

Increased Glutamate Decarboxylase mRNA Levels in the Striatum and Pallidum of MPTP-treated Primates

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The mRNA levels encoding for the enzyme glutamate decarboxylase (GAD67) were measured by computerized image analysis after *in situ* hybridization histochemistry and radioautography in the striatum and pallidum of normal squirrel monkeys (*Saimiri sciureus*), or after treatment with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). All MPTP-injected monkeys exhibited profound motor deficits including akinesia. The dopaminergic innervation, as visualized and quantified on x-ray films after ³H-mazindol binding on tissue sections, was uniformly lost throughout the striatum of MPTP-treated monkeys.

Brain sections processed with a probe synthesized from a feline or human GAD67 cDNA exhibited intense radioautographic labeling throughout the striatum. When measured on x-ray films, the intensity of GAD67 mRNA labeling was increased in the striatum of MPTP-treated versus control monkeys. Increased labeling reached statistical significance in the dorsolateral sector of the rostral putamen and throughout the putamen and the caudate at the caudal, postcommissural, level. Analysis of emulsion radioautographs demonstrated that the increase in GAD67 mRNA labeling in MPTP-treated monkeys occurred in individual neurons of the striatum. In the external and internal segments of the pallidum, numerous neurons labeled with the GAD67 cRNA probe were visualized on emulsion radioautographs. The intensity of GAD67 mRNA labeling in single neurons of both pallidal segments was increased in MPTP-treated versus control monkeys. Construction of the histograms of frequency distribution of labeling indicated that this increase occurred in a majority of labeled neurons.

The present study demonstrates that GAD67 mRNA levels are significantly altered in the striatum and pallidum of parkinsonian monkeys. The preferential increase of GAD67 mRNA labeling in the dorsolateral putamen, which receives afferents from the sensorimotor cortex, provides further evidence of the involvement of GABAergic transmission in the expression of the motor deficits elicited after MPTP. In ad-

dition, increased GAD67 mRNA levels in the internal segment of the pallidum support the hypothesis of an increased activity of GABAergic neurons in the output structures of the basal ganglia in parkinsonism.

[Key words: glutamate decarboxylase, striatum, pallidum, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), mRNA, Parkinson's disease]

The vast majority of neurons in the basal ganglia including the striatum and pallidum produce the enzyme glutamate decarboxylase (GAD) and use its catalytic product, GABA, as their neurotransmitter. Striatal GABAergic projection neurons send their axons to the substantia nigra and the internal (GPi) or the external (GPe) segment of the pallidum (reviewed in Parent, 1986). The pars reticulata of the substantia nigra (SNr) and GPi are the output structures of the basal ganglia and their GABAergic neurons project to the thalamus, habenula, and brainstem tegmentum. In rodents, striatal neurons that project to the SNr and entopeduncular nucleus (rodent homolog of the primate GPi) preferentially express the peptides substance P and dynorphin, while neurons projecting to the GPe preferentially express the peptide enkephalin (reviewed in Reiner and Anderson, 1990). The preferential distribution of enkephalin and substance P immunoreactivity in the GPe and GPi, respectively, has also been shown in the primate (Haber and Elde, 1982; Haber and Watson, 1985). The human GPe, however, exhibits intense dynorphin immunoreactivity (Haber and Watson, 1985).

Extensive evidence indicates that GABAergic neurons of the basal ganglia are under the control of dopaminergic afferents from the pars compacta of the substantia nigra (SNc). For instance, recent *in situ* hybridization and Northern blot experiments have shown that the levels of mRNA encoding for GAD67 are increased in neurons of the rat striatum following unilateral dopamine deafferentations in adults (Vernier et al., 1988; Lindfors et al., 1989b; Segovia et al., 1990; Soghomonian et al., 1992) or bilateral deafferentations in neonates (Soghomonian, 1993). Among the two GADs expressed in the rat striatum (Mercugliano et al., 1992), only the high-molecular-weight isoform (GAD67 as compared to GAD65) appears to be regulated by dopamine (Soghomonian et al., 1992). Other experimental results also indicate that GAD67 mRNA levels in the globus pallidus and the entopeduncular nucleus of the rat are regulated by dopamine (Soghomonian and Chesselet, 1992).

Alteration of GABAergic neuronal activity in the basal ganglia has been proposed as a major substrate for the expression of behavioral dysfunctions observed in experimental or pathological parkinsonism (reviewed in Albin et al., 1989). The role of

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GABA in parkinsonism is supported mainly by its increased levels in the striatum of patients (Kish et al., 1986). In addition, previous data have shown changes in the levels of GABA release in the GPe of primates rendered parkinsonians after injections of the neurotoxin MPTP (Robertson et al., 1991).

The goal of the present study was to investigate possible changes in GAD67 mRNA levels in the striatum and pallidum of primates rendered parkinsonians with single or repeated injections of MPTP. The mRNA levels were measured in different regions of the striatum and in the pallidum by radioactive *in situ* hybridization histochemistry with probes synthesized from the human or feline GAD67 cDNAs (Erlander et al., 1991; Bu et al., 1992).

Materials and Methods

Animals. Twelve adult squirrel monkeys (*Saimiri sciureus*) were used for this study. Animals were obtained from Charles River (Montreal, Canada), and housed two per cage with water and food available ad libitum. Seven monkeys (I–VII) were given subcutaneous injections of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP hydrochloride; Aldrich Chemical Co., Milwaukee, WI). Five control monkeys received injections of the vehicle only (0.9% sodium chloride and 1% ascorbic acid). Monkeys were injected once a week until showing profound parkinsonian signs such as akinesia, rigidity, and tremor. They received a total of 7 mg/kg (monkeys I and II; five injections), 4 mg/kg (monkeys III and VI; two injections), 3 mg/kg (monkeys IV and VII; two injections), or 2.5 mg/kg (monkey V; one injection) of MPTP. All animals were injected intravenously with an overdose of pentobarbital 10 d after the last injection of MPTP. At the time of death, all MPTP-injected monkeys exhibited severe parkinsonian signs. All the brains were removed quickly and kept frozen at -70°C . Coronal sections of the brains (10 μm thick) were cut on a cryostat, deposited on gelatin-chromalum-coated slides, and processed for *in situ* hybridization histochemistry.

In situ hybridization. ^{35}S -radiolabeled probes were synthesized by transcription *in vitro* from cDNA clones encoding for the feline or the human GAD67 cDNA (Kaufman et al., 1986; Bu et al., 1992). Transcription of the complementary RNAs (cRNAs) from the cDNAs was performed as previously described (Chesselet et al., 1987) in the presence of 2.5 μM ^{35}S -UTP (1000 Ci/mmol; DuPont–New England Nuclear) and 10 μM unlabeled UTP with ATP, CTP, and GTP in excess. The reaction was carried out for 2 hr at 37°C and then the template was digested with DNase I. The labeled cRNAs were purified by phenol/chloroform extraction and ethanol precipitation. The length of the GAD67 cRNA was reduced to 100–150 nucleotides by partial alkaline hydrolysis to improve accessibility of the probe (Cox et al., 1984).

For each experiment, brain sections at the level of the striatum or pallidum were quickly dried at room temperature under a flow of air and fixed for 5 min by immersion into a solution of 3% paraformaldehyde in phosphate buffer (pH 7.2; 1 M) containing 0.02% DEPC. The detailed procedure for the *in situ* hybridization protocol has been described elsewhere (Chesselet et al., 1987). Sections were treated for 10 min with 0.25% acetic anhydride and triethanolamine (0.1 M; pH 8.0) and for 30 min with Tris-glycine (1 M; pH 7.0), dehydrated in graded ethanol, and air dried. Each section was covered with 2–4 ng in 30 μl of radiolabeled cRNA probe (specific activity, 4×10^5 cpm/ng) diluted in hybridization solution containing 40% formamide, 10% dextran sulfate, $4 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M NaCl and 0.015 M sodium citrate), 10 mM dithiothreitol, 1% sheared salmon sperm DNA, 1% yeast tRNA, and 1 \times Denhardt's solution containing 1% of RNase-free bovine serum albumin. The sections were covered with Parafilm, placed in humidified boxes, and incubated for 4 hr in a pulsed-air oven at 50°C . Sections were then immersed in 50% formamide and $2 \times \text{SSC}$ at 52°C for 5 and 20 min, in RNase A (100 $\mu\text{g}/\text{ml}$; Sigma) and $2 \times \text{SSC}$ for 30 min at 37°C , in 50% formamide and $2 \times \text{SSC}$ for 5 min, and left overnight in $2 \times \text{SSC}$ and 0.05% Triton X-100 at room temperature under mild agitation. Sections were then dehydrated in ethanol, defatted in xylene for 30 min, rinsed in 100% ethanol, air dried, and stored in a desiccator until radioautographic processing.

^3H -mazindol binding. The density of dopamine reuptake sites in the striatum was measured after ^3H -mazindol binding as previously reported by Javitch et al. (1985). Frozen sections from control and MPTP-

treated monkeys were dried under a flow of air and rinsed for 5 min at 4°C in 50 mM Tris buffer with 120 mM NaCl and 5 mM KCl to wash off the endogenous ligand. They were then incubated for 40 min in 15 nM ^3H -mazindol (DuPont–New England Nuclear; specific activity, 22.7 Ci/mmol) in 50 mM Tris buffer containing 300 mM NaCl and 5 mM KCl. Desipramine (0.3 μM) was added to the incubation medium to block the norepinephrine transporter. Some sections from control and MPTP-treated animals were also incubated in the presence of 30 μM benztropine to measure the nonspecific binding. Sections were then rinsed 2×3 min in the incubation buffer, 10 sec in distilled water, and then dried under a flow of air at room temperature. Sections were then juxtaposed to x-ray films (LKB ultrafilm) for 20 d.

Radioautography. Sections of the striatum and pallidum labeled with the GAD67 probe were first processed for x-ray film radioautography and then emulsion radioautography. In the first case, sections were juxtaposed to Kodak X-OMAT AR x-ray films and exposed for 2–4 weeks. In the second case, sections were coated with a Kodak NTB3 nuclear emulsion diluted 1:1 with water containing 300 mM ammonium acetate, air dried, and stored at 4°C in light-tight boxes in the presence of desiccant. Following 15–20 d or 4–15 d of exposure for film or emulsion radioautography, respectively, the sections were developed in Kodak D-19 for 3.5 min at 14°C . Emulsion radioautographs were lightly counterstained with hematoxylin and eosin and mounted with Eukitt mounting media.

Analysis of labeling. Levels of radioautographic labeling with the GAD67 probe or with ^3H -mazindol in the caudate and putamen of control and MPTP-treated monkeys were first quantified on x-ray films by computerized densitometry with ULTIMAGE software (MacIntosh computer). The optical densities in each striatal sector were calculated after subtracting the optical density of the film and standardization against emulsion-coated filters (Kodak). Comparison of labeling between each experimental group of monkeys was carried out from sections processed in parallel, using three sections per striatal level per animal. The average level of labeling in different experimental groups was compared with a Mann–Whitney statistical rank sum test, with $p < 0.05$ considered significant.

The level of radioautographic labeling on emulsion radioautographs in individual neurons of the dorsal putamen and the pallidum was quantified by computerized image analysis (ULTIMAGE). Individual neurons were observed on a microscope at $100 \times$ magnification under bright-field illumination. The area covered by silver grains in individual neurons was calculated on the digitized image and expressed as a number of pixels per neuron. In each region investigated, labeling was measured on an average of 50–70 neurons per monkey from five MPTP and five control monkeys. The average level of labeling between control and MPTP-treated monkeys was compared with the Mann–Whitney U test. Histograms of frequency distribution of labeling were constructed from individual values of all labeled neurons in control and MPTP-treated monkeys.

Results

GAD67 mRNA labeling in the striatum and pallidum of control monkeys

As visualized on x-ray film radioautographs, brain sections from normal monkeys processed with the human GAD67 probe exhibited labeling in the striatum (caudate and putamen; Figs. 2A, 3A). The human and feline GAD67 cRNA probes produced a similar pattern of radioautographic labeling (Figs. 1A, 2A). Quantitative analysis of labeling in different sectors of the striatum revealed slightly lower values in the dorsal portion of the caudate nucleus than in the remaining portions of the striatum. The observation of emulsion radioautographs demonstrated that labeling in the striatum was distributed over neuronal profiles (Fig. 4A). As previously described in the rat (Chesselet et al., 1987), a majority of neurons in the striatum of the squirrel monkey exhibited a low density of labeling, whereas few neurons were densely labeled (Fig. 4A). In the pallidum, GAD67 mRNA labeling was distributed over a majority of neurons of the GPI and GPe and was more intense than in the striatum (Figs. 3A, 4). Quantification of labeling on emulsion radioautographs showed that neurons in the GPI and the GPe expressed similar

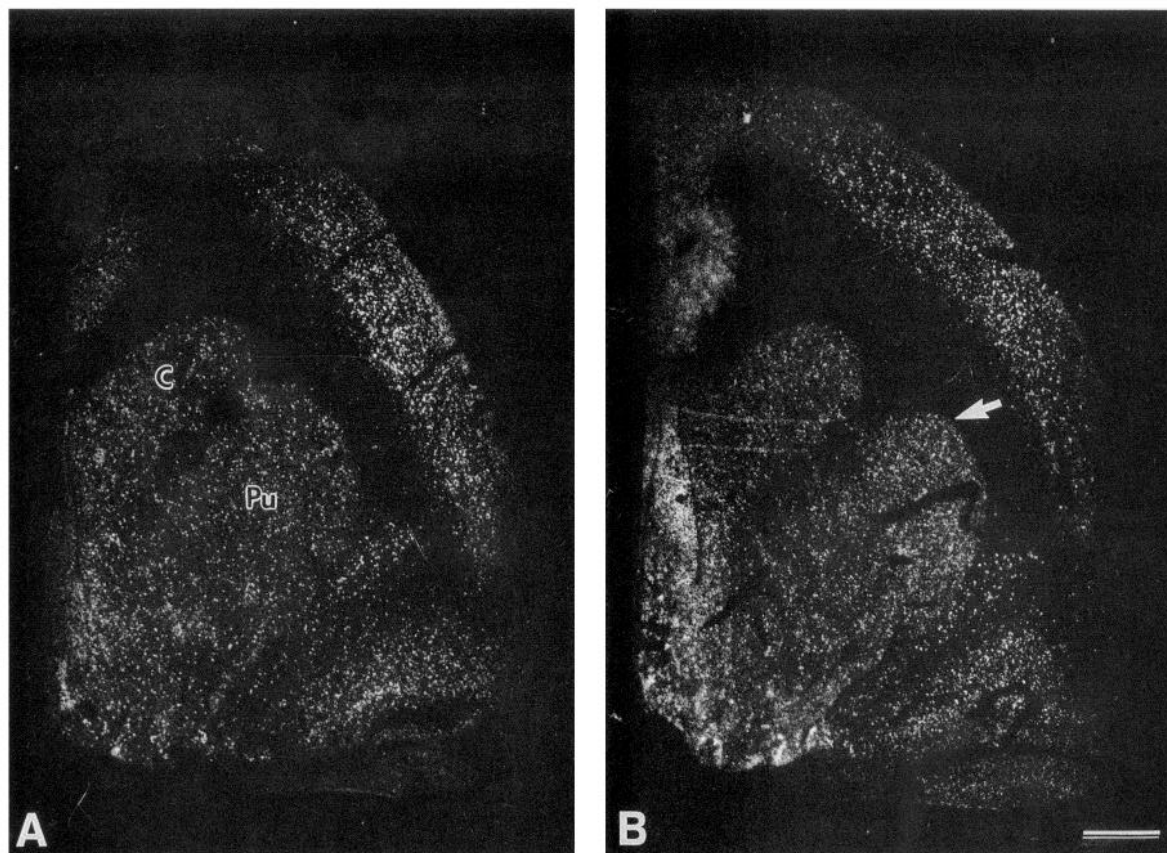


Figure 1. Negative images of x-ray films from frontal adjacent right brain sections processed for *in situ* hybridization with a ^{35}S -labeled cRNA probe for the feline GAD67 mRNA in a control (*A*) and an MPTP-treated (*B*) monkey. In the control, GAD67 mRNA labeling is found throughout the striatum (*C*, caudate; *Pu*, putamen). Section from the MPTP-treated monkey shows an increased labeling in the dorsolateral putamen (*arrow*). Scale bar, 100 μm .

GAD67 mRNA levels (699.0 ± 37.4 and 509.8 ± 38.5 pixels per neuron for the GPi and the GPe, respectively).

Effect of MPTP treatment on ^3H -mazindol binding

In order to characterize the extent of loss of dopaminergic axonal fibers in MPTP-treated monkeys, sections from both control and MPTP-treated animals were processed for ^3H -mazindol binding and radioautography. In control animals, intense ^3H -mazindol labeling was observed throughout the striatum (Figs. 2*C*, 3*C*). The intensity of labeling appeared lower in the dorsal caudate and the medial portion of the putamen. As a control of specificity, ^3H -mazindol labeling was no longer detectable when sections were incubated in the presence of the dopamine uptake blocker benztropine (not illustrated). In MPTP-treated primates, there was a dramatic loss of ^3H -mazindol binding throughout the striatum (Figs. 2*D*, 3*D*, 5).

GAD67 mRNA labeling in the striatum of MPTP-treated monkeys

The intensity of GAD67 mRNA labeling was first quantified on x-ray films in various sectors of the striatum at four frontal levels (rostral to caudal: A15, A14, A12.5, and A11.5 according to stereotaxis atlas of Emmers and Akert, 1963). In MPTP-treated monkeys, GAD67 mRNA labeling was increased when compared to controls (Figs. 2*A,B*; 3*A,B*). At frontal levels A15, A14, and A12.5, increased GAD67 mRNA labeling reached statistical significance only in the dorsolateral sector of the pu-

tamen (Fig. 6*A–C*). At the caudalmost, postcommissural level (level A11.5), GAD67 mRNA labeling was increased in the dorsal and ventral putamen and the caudate. Among the seven monkeys treated with MPTP, two (monkeys I and VII) exhibited only a slightly increased GAD67 mRNA labeling versus the controls. Although the increased GAD67 mRNA labeling was statistically significant in the dorsolateral putamen when these two MPTP-treated monkeys were included, the increased labeling in the putamen and caudate at the postcommissural level reached statistical significance only when these two were omitted (Fig. 6*D*).

Observation and quantitative analysis of emulsion radioautographs demonstrated that GAD67 mRNA labeling in MPTP-treated monkeys was increased over individual neurons of the striatum. The average surface area of silver grains overlying neurons in the dorsolateral putamen (level A14) was of 353.3 ± 25.9 pixels in controls versus 896.4 ± 47.7 pixels in MPTP-treated monkeys ($p < 0.05$ with a Mann–Whitney; $n = 5$). The histogram of frequency distribution of labeling in individual neurons of MPTP-treated versus control monkeys was homogeneously displaced to the right, indicating that increased GAD67 labeling occurred in most labeled neurons (Fig. 7).

GAD67 mRNA labeling in the pallidum of MPTP-treated monkeys

The average number of labeled neuronal profiles detected on emulsion radioautographs under dark-field microscopic illu-

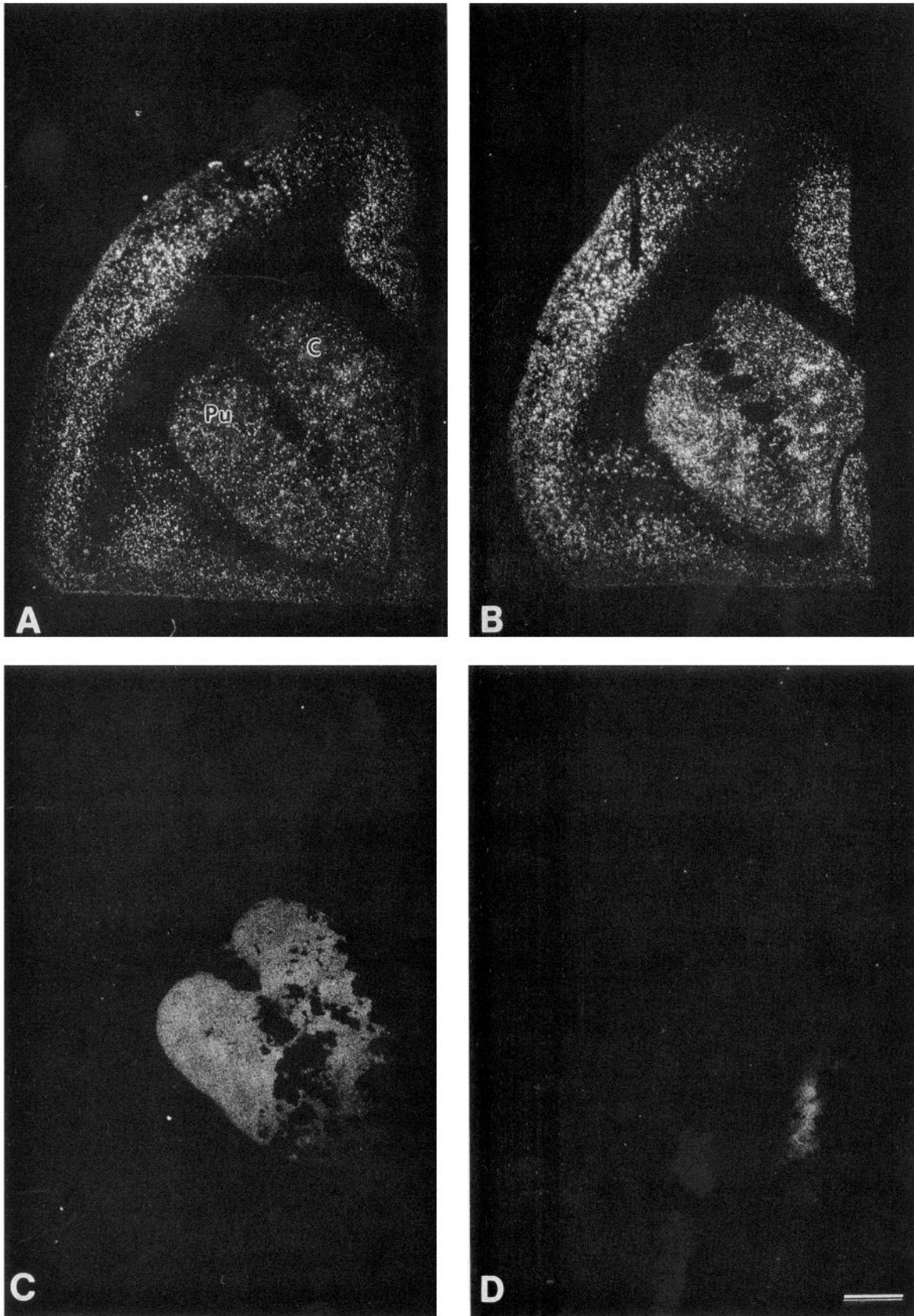
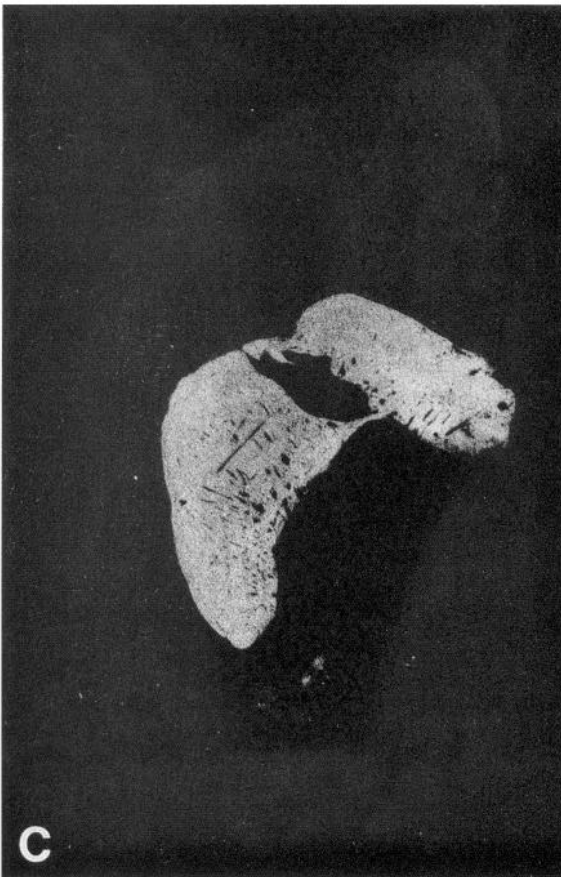
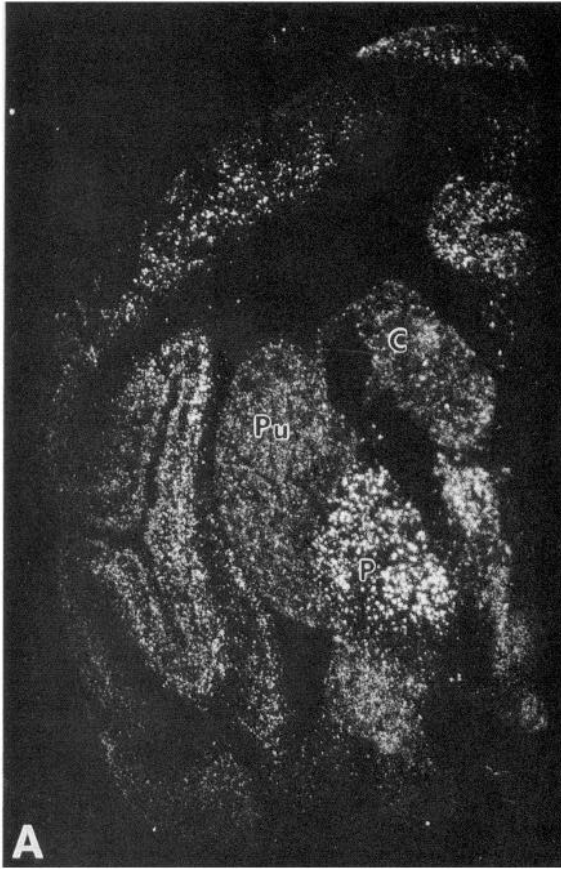


Figure 2. Negative images of x-ray films from frontal adjacent left brain sections processed for *in situ* hybridization with a ^{35}S -labeled cRNA probe from the human GAD67 (*A, B*) or processed for ^3H -mazindol binding (*C, D*). Sections are from a control (*A, C*) and an MPTP-treated (*B, D*) monkey and are from a frontal level A15 (rostral), according to Emmers and Akert's (1963) stereotaxic atlas. Note the increased GAD67 mRNA labeling and the absence of ^3H -mazindol labeling in the striatum of MPTP-treated monkeys (*B, D*). *C*, caudate; *Pu*, putamen. Scale bar, 100 μm .



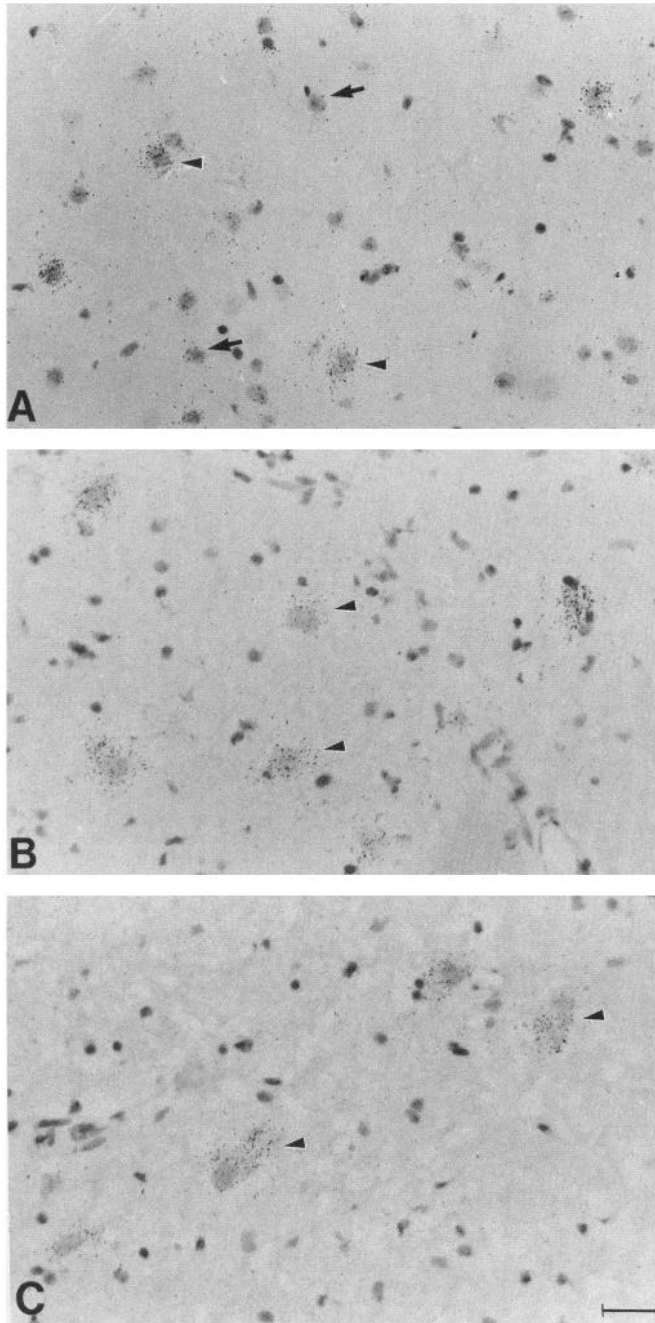


Figure 4. Bright-field photomicrographs of brain sections processed for *in situ* hybridization histochemistry with a ^{35}S -labeled human GAD67 cRNA probe and emulsion radioautography. Labeled neurons are shown in the putamen (*A*), the GPI (*B*), and the GPe (*C*) of a control monkey. In the putamen, note the presence of neurons intensely labeled (arrowheads) and lightly labeled (arrows). In the GPI and GPe, neurons exhibit relatively homogeneous intense labeling (arrowheads). Scale bar, 26 μm .

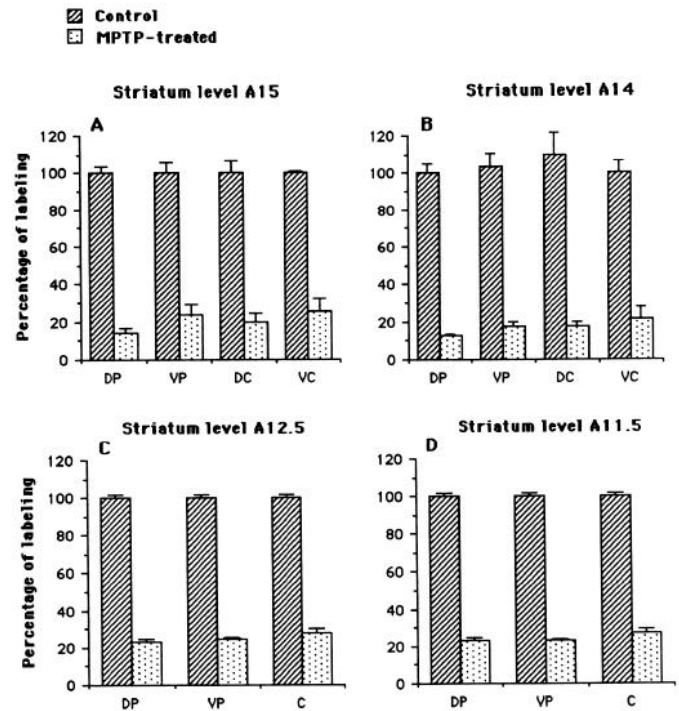


Figure 5. Level of ^3H -mazindol labeling in the striatum of adult control (hatched bars) and MPTP-treated (dotted bars) monkeys. The values represent the average intensity of labeling measured on x-ray films by computerized densitometry and expressed as a percentage of the controls at frontal levels A15 (*A*), A14 (*B*), A12.5 (*C*), and A11.5 (*D*). The data (mean \pm SEM) were obtained from five controls and five lesioned animals. Labeling was measured in the dorsal putamen (DP), the ventral putamen (VP), the dorsal caudate (DC), and the ventral caudate (VC). At caudal level A11.5 (Emmers and Akert, 1963), only one measurement was made in the caudate (C). $p < 0.005$ when comparing values in MPTP-treated monkeys with the corresponding controls with a Mann-Whitney *U* test ($n_2 = 5$).

mination was significantly higher in MPTP-treated than in control monkeys in both the GPI (86.7 ± 10.6 vs 45.3 ± 9.7 neuronal profiles per field; $p < 0.05$ with a Mann-Whitney test; $n = 5$) and the GPe (118.3 ± 9.9 vs 68.1 ± 12.8 neuronal profiles per field; $p < 0.05$; $n = 5$). Quantification of labeling at the single cell level in five control and five MPTP-treated monkeys showed that individual neurons of the pallidum in MPTP-treated monkeys had increased GAD67 mRNA labeling. Increased GAD67 mRNA labeling was higher in the GPI (1826.9 ± 94.6 vs 509.8 ± 38.5 pixels per neuron; $n = 5$; $p < 0.05$) than in the GPe (1257.8 ± 69.0 vs 699.0 ± 37.4 pixels per neuron; $n = 5$; $p < 0.05$). The histograms of frequency distribution of labeling in the GPI and GPe of controls and MPTP-treated monkeys are shown in Figure 8. For each control and MPTP-treated monkey, average values of GAD67 mRNA labeling in the GPe but not in the GPI were significantly correlated with average values of labeling in the dorsolateral putamen at levels A15, A14, and A11.5 (Fig. 9).

Figure 3. Negative images of x-ray films from frontal adjacent left brain sections processed for *in situ* hybridization with a ^{35}S -labeled cRNA probe from the human GAD67 (*A*, *B*) or processed for ^3H -mazindol binding (*C*, *D*). Sections are from a control (*A*, *C*) and an MPTP-treated (*B*, *D*) monkey and are from a frontal level A11.5 (caudal postcommissural), according to Emmers and Akert's (1963) stereotaxic atlas. Note the increased GAD67 mRNA labeling and the absence of ^3H -mazindol labeling in the striatum of MPTP-treated monkeys (*B*, *D*). Intense GAD67 mRNA labeling is also illustrated in the pallidum. C, caudate; Pu, putamen; P, pallidum. Scale bar, 100 μm .

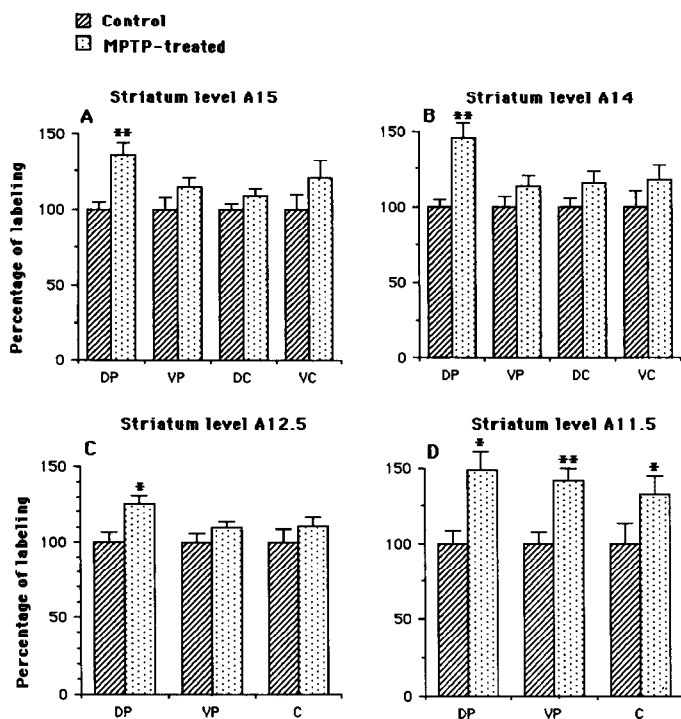


Figure 6. Level of GAD67 mRNA labeling in the striatum of adult control (hatched bars) and MPTP-treated (dotted bars) monkeys. The values represent the average intensity of labeling measured on x-ray films by computerized densitometry and expressed as a percentage of the controls at frontal levels A15 (A), A14.5 (B), A12.5 (C), and A11.5 (D). The data (mean \pm SEM) were obtained from five controls and five lesioned animals. Labeling was measured in the dorsal putamen (DP), the ventral putamen (VP), the dorsal caudate (DC), and the ventral caudate (VC). At caudal level A11.5 (Emmers and Akert, 1963), only one measure was made in the caudate (C). *, $p < 0.01$; **, $p < 0.005$; compared to the corresponding controls with a Mann-Whitney U test ($n_2 = 5$).

Discussion

The present results show an increase in GAD67 mRNA levels in neurons of the striatum and pallidum of parkinsonian, MPTP-treated squirrel monkeys (*Saimiri sciureus*). This increase was restricted to the dorsolateral sector of the putamen at rostral levels and throughout the rest of the striatum at caudal, post-commissural, levels. In the pallidum, GAD67 mRNA levels were increased in both the GPi and GPe. Previous studies in the rat striatum have shown that increased GAD67 mRNA levels after 6-hydroxydopamine lesions of dopamine neurons are associated with increased GAD level, GAD activity, and GABA release (Lindfors et al., 1989a,b; Segovia et al., 1990; Soghomonian et al., 1992). Thus, it can be hypothesized that the GAD67 mRNA increases in these MPTP-treated monkeys reflect an increased GABAergic activity. It must be emphasized, however, that the regulation of GAD67 gene expression does not always parallel the regulation of other indexes of GABAergic activity in various neuronal systems (see Martin and Kimwall, 1993).

Glutamate decarboxylase mRNA regulation in the striatum

The distribution of GAD67 mRNA labeling in the monkey striatum was similar using cRNA probes synthesized from feline or human cDNA. This observation is consistent with the high homology between the cDNA sequences in these two species (Erlander et al., 1991; Bu et al., 1992). The distribution of GAD67 mRNA labeling at cellular level in the squirrel monkey appeared

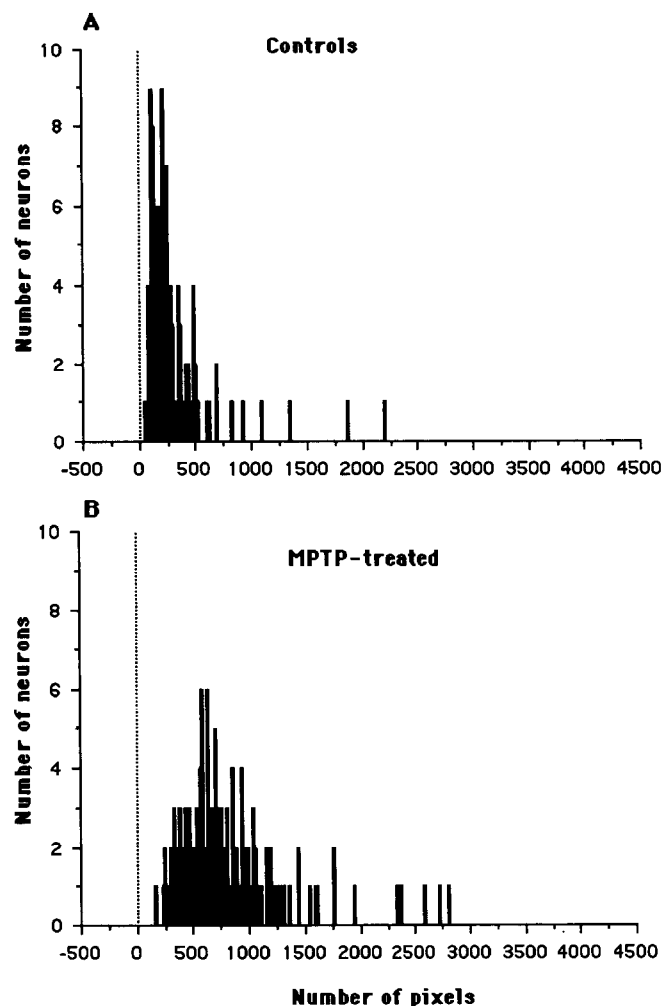


Figure 7. Histograms of frequency distributions of labeling for GAD67 mRNA in neurons of the dorsal putamen in control (A) and MPTP-injected (B) monkeys. Quantification of silver grains over individual neurons was done by computerized image analysis on sections (level A14) processed for emulsion radioautography and exposed for 8 d. Data are from 50 neurons per monkey from five control and five lesioned monkeys. The area covered by silver grains is expressed in number of pixels per neuron.

similar to that previously reported in the rat striatum (Chesselet et al., 1987; Chesselet and Robbins, 1989). In both species, most neurons were found to express low levels of GAD67. Intensely labeled neurons may correspond to the GABAergic interneurons of the striatum that coexpress the calcium binding protein parvalbumin (Chesselet et al., 1987; Soghomonian et al., 1992).

The selective regional increase in GAD67 mRNA labeling in MPTP-treated monkeys is at variance with studies on rats where 6-hydroxydopamine lesions of dopamine neurons result in increased GAD67 mRNA levels throughout the striatum (Lindfors et al., 1989b; Soghomonian et al., 1992). Because ^3H -mazindol binding was evenly lost in the striatum of MPTP-treated monkeys, it is unlikely that the regional increase in GAD67 mRNA levels was due to an uneven dopamine denervation in various striatal sectors. One MPTP-treated monkey in our study exhibited patches of GAD67 mRNA labeling in the caudate (e.g., Fig. 2B). Careful examination of labeling in other MPTP-treated monkeys, however, did not confirm this observation. Thus, our results do not suggest that the increase in GAD67 mRNA levels corresponds to the patch-matrix organization of the striatum (Graybiel and Ragsdale, 1983). The

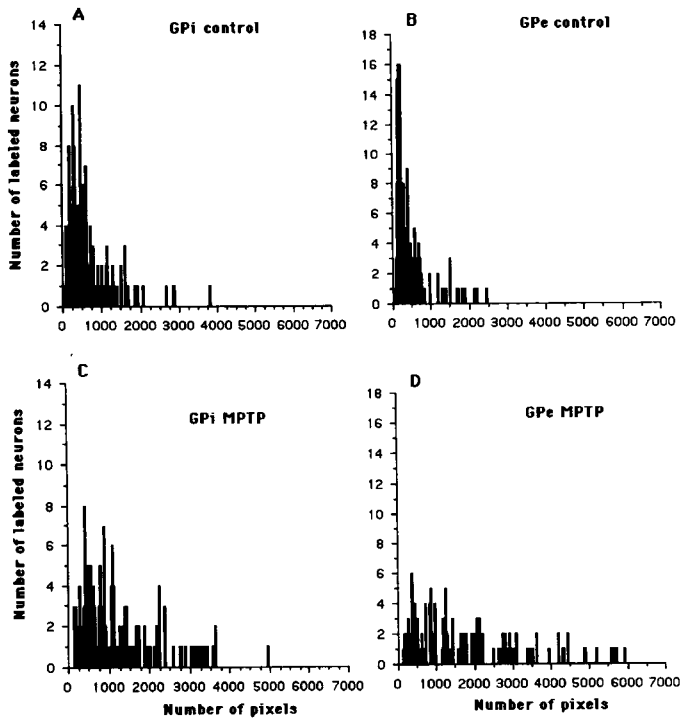


Figure 8. Histograms of frequency distributions of labeling for GAD67 mRNA in neurons of the internal pallidum (GPi) and external pallidum (GPe) in vehicle-injected (A, B) and MPTP-injected (C, D) monkeys. Quantification of silver grains over individual neurons was done by computerized image analysis on sections processed for emulsion radioautography and exposed for 8 d. Data are from 50 neurons per monkey from five control and five lesioned monkeys. The area covered by silver grains is expressed in number of pixels per neuron.

striatal region exhibiting the highest increase in GAD67 labeling, that is, the dorsolateral putamen, is known to receive afferents from sensorimotor areas of the cerebral cortex (reviewed in Parent, 1986, 1990). It is therefore possible that these cortical afferents contributed to the selective alteration of GAD67 gene expression in MPTP-treated monkeys. In fact, previous studies have shown that cortical afferents regulate the expression of GAD67 (Salin and Chesselet, 1993) and enkephalin (Somers and Beckstead, 1990; Salin and Chesselet, 1992) mRNA levels in striatal neurons of the rat. In keeping with reports of a colocalization of GABA and enkephalin in some striatal neurons, it is also of interest to note that cortical lesions were found to reduce the increase in preproenkephalin mRNA levels observed in 6-hydroxydopamine-treated rats (Campbell and Björklund, 1993).

As indicated by the shift in the histogram of frequency distribution of labeling, GAD67 mRNA levels in the dorsolateral putamen of MPTP-treated monkeys were increased in a significant number of labeled neurons. Since GABAergic projection neurons of the striatum form two major subpopulations that can be distinguished on the basis of their projections to the GPi/SNr or GPe and/or on the basis of their peptide content, the identity of neurons that exhibited increased GAD67 mRNA labeling after MPTP treatments remains hypothetical. We have recently reported that the dorsolateral putamen in MPTP-treated monkeys also exhibits increased preproenkephalin mRNA levels (Soghomonian et al., 1993). The similar regional increase in GAD67 and preproenkephalin mRNA levels after MPTP suggests that these effects occur in striatopallidal neurons that coexpress GABA and enkephalin. This interpretation is con-

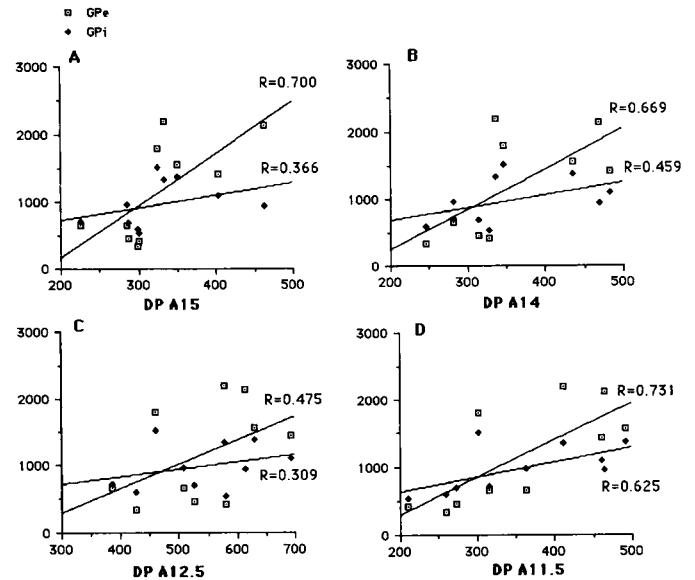


Figure 9. Values of GAD67 mRNA labeling in the dorsolateral putamen (DP) at frontal levels A15, A14, A12, and A11.5, according to the stereotaxic atlas of Emmers and Akert (1963), are plotted against values of GAD67 mRNA labeling in the GPe and GPi. Values are from five control and five MPTP-treated animals. *R*, Pearson product-moment correlation coefficient, GPe versus DP A15, DP A14, or DP A11.5; *p* < 0.05.

tent with a previous report showing increased GABA release in the GPe of MPTP-treated macaques (Robertson et al., 1991). An opposite regulation of GAD67 mRNA levels in presumed projection neurons and interneurons of the rat striatum has also been reported (Soghomonian et al., 1992). The effects of MPTP treatment on GAD67 mRNA levels in GABAergic interneurons were not investigated in this study.

Glutamate decarboxylase mRNA regulation in the pallidum

Many neurons of the GPe and GPi were found to express high levels of GAD67 mRNA. This result is consistent with previous studies in the rat pallidum (Mercugliano et al., 1992) and supports the hypothesis that most, if not all, pallidal neurons in both species are GABAergic (Smith et al., 1987). In the squirrel monkey, individual neurons of the GPi and GPe were found to express similar levels of GAD67 mRNA. In the rat, higher GAD67 mRNA levels have been previously reported in the globus pallidus than in the entopeduncular nucleus (Mercugliano et al., 1992). Such species differences could be of functional importance in view of the preferential distribution of GAD67 in neurons with a tonic versus phasic firing activity (Feldblum et al., 1993).

Our results in the GPe of MPTP-treated monkeys are consistent with previous reports of increased GAD67 mRNA levels in the rat globus pallidus after unilateral 6-hydroxydopamine injections (Kincaid et al., 1992; Soghomonian and Chesselet, 1992). These data demonstrate that dopaminergic depletion alters GAD67 gene expression in the external pallidum. Whether or not this effect is mediated by direct dopaminergic afferents or through other afferents to the GPe remains to be determined. The globus pallidus or GPe is known to receive a major projection from the striatum. The correlation of GAD67 labeling in the striatum and the GPe reported in the present study supports the hypothesis that GABAergic striatal neurons play a prominent role in the control of GABAergic neurons of the GPe.

On the other hand, the GPe also receives a strong excitatory input from the subthalamic nucleus. Previous data have shown increased activity of the subthalamic nucleus in MPTP-treated monkeys (Miller and DeLong, 1986). Thus, the subthalamic input appears to be in a strategic position to activate GPe neurons and elicit an increase in GAD67 gene expression after MPTP. This hypothesis is further supported by recent experimental evidence that lesions of the subthalamic nucleus prevent the increase in GAD67 mRNA levels in the rat globus pallidus after 6-hydroxydopamine (Chesselet et al., unpublished observations).

Another finding in this study was that GAD67 mRNA labeling was increased in the GPi of MPTP-treated squirrel monkeys. This is in agreement with a preliminary report showing such an increase in the GPi of MPTP-treated *Macaca fascicularis* monkeys (Herrero et al., 1993). In the rat unilaterally lesioned with 6-hydroxydopamine, decreased levels of GAD67 mRNA have been previously reported in neurons of the contralateral entopeduncular nucleus (Soghomonian and Chesselet, 1992). Together, these results indicate that dopamine plays an important role in the regulation of GAD67 gene expression in neurons of the internal segment of the pallidum. The GPi receives its main afferents from the striatum (Parent, 1986). Dopamine receptors in the rat entopeduncular nucleus (internal pallidum) are of the D₁ type and are mainly found on striatal afferents (Barone et al., 1987). Thus, it is likely that dopamine regulates GABAergic neurons of the GPi primarily through post- or presynaptic interactions with striatal neurons. In contrast to the GPe, GAD67 mRNA labeling in the GPi was not correlated with GAD67 mRNA labeling in the striatum. This can be viewed as further evidence that striatal GABAergic neurons showing a regulation in GAD67 gene expression after MPTP do not correspond to those projecting to the GPi.

It remains to be determined how GAD67 mRNA regulation in the GPe and GPi of MPTP-treated monkeys correlates with the electrophysiological activity of these pallidal neurons. Indeed, earlier and more recent reports have indicated that dopamine denervations result in a net decreased firing rate and increased bursting activity of GPe neurons (Miller and DeLong, 1986; Pan and Walters, 1988; Fillion and Tremblay, 1991). Increased GAD67 gene expression in MPTP-treated monkeys, however, appears consistent with the previously reported increased firing rate of GPi neurons (Miller and DeLong, 1986).

Conclusions

The present study demonstrates that GAD67 gene expression in neurons of the basal ganglia is significantly altered in parkinsonian monkeys. The importance of GABA in parkinsonism was initially suspected on the basis of higher levels of the amino acid found in symptomatic humans (Kish et al., 1986). The regional regulation of GAD67 gene expression in striatal regions (i.e., dorsolateral putamen) involved in sensorimotor processing further indicates that GABA is involved in some of the motor dysfunctions observed in parkinsonism. Involvement of the dorsal putamen in parkinsonian symptoms is also suggested by previous reports showing a preferential loss of dopaminergic innervation in that region in humans and MPTP-treated monkeys (Kish et al., 1988; Lavoie et al., 1992). Together, these data support the idea that therapeutic interventions, that is, intrastriatal grafting of dopamine-producing neurons in dopamine-depleted brains, should take into account adequate anatomical boundaries. Our results also provide further evidence

that MPTP-treated primates reproduce the biochemical and molecular alterations observed in cases of idiopathic parkinsonism.

Anatomical, electrophysiological, and pharmacological evidence indicates that dopamine exerts an opposite regulation of striatal GABAergic neurons projecting to the substantia nigra/GPi or to the GPe. After experimental dopamine deafferentations, this dual regulation is evidenced by increased enkephalin and decreased substance P/dynorphin peptide levels in striatopallidal and striatonigral neurons, respectively (for reviews, see Albin et al., 1989; Gerfen, 1992). It has been proposed that such a differential regulation results in increased activity in the GPi/substantia nigra and decreased activity in the GPe. Ultimately, these changes may result in increased inhibition of target structures such as the thalamus, thereby inhibiting motor activity. Although the regulation of GPe neurons may be more complex than previously thought, the present results in the GPi support the hypothesis of an increased GABAergic activity in the output structures of the basal ganglia in parkinsonism.

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