significantly higher levels of GAD₆₇ mRNA in PV– than in PV+ GP neurons [$F_{(1,21)} = 14.4$; $p < 0.01$], but the levels of GAD₆₇ mRNA in PV+ and PV– pallidal neurons in animals treated with SCH-23390 or vehicle did not differ.

Seven day treatment

A similar overall pattern was seen in animals treated with eticlopride, SCH-23390, or vehicle for 7 d (Fig. 4). The drug treatment condition significantly affected pallidal GAD₆₇ mRNA levels

[$F_{(1,21)} = 5287.6$; $p < 0.001$], with the GAD₆₇ mRNA levels in both cell types of eticlopride-treated animals being elevated relative to SCH-23390-treated [$F_{(1,21)} = 11769.2$; $p < 0.001$ for PV+ neurons; $F_{(1,21)} = 16589.2$; $p < 0.001$ for PV– neurons] or vehicle-treated animals [$F_{(1,21)} = 11137.9$; $p < 0.001$ for PV+ neurons; $F_{(1,21)} = 15626.3$; $p < 0.001$ for PV– neurons]. In addition, after 7 d of treatment, GAD₆₇ mRNA levels in both cell populations of SCH-23390-treated animals were significantly lower than GAD₆₇ mRNA levels in corresponding cell types of vehicle-treated controls [$F_{(1,21)} = 8.7$; $p < 0.01$ for PV+ neurons; $F_{(1,21)} = 14.4$; $p < 0.01$ for PV– neurons].

In animals injected for 7 d, GAD₆₇ mRNA levels differed between PV+ and PV– cell types [$F_{(1,21)} = 218.9$; $p < 0.001$], and a significant treatment $\times$ cell type interaction was observed [$F_{(2,21)} = 198.5$; $p < 0.001$]. In the group receiving eticlopride, GAD₆₇ mRNA levels in PV– GP neurons were significantly higher than in PV+ GP neurons [$F_{(1,21)} = 574.7$; $p <$

0.001]; by contrast, there were no cell population differences in animals treated for 7 d with SCH-23390 or vehicle. Figure 5 shows a frequency histogram of GAD₆₇ mRNA distribution in PV+ and PV– neurons of representative 7 d eticlopride and 7 d vehicle animals.

Twenty-one day treatment

A similar pattern was observed in animals treated for 21 d with eticlopride, SCH-23390, or vehicle (Fig. 4). The drug treatment condition significantly influenced pallidal GAD₆₇ mRNA levels [$F_{(1,21)} = 575.7$; $p < 0.001$], with GAD₆₇ mRNA levels being significantly higher in both cell populations of eticlopride-treated animals compared with SCH-23390-treated animals [$F_{(1,21)} = 380.9$; $p < 0.001$ for PV+ neurons; $F_{(1,21)} = 2421.4$; $p < 0.001$ for PV– neurons] or vehicle-treated groups [$F_{(1,21)} = 400.3$; $p < 0.001$ for PV+ neurons; $F_{(1,21)} = 2398.2$; $p < 0.001$ for PV– neurons]. The GAD₆₇ mRNA levels of SCH-23390- and vehicle-treated animals did not differ.

In addition, ANOVA revealed a significant effect of cell type [$F_{(1,21)} = 98.3$; $p < 0.001$] as well as a significant treatment $\times$ cell type interaction [$F_{(2,21)} = 110.4$; $p < 0.001$] in animals treated for 21 d. Although no population differences occurred in SCH-23390- or vehicle-treated animals, GAD₆₇ mRNA in eticlopride-treated animals was significantly higher in PV– than PV+ pallidal neurons [$F_{(1,21)} = 533.1$; $p < 0.001$].

Comparisons between time points

Additional comparisons revealed that overall pallidal GAD₆₇ mRNA levels of eticlopride-treated animals differed across the time span of the treatments [$F_{(2,23)} = 1313.3$; $p < 0.001$]. *Post hoc* Scheffé tests indicated that GAD₆₇ mRNA levels after both 3 and 21 d of eticlopride administration were lower than after 7 d of this treatment.

Because overall GAD₆₇ mRNA levels vary between each injection group, and because population differences emerged at all three time points in eticlopride-treated animals, it was important to determine whether differences between PV+ and PV– palli-

Figure 3. 6-OHDA lesion-induced increases in GP GAD₆₇ mRNA expression. Although 6-OHDA lesion increased GAD₆₇ mRNA expression in both PV+ and PV− GP neurons when compared with the unlesioned hemisphere (*), at both times (5 and 21 d), this increase was more pronounced in PV− GP neurons than PV+ GP neurons (◇). Note that this graph is expressed as percent increase from unlesioned hemisphere attributable to differences in background (and subsequent GAD₆₇ mRNA labeling) between the *in situ* runs. No differences were found between the initial measured values of GAD₆₇ mRNA expression in PV+ and PV− neurons in the unlesioned hemisphere; $n = 8$ for all groups. Les'd Hemi, Lesioned hemisphere; UnLes'd Hemi, unlesioned hemisphere. CPu, Caudate putamen; C, central sector; M, medial sector; D, distal sector; IC, internal capsule.

dal GAD₆₇ mRNA levels in eticlopride-treated animals varied at the three time points. To examine this possibility, difference scores were computed for each eticlopride-treated animal (PV− values − PV+ values) at each of the three time points. ANOVA revealed a significant difference between these difference scores for the three time points [$F_{(2,23)} = 56.8$; $p < 0.001$]. *Post hoc* Scheffé tests revealed that, whereas 3 d eticlopride-treated animals had significantly smaller difference scores than 7 or 21 d eticlopride-treated animals, the 7 and 21 d groups did not differ. Therefore, although overall GAD₆₇ mRNA levels were significantly lower in 21 d animals than in 7 d eticlopride-treated animals, the cell population difference was maintained for at least 3 weeks postoperatively (see Fig. 4).

Experiments 3 and 4: combined subthalamic nucleus lesion and 6-OHDA or repeated D2 antagonist administration

Experiment 3: combined STN lesion and 6-hydroxydopamine lesion

Animals received a subthalamic nucleus or sham lesion followed 6 d later by a SNc 6-OHDA or sham (vehicle) lesion, yielding four groups: LL, LS, SL, and SS. No differences were noted in GAD₆₇

Figure 4. Repeated administration of the D2 antagonist eticlopride (Etic) increases GAD₆₇ mRNA more markedly in PV− than PV+ GP neurons. Although GAD₆₇ mRNA expression increased in both PV+ and PV− GP neurons when compared with vehicle (Veh) controls (*), this increase was more pronounced in PV− GP neurons when compared to PV+ GP neurons (◇). In addition, at 7 d, SCH-23390 (SCH)-treated animals had significantly lower GAD₆₇ mRNA expression levels when compared with vehicle controls (#). No neuron population differences in GAD₆₇ mRNA expression were seen in animals treated with vehicle or the D1 antagonist SCH-23390; $n = 8$ for all groups. Top panel, Dark-field photomicrograph showing silver grain clusters from a 7 d eticlopride-treated animal. Gray arrows indicate PV+ GP neurons, and white arrows indicate PV− GP neurons.

mRNA expression levels between PV+ and PV− cells in the SS group.

Overall, clear trends were evident toward both 6-OHDA-induced increases and STN lesion-induced decreases in GAD₆₇ mRNA, with the PV− and PV+ cell populations being distinctly affected (Fig. 6). For both PV+ and PV− pallidal neurons, ANOVA revealed a significant interaction between lesion type [LL, LS, SL, SS groups, $F_{(1,28)} = 59.1$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 116.9$; $p < 0.001$ for PV− cells; Fig. 7]. GAD₆₇ mRNA levels in PV+ cells were significantly lower in the LL group compared with the SS group [$F_{(1,14)} = 11.1$; $p < 0.01$]. By contrast, GAD₆₇ mRNA levels in PV− pallidal neurons were significantly increased in the LL group compared with the SS group [$F_{(1,14)} = 30.7$; $p < 0.001$]. Indeed, whereas the GAD₆₇ mRNA levels in PV+ and PV− neurons of the SS group did not differ, GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons of the LL group [$F_{(1,7)} = 150.9$; $p < 0.001$].

As originally described by Delfs et al. (1995b), the STN lesion

Figure 5. Frequency histograms demonstrating GAD₆₇ mRNA distribution in PV+ and PV− cells of 7 d vehicle-treated (top) and 7 d eticlopride-treated (bottom) animals. Note (1) the overall increase in GAD₆₇ mRNA and (2) the greater GAD₆₇ mRNA expression in PV− GP neurons with respect to PV+ GP neurons for the eticlopride-treated rat.

significantly affected pallidal GAD₆₇ mRNA levels [$F_{(1,28)} = 210.502$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 227.370$; $p < 0.001$ for PV− cells]. STN lesion alone (LS group) resulted in a slight decrease in GAD₆₇ mRNA labeling in both GP cell populations when compared with sham controls [SS group, $F_{(1,14)} = 20.4$; $p < 0.001$ for PV+ neurons; $F_{(1,14)} = 9.5$; $p < 0.01$ for PV− neurons]. This decline in GAD₆₇ mRNA after STN lesion alone was more pronounced in PV+ than in PV− pallidal neurons. Although GAD₆₇ mRNA levels in PV+ and PV− neurons of the SS group did not differ, GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons of the LS group [$F_{(1,7)} = 16.0$; $p < 0.01$].

The SNc 6-OHDA lesion also significantly affected pallidal GAD₆₇ mRNA levels [$F_{(1,28)} = 137.0$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 465.6$; $p < 0.001$ for PV− cells]. As shown previously (Kincaid et al., 1992; Soghomonian and Chesselet, 1992) (experiment 1), an SNc 6-OHDA lesion alone (SL group) significantly increased GAD₆₇ mRNA in both GP cell populations compared with the sham control [SS group, $F_{(1,14)} = 190.7$; $p < 0.001$ for PV+ neurons; $F_{(1,14)} = 562.2$; $p < 0.001$ for PV− pallidal neurons]. This 6-OHDA-induced increase in GAD₆₇ mRNA was greater in PV− than PV+ pallidal neurons. Although GAD₆₇ mRNA levels in PV+ and PV− neurons of the SS group did not differ, GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons of the SL group [$F_{(1,7)} = 123.0$; $p < 0.001$].

Experiment 4: combined STN lesion and repeated D2 antagonist injections

Animals received a subthalamic nucleus or sham lesion followed 6 d later by 3 or 7 d of twice-daily injections of the D2 antagonist

eticlopride or vehicle saline. This yielded eight groups: L-3E, L-3V, S-3E, S-3V, L-7E, L-7V, S-7E, and S-7V. No differences were noted in GAD₆₇ mRNA expression levels between PV+ and PV− cells in the S-3V or S-7V groups.

Overall, clear trends were seen toward eticlopride-induced increases in GAD₆₇ mRNA expression at both time points, as well as STN lesion-induced decreases in GAD₆₇ mRNA, with the PV+ and PV− cell populations being distinctly affected (Figs. 6, 8). For groups given 3 d of treatment, ANOVA revealed a significant treatment $\times$ lesion interaction [$F_{(1,28)} = 125.2$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 132.3$; $p < 0.001$ for PV− cells; Fig. 8]. When compared with animals in the S-3V group, animals in the L-3E group showed significantly decreased levels of GAD₆₇ mRNA in PV+ pallidal neurons [$F_{(1,14)} = 14.1$; $p < 0.01$] but increased levels of GAD₆₇ mRNA expression in PV− pallidal neurons [$F_{(1,14)} = 104.9$; $p < 0.001$]. Indeed, GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons in animals of the L-3E group [$F_{(1,7)} = 136.4$; $p < 0.001$].

As in the previous experiment, the STN lesion significantly affected pallidal GAD₆₇ mRNA in the two populations of cells [$F_{(1,28)} = 723.7$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 203.1$; $p < 0.001$ for PV− cells]. GAD₆₇ mRNA levels in PV+ pallidal neurons were lower in animals of the L-3V group compared with the S-3V group [$F_{(1,14)} = 84.6$; $p < 0.001$]. By contrast, the GAD₆₇ mRNA levels in PV− pallidal neurons of the L-3V and S-3V groups did not differ [$F_{(1,14)} = 2.6$; $p > 0.05$]. Indeed, GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons of the L-3V group [$F_{(1,7)} = 137.6$; $p < 0.001$]. Therefore, the STN lesion alone appeared to preferentially affect the PV+ pallidal neurons.

Also, the repeated drug treatment condition (eticlopride vs vehicle) significantly affected GAD₆₇ mRNA expression in both populations of pallidal neurons [$F_{(1,28)} = 459.9$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 603.4$; $p < 0.001$ for PV− cells]. GAD₆₇ mRNA levels were significantly elevated in both PV+ and PV− pallidal neurons of S-3E animals when compared with S-3V animals [$F_{(1,14)} = 549.3$; $p < 0.001$ for PV+ neurons; $F_{(1,14)} = 1199.7$; $p < 0.001$ for PV− neurons]. Simple effects comparisons indicated that GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons in S-3E animals [$F_{(1,7)} = 36.1$; $p < 0.001$].

A similar analysis was conducted on GAD₆₇ mRNA levels in GP sections derived from the 7 d treatment group. ANOVA revealed a significant lesion $\times$ treatment interaction for both cell types [$F_{(1,28)} = 1085.5$; $p < 0.001$ for PV+ neurons; $F_{(1,28)} = 1595.1$; $p < 0.001$ for PV− neurons]. GAD₆₇ mRNA levels in tissue from L-7E animals differed significantly from GAD₆₇ mRNA levels in corresponding cell types from S-7V animals: GAD₆₇ mRNA levels in PV+ cells in L-7E animals were significantly lower than GAD₆₇ mRNA levels in S-7V PV+ cells [$F_{(1,14)} = 6.9$; $p < 0.05$], whereas GAD₆₇ mRNA levels in PV− pallidal neurons were significantly increased when compared with PV− cells from S-7V-treated tissue [$F_{(1,14)} = 136.2$; $p < 0.001$]. Indeed, simple effects comparisons indicated that GAD₆₇ mRNA was significantly higher in PV− than PV+ neurons in L-7E animals [$F_{(1,7)} = 236.7$; $p < 0.001$].

The STN lesion significantly affected pallidal GAD₆₇ mRNA in both PV+ and PV− cell populations [$F_{(1,28)} = 1989.4$; $p < 0.001$ for PV+ cells; $F_{(1,28)} = 1757.9$; $p < 0.001$ for PV− cells]. Pallidal PV+ GAD₆₇ mRNA levels were significantly lower in L-7V animals compared with S-7V animals [$F_{(1,14)} = 54.8$; $p < 0.001$]. By contrast, pallidal PV− GAD₆₇ mRNA levels of L-7V animals were no different from those of S-7V animals [$F_{(1,14)} = 1.5$; $p > 0.05$]. Simple effects comparisons indicated that GAD₆₇ mRNA was

significantly higher in PV[−] than PV⁺ neurons in L-7V animals [$F_{(1,7)} = 36.2$; $p < 0.001$].

In addition, treatment of sham STN-lesioned rats with eticlopride for 7 d increased GAD₆₇ mRNA levels in both GP cell types [$F_{(1,28)} = 1634.8$; $p < 0.001$ for PV⁺ cells; $F_{(1,28)} = 3414.0$ for PV[−] cells]. A simple main effects analysis revealed that, for S-7E animals, both cell types demonstrated dramatically increased levels of GAD₆₇ mRNA when compared with S-7V controls [$F_{(1,14)} = 1965.6$; $p < 0.001$ for PV⁺ neurons; $F_{(1,14)} = 5473.9$; $p < 0.001$ for PV[−] neurons]. This increase at 7 d was significantly greater than the corresponding group increase in 3 d eticlopride-treated animals ($t_{(14)} = -69.0$; $p < 0.001$). Furthermore, it appeared that the eticlopride-induced increase in S-7E animals was more pronounced in PV[−] than PV⁺ pallidal neurons. Although GAD₆₇ mRNA levels were found not to differ between PV⁺ and PV[−] neurons in the S-7V condition, the elevation in GAD₆₇ mRNA in the S-7E condition was significantly greater in PV[−] pallidal neurons than in PV⁺ pallidal neurons [$F_{(1,7)} = 283.2$; $p < 0.001$].

Discussion

These findings demonstrate that the PV⁺ and PV[−] neurons of the rodent GP are influenced in qualitatively similar ways by 6-OHDA-induced nigrostriatal injury, repeated D2 antagonist administration, and STN lesions. Overall, 6-OHDA lesion or D2 antagonist administration markedly increased GAD₆₇ mRNA in both PV⁺ and PV[−] pallidal neurons. However, after either 6-OHDA or D2 antagonist treatment, the increase in GAD₆₇ mRNA was more pronounced in PV[−] than PV⁺ pallidal neurons. Although other investigators have reported increased pallidal GAD₆₇ mRNA after nigral 6-OHDA lesion or repeated D2-class antagonist injections (Kincaid et al., 1992; Soghomonian and Chesselet, 1992; Delfs et al., 1995a), this represents the first report of GAD₆₇ mRNA regulation in these two cell populations.

These experiments also demonstrate that the STN lesion decreased GAD₆₇ mRNA levels in both PV⁺ and PV[−] pallidal neurons. In contrast to the influences of 6-OHDA lesion or eticlopride administration, the impact of the STN lesion on pallidal GAD₆₇ mRNA expression was greater for the PV⁺ than PV[−] neurons

Pallidal GAD₆₇ mRNA after nigrostriatal lesion or repeated D2 antagonist: functional implications

In the basal ganglia, changes in GAD₆₇ mRNA expression correlate with increased GAD protein levels, electrophysiological ac-

tivity, and compensatory downregulation of GABA receptors (Segovia et al., 1990, 1991; Chada et al., 2000). In other systems (e.g., cerebellum and hippocampus), changes in GAD₆₇ mRNA expression correlate with increased GAD protein in terminals, increased GABA release, and increases in firing rates of GABAergic neurons (Litwak et al., 1990; Drengler and Oltmans, 1993; Falkenberg et al., 1997). Therefore, although the increase in the GAD₆₇ transcript does not necessarily predict a linear increase in GAD protein levels and subsequent GABA release, the increases in GAD₆₇ mRNA described here are likely to correspond to increased GABAergic transmission within GP neurons.

Figure 6. Photomicrographs of GAD₆₇ mRNA in PV⁺ (brown cells) and PV[−] (yellow arrows) in neurons of the GP of SS, LS, LL, and SL animals and in the GP of S-7V, L-7V, L-7E, and S-7E animals. Scale bar, 50 μ m.

Figure 7. Combined STN and 6-OHDA lesions interact to differentially regulate GAD₆₇ mRNA expression in PV+ and PV− GP neurons. *, Significant differences compared with corresponding cell types in Sham+Sham controls. ◇, Significantly increased GAD₆₇ mRNA levels compared with PV+ GP neurons for corresponding treatment. The dashed line represents the approximate control levels for comparison purposes; *n* = 8 for all groups.

Figure 8. STN and repeated D2 antagonist administration interact to differentially regulate GAD₆₇ mRNA expression in PV+ and PV− GP neurons. *, Differences from corresponding cell types in sham vehicle (Veh) groups. ◇, Significantly increased levels of GAD₆₇ mRNA compared with PV+ GP neurons within groups. The dashed line indicates approximate control levels for comparison purposes; *n* = 8 for all groups. Etic, Eticlopride.

Accordingly, the present results support the proposal that 6-OHDA lesion or D2 antagonist administration triggers a series of events beginning with disinhibition of striatopallidal neurons and resulting in increased subthalamopallidal activity (Chesselet and Delfs, 1996). Increased STN activity, in turn, can result in GP burst firing (Ni et al., 2000) and enhanced GP GAD₆₇ mRNA expression levels. In addition, our results suggest that the DA cell lesions or D2 antagonist administration have a second influence, to remove a direct dopaminergic influence on GP neurons. Intrapallidal DA appears normally to suppress gene expression preferentially in PV− neurons through D2-class receptors (Billings and Marshall, 2003).

The similar effects of 6-OHDA and D2 antagonist administration indicate that the loss of DA neurons influences GP GAD₆₇ mRNA primarily through D2-class, not D1-class, receptors. Administration of the D1-class antagonist SCH-23390 for 3, 7, or

21 d did not increase GAD₆₇ mRNA in GP neurons. To the contrary, a small decline was observed after 7 d of treatment. Because a D1 autoradiographic signal, but not D1 mRNA, is found in the GP, pallidal D1 receptors may be located on terminals of striatal and subthalamic neurons (Fremeau et al., 1991; Flores et al., 1999). An influence of D1 antagonist on glutamate release from subthalamopallidal terminals might explain the observed decrease in GP GAD₆₇ mRNA expression.

Neuron population specificity of effects of nigrostriatal lesion or repeated D2 antagonist

Previous work demonstrated a differential regulation of neurochemical markers by DA in PV+ and PV− GP neurons. Systemic or local application of a D2-class antagonist induces Fos almost exclusively within PV− pallidal neurons (Ruskin and Marshall, 1997; Billings and Marshall, 2003). PPE mRNA, which seldom colocalizes with PV and is found predominantly in pallidostriatal neurons, also increases after 6-OHDA lesion or repeated D2-class antagonist administration (Schuller et al., 1999). DA D₂ receptor mRNA occurs at higher levels in PPE mRNA-containing (PPE+) pallidal neurons, suggesting that this population of PPE+/PV− neurons may be more sensitive to alterations in pallidal DA tone (Hoover and Marshall, 2002).

Electrophysiological recordings also indicate that DA may have differing effects on pallidal neuron populations. Microiontophoretic application of DA into the GP causes both increases and decreases in cell firing rates (Bergstrom and Walters, 1984; Napier et al., 1991; Kelland et al., 1995).

In animals receiving the D2-class antagonist eticlopride, an interesting time course of altered GAD₆₇ mRNA expression emerged. In both neuron populations, expression levels increased initially and then returned toward baseline (see Fig. 4). The population difference in GAD₆₇ expression was emerging after 3 d and was completely developed after 7 d of eticlopride administration. After 21 d of treatment, overall GAD₆₇ mRNA levels were lower than at 7 d, but the population difference was undiminished. Thus, it seems that pallidal GAD₆₇ mRNA levels are initially upregulated but are later normalized or even decreased below baseline (Mercugliano et al., 1992; Delfs et al., 1995; Mavridis and Besson, 1999). This time course contrasts with that observed after 6-OHDA-induced lesion of the dopaminergic neurons, in which the GAD₆₇ mRNA upregulation was the same at 5 and 21 d postoperatively.

Dual processes by which DA and STN inputs regulate GP GAD₆₇ mRNA

In all experiments, GAD₆₇ mRNA expression in the GP PV+ and PV− neurons of sham lesion or vehicle-treated controls did not differ. Although the direction of GAD₆₇ mRNA expression changes was similar in PV+ and PV− neurons after either 6-OHDA lesion alone or STN lesion alone, the effects of the 6-OHDA lesion (or D2 antagonist treatment) were greater for the PV− cells than the PV+ cells, whereas the effects of the STN lesion were greater for the PV+ than PV− neurons (Fig. 9). This double dissociation, in addition to the finding that the direction of changes in GAD₆₇ mRNA expression of the two cell populations differed after combined 6-OHDA and STN lesions, argues for the distinctiveness of GAD₆₇ mRNA regulation in PV+ and PV− GP neurons. This suggests that the influences of dopamine on pallidal gene expression occur through at least two processes: one involving the activation of STN inputs to the GP neurons and a second process involving the removal of nigropallidal dopamine (D2-class) transmission. We propose that the elevation of

Figure 9. Combined DA and STN manipulations indicate a double dissociation of the effects of these treatments on PV+ and PV− GP neurons. DA manipulations: A, 7 d eticlopride; B, sham STN and 7 d eticlopride; C, 5 d 6-OHDA lesion; D, 21 d 6-OHDA lesion; E, 3 d eticlopride; F, sham STN and 3 d eticlopride; G, sham STN and 6-OHDA lesion; H, 21 d eticlopride. STN manipulations: I, STN lesion and 7 d vehicle; J, STN lesion and sham 6-OHDA lesion; K, STN lesion and 3 d vehicle. Combined manipulations: L, STN lesion and 3 d eticlopride; M, STN lesion and 7 d eticlopride; N, STN lesion and 6-OHDA lesion.

GAD₆₇ mRNA in PV+ pallidal neurons depends exclusively on STN activation, whereas the increase in PV− neurons in this transcript depends on both processes. The rationale for proposing that decreased pallidal DA transmission preferentially affects the gene expression of PV− GP neurons was cited above.

It remains unclear what drives the increase in GAD₆₇ mRNA expression in PV− neurons of the GP in animals with an STN lesion. Although the STN provides the greatest excitatory input to the GP, other areas may contribute to pallidal gene expression. For example, the parafascicular nucleus projects directly to the GP (Kincaid et al., 1991; Deschenes et al., 1996; Orioux et al., 2000; Gonzalo et al., 2002) and may importantly affect pallidal neuronal activity independently of STN inputs (Mouroux et al., 1997). Thus, parafascicular inputs to the GP may account for the remaining elevation in GAD₆₇ mRNA expression levels in 6-OHDA- (or eticlopride)-treated, STN-lesioned animals.

Implications for basal ganglia circuit function of changes in GP GAD₆₇ mRNA expression

The GP is a highly heterogeneous structure, with population distinctions based on electrophysiological characteristics, projection target, chemical phenotype, receptor transcript density, and dendritic branching patterns (Park et al., 1982; Napier et al., 1991; Kita and Kitai, 1994; Nambu and Llinas, 1994; Rajakumar et al., 1994; Kelland et al., 1995; Ruskin and Marshall, 1997; Hoover and Marshall, 1999, 2002; Sato et al., 2000). The separation of pallidal neurons based on chemical markers has proven to be particularly useful because such markers correlate with projection targets. Tracer studies indicate that most pallidal neurons that are retrogradely labeled from the STN or substantia nigra pars reticulata contain PV, whereas most pallidal neurons retrogradely labeled from the striatum lack PV (Ruskin and Marshall, 1997; Hoover and Marshall, 1999, 2002). It may be that most GP neurons containing PV project to “downstream” structures, and a few project to the striatum; alternatively, most or all PV+ GP neurons may project both to the striatum and downstream structures, with the density of projections to each target differing. In either case, the patterns of projections from the PV+ and PV− cell populations are distinct.

Pallidostriatal neurons appear to terminate primarily on PV−-containing striatal interneurons (Bevan et al., 1998), which in turn powerfully inhibit striatofugal projection neurons (Koos and Tepper, 1999). Striatal PV+ interneurons integrate information from many cortical areas (Bennett and Bolam, 1994; Ramanathan et al., 2002) as well as from other interneurons through

dendritic gap junctions (Kita and Kitai, 1990; Koos and Tepper, 1999). Striatal PV+ interneurons receive multiple synaptic contacts from a single cortical neuron (Ramanathan et al., 2002), and the PV+ striatal interneurons may be more responsive to cortical inputs than are striatal projection neurons (Parthasarathy and Graybiel, 1997; Stern et al., 1997; Ramanathan et al., 2002). Together, these observations indicate that pallidostriatal neurons are critically positioned to regulate striatal activity. Indeed, lesion-induced alterations in activity of the pallidostriatal projection might explain the decrease in striatal GAD₆₇ mRNA expression observed in 6-OHDA-lesioned rats (Soghomonian et al., 1992). Under conditions of D2 antagonist treatment or nigrostriatal injury, activation of inhibitory pallidostriatal neurons could result in the subsequent disinhibition of striatal projection neurons, exacerbating hypokinetic symptoms. Thus, the nigropallidal projection provides an important target in the study of the genesis and expression of motor symptoms in animal models of Parkinson's disease.

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