

Local circuit neurons in the striatum regulate neural and behavioral responses to dopaminergic stimulation

E. Saka*, M. Iadarola†, D. J. Fitzgerald‡, and A. M. Graybiel*§

*Department of Brain and Cognitive Sciences and The McGovern Institute for Brain Research, Massachusetts Institute of Technology, E25-618, 45 Carleton Street, Cambridge, MA 02139; †Neural Gene Expression Unit, National Institutes of Health, Pain and Neurosensory Mechanisms Branch, Building 49, Room 1A08, 49 Convent Drive, MSC-4410, Bethesda, MD 20892-4410; and ‡Biotherapy Section, Laboratory of Molecular Biology, National Cancer Institute, Building 37, Room 5124, 37 Convent Drive, MSC-4264, Bethesda, MD 20892-4264

Contributed by A. M. Graybiel, April 9, 2002

Interneurons are critical for shaping neuronal circuit activity in many parts of the central nervous system. To study interneuron function in the basal ganglia, we tested and characterized an NK-1 receptor-based method for targeted ablation of specific classes of interneuron in the striatum. Our findings demonstrate that the neurotoxin SP-PE35, a substance P–*Pseudomonas* exotoxin conjugate, selectively targets striatal cholinergic and nitric oxide synthase/somatostatinergic interneurons when injected locally into the striatum. The effects of this selective cell targeting encompassed alterations in both behavioral and neural responses to dopaminergic stimulation, including altered patterns of early-gene response in striosomes and matrix. We conclude that NK-1-bearing local circuit neurons of the striatum regulate the differential responses of striatal projection neurons to dopamine-mediated signaling.

The striosome and matrix compartments of the striatum are vividly demarcated by their differential expression of neurotransmitter-related compounds ranging from second messengers to neurotransmitters, neuropeptides, and their receptors (1, 2). The different connections of striosomes and matrix suggest that they participate differentially in limbic-based (striosome) and sensorimotor/associative (matrix) forebrain circuits (3–6). No behavioral or electrophysiological assays have yet identified specific functions for these compartments, but indirect assays with intracranial self-stimulation (7), early-gene activity (8), and metabolic activity (9) suggest different neural operations for striosome-based and matrix-based circuits.

How such differential functional activity is brought about in the two compartments is not known, but much interest is focused on the possibility that striatal interneurons differentially regulate activity in the striosomes and surrounding matrix (10–13). Two classes of interneuron have been implicated in such differential regulation: the cholinergic interneurons and the interneurons coexpressing nitric oxide synthase (NOS), somatostatin, and neuropeptide Y (NOS neurons). Both have been implicated in striatal plasticity, including the induction of long-term depression and long-term potentiation (14). Both lie mainly in the matrix and tend to lie at striosome-matrix borders (15–17).

These two classes of striatal interneuron both express high levels of NK-1 receptor, the tachykinin receptor at which substance P (SP) acts (18). This differential NK-1 expression pattern suggested the possibility of using a toxin-induced ablation technique to destroy these interneurons selectively (19, 20). We implemented this technology by injecting a neuronal toxin that in other systems selectively destroys neurons bearing SP (NK-1) receptors.[¶] SP-PE35 is a site-specific conjugate between an N-terminally derivatized SP peptide and an N-terminally truncated *Pseudomonas* exotoxin (PE35). SP-PE35 does not contain the native binding domain of the exotoxin (present on the amino end), but it does contain its endosome-release and enzymatic domains, thereby targeting the conjugate to the NK-1 receptor. After binding to the receptor, the molecule is internalized and leads to cytotoxicity by blocking protein synthesis. In the experiments reported here, we assessed the efficacy of SP-PE35 in

selectively ablating striatal cholinergic and somatostatinergic interneurons and tested for neural and functional effects of such cell-specific lesions.

Materials and Methods

Experiments were carried out on 59 male Sprague–Dawley rats (250–350 g) and 10 male C57BL/6 mice that were housed in pairs under a 12-h light/dark cycle with free access to food and water. All procedures were approved by the Massachusetts Institute of Technology Committee on Animal Care. Animals received unilateral or bilateral intra-striatal injections of SP-PE35 or, as a vehicle control, 0.9% saline. For surgery, the animals were anesthetized with ketamine hydrochloride (75 mg/kg for rats, 12 mg/kg for mice) and xylazine (10 mg/kg for rats, 16 mg/kg for mice). Intra-striatal stereotaxic injections of 25–100 ng (rat) or 10 ng (mouse) of SP-PE35 in 25 ng/ μ l 0.9% NaCl solution, or for controls, 0.9% NaCl injections, were made at a rate of 0.04 μ l/min into the right side or bilaterally, at anterior = 1.2 mm, lateral = 2.6 mm, ventral = 4.8 mm (rat caudoputamen) or anterior = 0.8 mm, lateral = 2.0 mm, ventral = 2.2 mm (mouse caudoputamen) relative to bregma and the dural surface.

Retrograde tracing experiments to label striatal projection neurons in the caudoputamen were carried out in six of the rats, 10 days after unilateral SP-PE35 injection. In each rat, 1 μ l of cholera beta toxin (List Biological Laboratories, Campbell, CA, 500 ng/ μ l), was injected into the substantia nigra of each side (posterior = 5.6 mm, lateral = \pm 2.6 mm, ventral = 7.6 mm).

The home-cage behavior of all animals was observed daily. In addition, we scored the amount of ipsilateral and contralateral rotation induced by dopaminergic challenge in rats ($n = 28$) and mice ($n = 5$) with unilateral toxin injections and the amount of motor stereotypy induced by dopaminergic challenge in rats with bilateral toxin ($n = 8$) or vehicle ($n = 7$) injections. To test for the development of rotational behavior indicative of a striatal lesion, the dopamine receptor agonist, apomorphine (3 mg/kg), or saline, was administered i.p. 1–10 days after unilateral SP-PE35 injection. The home-cage behavior of the rats was observed for 15 min before and 1 h after the apomorphine treatment. Rotations per minute ipsilateral and contralateral to SP-PE35 injection were counted during the 15-min period after administration of drug or saline.

Other groups of rats ($n = 20$) and mice ($n = 5$) with unilateral lesions were treated acutely 10 days after SP-PE35 injection either with i.p. injections of apomorphine (5 mg/kg), a combination of a D1-class dopamine receptor agonist (SKF-81297, 3

Abbreviations: NOS, nitric oxide synthase; SP, substance P; PE, *Pseudomonas* exotoxin; ChAT, choline-acetyltransferase; ISMP, index of striosome to matrix predominance.

§To whom reprint requests should be addressed. E-mail: graybiel@mit.edu.

¶Iadarola, M. J., Wang, X., Caudle, R. M., Perez, F., Carrero, H., Yang, H.-Y. T. & Fitzgerald, D. (1999) *Soc. Neurosci. Abstr.* 25, 679.

mg/kg) and a D2-class dopamine receptor agonist (quinpirole, 3 mg/kg), or saline.

To test for the effects of SP-PE35 on behavioral stereotypy in response to repeated psychomotor stimulant exposure, rats with bilateral SP-PE35 or vehicle injections of 10 days standing were given apomorphine i.p. 5 mg/kg twice a day for 7 days (10 a.m. and 5 p.m.), followed by 7 days of withdrawal from the drug and a final 10 a.m. challenge with apomorphine. Their behavior was monitored after the 10 a.m. injections and after the final challenge. An observer blind to the experimental conditions observed each rat for 1 min every 10 min for 50 min. For each sampling time, a stereotypy score from 1 to 10 was given based on estimates of the repetitiveness, frequency, duration, and spatial distribution of behavioral responses (8), and the average of these scores was taken as the session score. Scores for the SP-PE35-injected and vehicle-injected groups were compared by Mann-Whitney *U* tests.

Immunohistochemistry. Two hours after final drug or saline injection, or 5 days after cholera toxin injection, animals were deeply anaesthetized with pentobarbital (>50 mg/kg) and perfused transcardially with 4% paraformaldehyde in 0.1 M NaKPO₄ buffer. Frozen 24- μ m-thick transverse sections were stained free-floating by single- or dual-antigen immunohistochemical methods (8, 21) with antisera including rabbit anti-NK-1 (Chemicon; 1:5,000), anticalretinin (Swant, Bellinzona, Switzerland; 1:4,000), antimethionine enkephalin (Dia Sorin, Stillwater, MN; 1:2,000), antidynorphin (leumorphine, kind gift from S. Watson, University of Michigan, Ann Arbor; 1:10,000), goat anticholine-acetyltransferase (ChAT, Chemicon; 1:2,000), antivesicular acetylcholine transporter (Chemicon; 1:8,000), rat antisomatostatin (Fitzgerald Industries, Concord, MA; 1:100), mouse antiparvalbumin (Sigma; 1:1,000), and antityrosine hydroxylase (Dia Sorin; 1:1,000). NADPH-diaphorase histochemistry was used to mark NOS neurons (21). Retrogradely labeled projection neurons were stained with goat anti-cholera toxin antiserum (List Biological Labs; 1:3,500).

Neurons with early-gene responses to final drug challenges were analyzed with rabbit antisera raised against c-Fos (Ab-5, Calbiochem; 1:40,000), FosB (Santa Cruz Biotechnology; 1:10,000), JunB (kind gift of R. Bravo, Bristol-Myers Squibb; 1:10,000), and Fra (1:10,000) (21). Dual immunostaining was carried out to detect simultaneously Fos-positive parvalbumin-, calretinin-, and enkephalin-immunoreactive neurons (21) and striosomes (rabbit anti-mu opiate receptor, MOR1, Dia Sorin; 1:20,000). Mouse tissues were stained for NK-1, ChAT, NADPH diaphorase, c-Fos, and MOR1.

Data Analysis. All sections were analyzed by light microscopy. Regions with totally depleted or diminished NK-1 staining were charted with the aid of a computerized imaging system (Biocom, Les Ulis, France). Counts of immunopositive neurons in two sections from each rat brain (anterior +1.6 to 1.8 mm and midanterioposterior level +0.0 to 0.2 mm, relative to bregma) were made within a 4-mm² sample region in the medial and lateral caudoputamen. Neurons immunoreactive for ChAT, NADPH diaphorase, parvalbumin, calretinin, dynorphin, and enkephalin, and neuronal nuclei immunoreactive for Fos, were counted within NK-1-depleted zones and corresponding zones in the intact caudoputamen. A thresholding technique was applied for consistency of counting along with rechecks of previous counts (21).

For each sample region, the density of immunopositive neurons and the densities of Fos-positive nuclei in striosomes (MOR1-positive) and matrix (MOR1-negative) were determined and ISMP (index of striosome to matrix predominance) values were calculated (8). Data for the SP-PE35-injected and control hemispheres were compared by two-tailed Student's *t* tests.

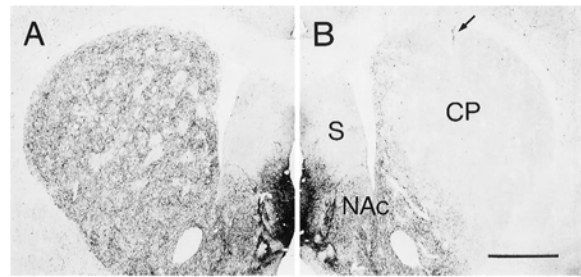


Fig. 1. Immunohistochemical staining for NK-1 receptor distribution in the rat caudoputamen (CP) 10 days after unilateral injection of SP-PE35 demonstrates loss of NK-1 receptors on the injected side (B) compared to normal side (A). Arrow in B points to needle track. NAC, nucleus accumbens; S, septum. (Scale bar = 1 mm.)

Results

Intrastriatal Administration of SP-PE35 in the Rodent Striatum Induces Selective Degeneration of Cholinergic and Somatostatinergic Interneurons.

Even the lowest dose of SP-PE35 resulted in a total disappearance of NK-1 immunostaining by day 10 postinjection (Fig. 1). A time-course study of sections from rats perfused at 2 h to 28 days indicated that NK-1 immunostaining at the injection site first increased and then decreased sharply. By postinjection day 4, both dendritic and cell body immunoreactivity was enhanced over control levels (data not shown), but by postlesion day 6 and thereafter, the injection sites were demarcated as regions devoid of NK-1 receptor immunoreactivity (Fig. 1).

Clear declines in immunostaining for cholinergic and NOS-containing interneurons were visible by postlesion day 4; both populations had decreased by half relative to the numbers on the contralateral side ($P < 0.001$). By postlesion day 10, no cholinergic interneurons or NOS interneurons remained within the zones of NK-1 depletion, as assayed by ChAT, vesicular acetylcholine transporter, NADPH diaphorase, and somatostatin im-

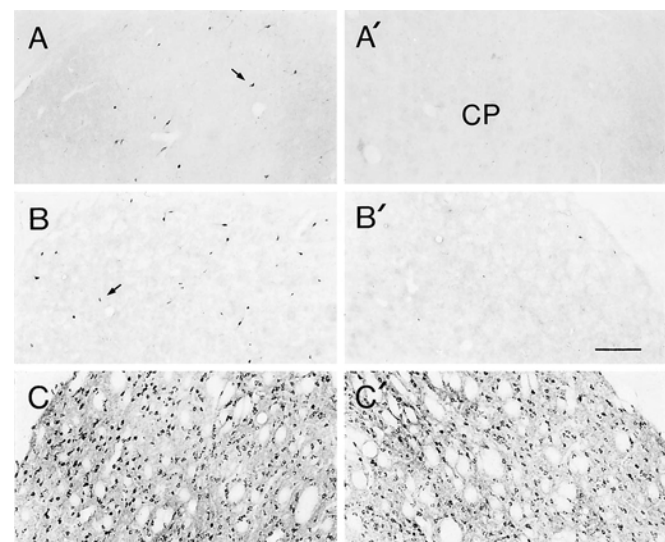


Fig. 2. SP-PE35 injections produce cell-specific ablation of striatal cholinergic and NOS interneurons in the rat striatum. (A and B) Normal levels of expression of (A) ChAT immunoreactivity and (B) NADPH diaphorase activity. Arrows point to examples of stained neurons. (A' and B') Shown is loss of these markers on the side of the SP-PE35 injection. (C and C') Shown are similar levels of retrograde labeling of striatonigral neurons with cholera toxin in the control (C) and SP-PE35-injected (C') caudoputamen (CP). (Scale bar = 200 μ m.)

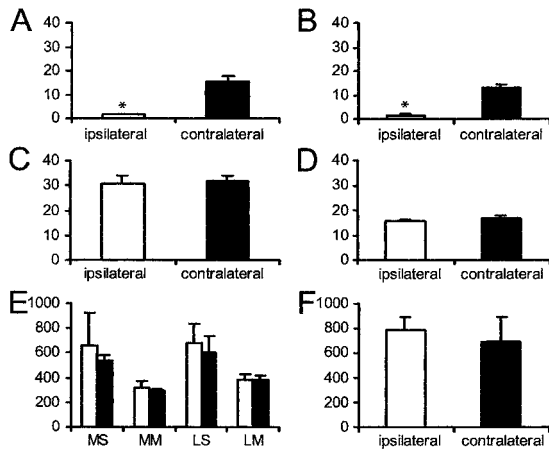


Fig. 3. The loss of cholinergic and NOS interneurons in the caudoputamen induced by SP-PE35 injection is selective. Graphs illustrate densities of neuronal subtypes (cell number/mm², \pm standard errors) calculated from counts in 4–6 rats per subtype in regions of SP-PE35 depletion and mirror image sites in contralateral (control) striatum. Note the almost complete loss of (A) cholinergic and (B) NOS interneurons, with preservation of normal densities of (C) parvalbumin-containing and (D) calretinin-containing interneurons as well as (E) dynorphin-positive and (F) enkephalin-positive projection neurons. *, $P < 0.0001$. MS, medial striosomes; MM, medial matrix; LS, lateral striosomes; LM, lateral matrix.

munostaining (Fig. 2). No decreases were detectable in parvalbumin-positive or calretinin-positive interneurons (Fig. 3).

NK-1 receptors in the striatum are expressed principally by the cholinergic and NOS interneurons, but low levels of NK-1 receptor expression (22) and some direct NK-1 receptor-mediated effects (23) have been reported for SP-immunoreactive projection neurons themselves, suggesting the presence of NK-1 autoreceptors. We therefore tested for changes in the numbers of striatal projection neurons within the SP-PE35 injection sites. In rats with unilateral SP-PE35 injections, we counted the numbers of enkephalin-immunoreactive and dynorphin-immunoreactive projection neurons at the sites of NK-1 depletion relative to corresponding regions in the contralateral striatum. We used immunohistochemistry for the coexpressed neuropeptide dynorphin (24) to mark SP-containing neurons, because adequate SP perikaryal immunostaining was not possible with available antisera. We found no change in the density for the enkephalin-immunoreactive neurons, but a slight, although statistically insignificant, increase in the numbers of dynorphin-immunoreactive neurons in striosomes on the side of SP-PE35 injection (Fig. 3).

Based on the fact that striatal SP/dynorphin-immunoreactive neurons project to the substantia nigra (4), we retrogradely labeled striatonigral projection neurons by injecting cholera beta toxin bilaterally into the substantia nigra of rats with unilateral SP-PE35 lesions, reasoning that the capacity for retrograde transport would indicate physiological viability of the resulting retrogradely labeled neurons. We observed abundant and apparently equivalent labeling of projection neurons within the sites of SP-PE35 injection in both striosomes and matrix, except along the tract of the injection needle (Fig. 2 C and C'). Finally, we observed no change in tyrosine hydroxylase immunostaining, a marker for dopamine-containing striatal afferents, in the regions of NK-1 depletion.

Ablation of Striatal Cholinergic and NOS Interneurons Alters the Relative Excitability of Striatal Projection Neurons in Striosome and Matrix Compartments. A clear change in the functional response of striatal neurons was evident in the expression of Fos-Jun

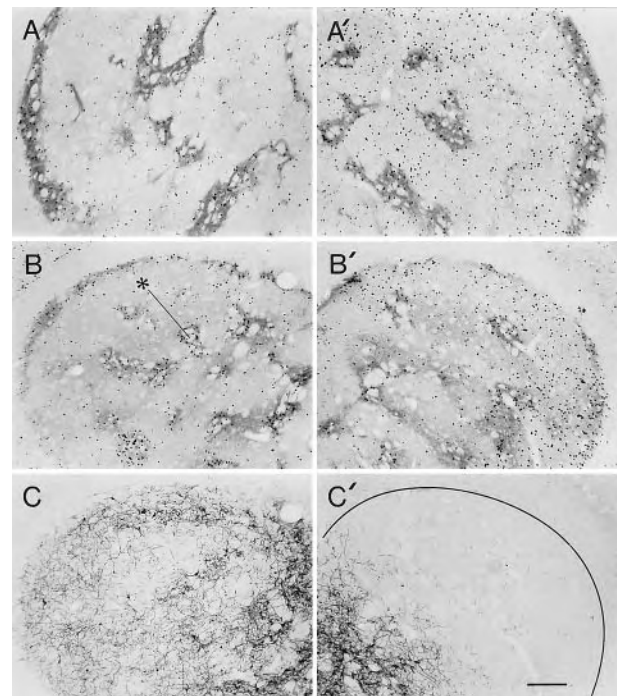


Fig. 4. Striosome-predominant expression of c-Fos induced by combined D1-D2 dopamine receptor agonist treatments is reduced by SP-PE35-mediated ablation of striatal cholinergic and NOS interneurons. Animals were treated with SKF-81297 and quinpirole (3 mg/kg each). Striosome-predominant Fos induction in intact striatum of (A) rat and (B) mouse, and loss of this pattern caused by increased Fos expression in the matrix on the side of SP-PE35 injection (A' and B'). * in B indicates example of a MOR1-positive striosome. (C and C') NK-1 immunostaining in sections adjacent to those shown in B and B'. Note that the zone of NK-1 depletion (C') is similar to the zone in which there is greater Fos induction in the matrix (B'), compared with Fos induction in the control striatum (B). (Scale bar = 200 μ m.)

family early-response genes (*c-Fos*, *FosB*, *Fra*, and *JunB*) evoked by acute administration of D1-class and D2-class dopamine receptor agonist combinations. In normal rats and mice, such treatments induce a striosome-predominant pattern of early gene expression (8, 21, 25, 26), and they did so in the caudoputamen contralateral to SP-PE35 injection (Fig. 4 A and B). By contrast, this pattern of greater striosome than matrix gene expression was greatly blurred within the zones of NK1 depletion (Fig. 4 A' and B').

To analyze this abnormality, we counted the numbers of Fos-positive nuclei in striosomes and matrix within the NK-1-depleted zones of the caudoputamen and in mirror-image zones on the contralateral side, and calculated the relative densities of Fos-positive nuclei in each compartment (ISMP values) for the sampled regions.

There were no significant differences in the numbers of Fos-positive neurons in striosomes on the two sides, but there were significantly larger numbers of Fos-positive neurons in the matrix of the NK-1-depleted zones than in the matrix on the contralateral side (Table 1). The strongest effect of the SP-PE35 injections was in the rostral striatum, where densities of Fos-positive neurons in the matrix were about 150% greater than those on the uninjected side ($P < 0.05$). The increases in matrix excitability were smaller, but also significant, in the more caudal sections (about 70% increases, $P < 0.05$). This differential effect of the SP-PE35 injections on the Fos response of the matrix compartment was reflected in lower ISMP values for the caudoputamen exposed to SP-PE35 than for the control caudoputamen (Fig. 5, $P < 0.0001$ in rostralateral striatum). These results

Table 1. Acute (SKF-81297/quinpirole, 3 mg/kg) and chronic (apomorphine, 5 mg/kg) dopamine receptor agonist treatments induce reduced striosome-predominant patterns of Fos expression after intrastriatal SP-PE35 injections

Groups	Rostral caudoputamen				Mid-anteroposterior caudoputamen			
	Striosomes		Matrix		Striosomes		Matrix	
	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral
Acute								
A	872 ± 130	680 ± 130	487 ± 107*	395 ± 82*	945 ± 77	1,083 ± 86	516 ± 85*	313 ± 52
B	874 ± 70	888 ± 161	198 ± 46	157 ± 34	978 ± 124	1,157 ± 132	279 ± 54	193 ± 45
Chronic								
A	871 ± 61	740 ± 69	108 ± 8**	136 ± 16*	895 ± 63	834 ± 76	123 ± 6**	144 ± 16
B	773 ± 59	660 ± 71	72 ± 5	86 ± 13	956 ± 59	858 ± 39	91 ± 6	103 ± 15

Values represent densities of Fos-positive neurons (neurons/mm²) in SP-PE35-injected (A) or control (B) striatum ± standard errors. *n* = 7–8; *, *P* < 0.05; **, *P* < 0.01.

suggest that the loss of clear striosome predominance after SP-PE35 treatment was the result of increased responsiveness of matrix neurons to dopamine agonist challenge. Such increased matrix responsiveness was also evident by visual inspection in sections immunostained for JunB, FosB, and Fra (data not shown).

Chronic intermittent treatment with D1-D2 dopamine receptor agonist combinations produces long-term changes in the functional responses of striatal neurons, including a heightened inducibility of a range of early-response genes in the striosomal compartment relative to their inducibility in the matrix even after weeks of drug withdrawal (8, 21). This change reflects depressed inducibility in the matrix (8). We tested whether the capacity for such long-term plasticity remains in the striatum after depletion of its cholinergic and NOS neurons. The typical pattern of striosome-predominant Fos expression emerged in control animals with intrastriatal saline injections challenged with apomorphine after chronic-intermittent apomorphine treatment and withdrawal. In the SP-PE35-injected animals this pattern was significantly reduced within zones of NK-1 depletion (Fig. 6, Table 1). As in the acutely treated animals, the reduced striosome predominance reflected greater Fos expression in the matrix than in the controls (58% increase rostrally, 37% farther caudally, *P* < 0.05 or *P* < 0.01). The SP-PE35 injections had almost no effect on Fos expression in striosomes. The increased matrix activation, in the absence of increased striosome activation, led to significant declines in the corresponding ISMP values (*P* < 0.01).

In normal animals, treatments with D1-D2 agonist combinations induce Fos expression almost exclusively in the dynorphin-expressing projection neurons of the striatum. We tested whether the increased Fos expression in the matrix compartment after SP-PE35 treatment reflected recruitment of Fos expression in enkephalinergic projection neurons. It was not. We found no

Fos expression in enkephalin-positive neurons with either acute or chronic apomorphine treatment.

The cholinergic interneurons of the striatum, destroyed by the SP-PE35 lesions in our experiments, influence the population of parvalbumin-containing interneurons in the striatum (27). We therefore tested whether the SP-PE35 injections altered the Fos response of these parvalbumin neurons to dopamine receptor agonists (*n* = 7 for each group). Whereas the Fos response in parvalbumin interneurons was effectively nil in the controls, in the SP-PE35-injected caudoputamen Fos-positive parvalbumin neurons appeared in every section in the rats chronically treated with apomorphine (Fig. 7), amounting to about a 13-fold increase relative to values in saline-injected caudoputamen of the controls (*P* < 0.01). There was no Fos expression in calretinin-containing interneurons.

Depletion of Cholinergic and NOS Interneurons in the Striatum Leads to Altered Rotational Responses to Dopamine Receptor Agonist Treatments but Does Not Prevent the Induction of Behavioral Stereotypy.

Rats with unilateral SP-PE35 injections were tested for the development of rotational behavior in response to i.p. apomorphine (3 mg/kg) or saline, starting from the first day of SP-PE35 injection. Before apomorphine treatment and after saline injections, levels of turning were almost nil, and no remarkable behavioral signs were observed. Apomorphine injections led to slow, but tight, turning toward the ipsilateral side (about two turns/min on postinjection days 1–6) in seven of eight SP-PE35-injected animals. From day 7 (*n* = 6), day 8 (*n* = 1), or day 9 (*n* = 1), the direction of turning reversed, and turning toward the contralateral side gradually increased to about two turns/min (Fig. 8). The late-stage contralateral turning response was confirmed in another group of 12 rats and in five mice with unilateral SP-PE35 injections, treated on postinjection day

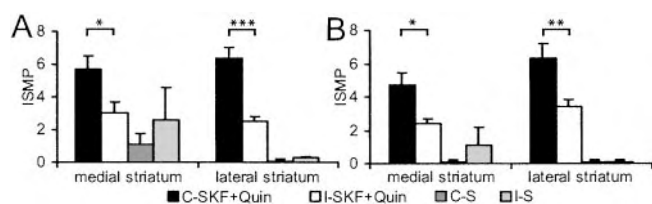


Fig. 5. Striosome-predominant Fos expression induced by combined D1-D2 dopamine receptor agonist treatment is diminished after SP-PE35 treatments. Graphs illustrate the ISMP values ± standard errors for Fos induction in SP-PE35-injected caudoputamen (I) at rostral (A) and midanteroposterior (B) levels compared with ISMP values in the caudoputamen contralateral to the injection (C). Rats (*n* = 5–8) were given SKF-81297 plus quinpirole (3 mg/kg each) or saline (S). *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

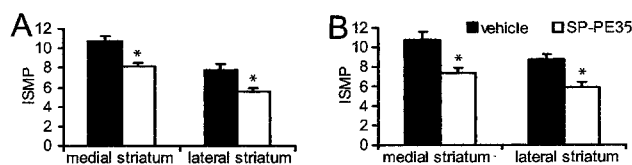


Fig. 6. Intrastriatal SP-PE35 injections prevent augmented striosome-predominant pattern of early gene expression typical of striatal response to chronic intermittent D1-D2 dopamine receptor agonist treatments. Graphs show that after chronic intermittent apomorphine treatment (5 mg/kg) and final apomorphine challenge, ISMP values (± standard errors) rise to high levels in the caudoputamen of controls (filled bars), but do not reach such high levels in the rats previously given intrastriatal injections of SP-PE35 (empty bars). (A) Rostral caudoputamen. (B) Midanteroposterior caudoputamen. *n* = 7–8; *, *P* < 0.01.

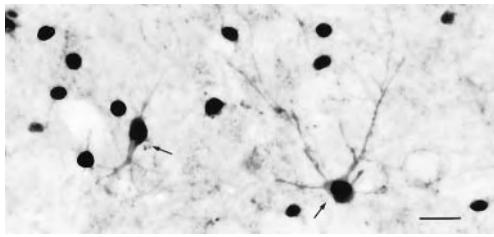


Fig. 7. SP-PE35 toxin-induced ablation of striatal cholinergic and NOS interneurons increases the early-gene responsiveness of striatal parvalbumin-containing interneurons to dopamine receptor agonist treatments. Photomicrograph illustrates Fos-positive parvalbumin-containing interneurons (arrows) in the SP-PE35-injected caudoputamen of a rat chronically treated with apomorphine (5 mg/kg) before final apomorphine challenge. (Scale bar = 20 μ m.)

10 with either apomorphine or the SKF-81297-quinpirole combination.

In addition to the increased turning, both the rats and the mice exhibited stereotypies, mainly repetitive nibbling and licking, in response to the drug treatments. To test formally for the development of stereotypy, we made bilateral injections of SP-PE35 in eight rats and scored the amount of stereotypy induced by the initial and final doses of apomorphine in the 7-day chronic-intermittent protocol, compared with values induced in rats with bilateral intrastriatal saline injection (Fig. 9). We found no differences between the saline and SP-PE35 groups: stereotypy scores rose from about 7.5 and 8 to about 9 and 9.5, respectively.

Discussion

The first functional marker of striosome-matrix compartmentalization in the striatum was a disproportionate increase in early-gene expression in striosomes after exposure to amphetamine, an indirect dopamine receptor agonist (25). It is now known that such relative enhancement of activation in striosomes is correlated with the development of behavioral stereotypy (8), that it depends on coordinate activation of D1-class and D2-class dopamine receptors (38), and that a similar enhancement of early-gene responses in striosomes occurs in response to dopaminergic challenge in animal models of Parkinson's disease exhibiting abnormal movement (28, 29). We show here that interneurons in the striatum function in the development of this differential compartmental response of striatal neurons and raise the possibility that these interneurons could influence the linkage between compartmental activation patterns and the expression of behavioral stereotypy.

Receptor-Based Cell Targeting Represents a Model for Analyzing the Functions of Interneurons in the Striatum. Selective ablation of subclasses of neurons is only now becoming possible with the development of molecularly based cell targeting techniques (19,

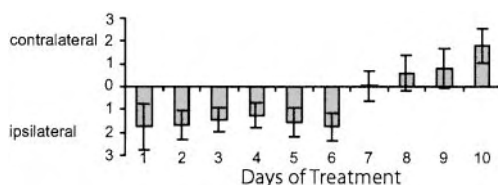


Fig. 8. Unilateral SP-PE35-mediated ablation of striatal cholinergic and NOS interneurons results in changes in rotational responses to dopamine receptor agonist challenge. The graph illustrates the time course of apomorphine-induced home-cage ipsilateral and contralateral turning behavior of rats with unilateral SP-PE35 injection ($n = 8$). Bars indicate standard errors of the mean.

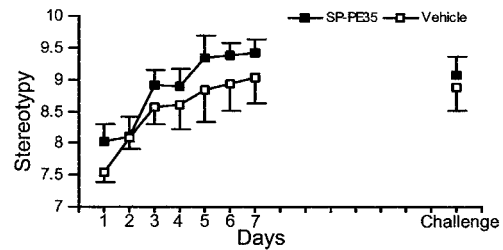


Fig. 9. SP-PE35 toxin-induced ablation of cholinergic and NOS interneurons in the caudoputamen does not prevent the development of stereotypy in rats administered chronic intermittent apomorphine. Curves show stereotypy scores (\pm standard errors) for rats given bilateral SP-PE35 injections (■) or bilateral saline injections (□) in the caudoputamen. Note that both during the development of sensitization and after a week of withdrawal, the expression of stereotypy is similar in the two groups.

20). Our experiments demonstrate that SP-PE35, a SP receptor-based toxin, when injected into the rodent striatum, selectively targets and eliminates the two classes of striatal interneuron that abundantly express SP (NK-1) receptors. The time course for the toxic effect was divided into an initial phase in which NK-1 expression was elevated as the cell loss began and a later phase in which both NK-1 expression and the cholinergic and NOS interneurons disappeared from the zones of NK-1 depletion. The targeted cell ablation was specific to the cholinergic and NOS interneurons and resulted in their permanent loss. The SP-PE35 dose and injection parameters could be adjusted to accomplish ablation of nearly all of these interneurons in the dorsal striatum or to accomplish their ablation in a particular striatal subregion. These results suggest that the SP-PE35 toxin treatment holds promise as a model for studying striatal circuit function.

A special feature of this cell targeting method is that it is based on differential endogenous expression of receptors competent to bind ligand and so can be used to assess the function of the subsets of neurons normally targeted by a particular ligand. SP, the endogenous ligand activating NK-1 receptors, is colocalized with γ -aminobutyric acid in striatal neurons, giving rise to the direct and striosomal output pathways of the basal ganglia. The SP-PE35 cell targeting thus represents an experimental model for studying not only the functions of the cholinergic and NOS interneurons themselves, but also the local intrastriatal effects of SP-mediated direct and striosomal pathways under conditions leaving their long-axon connections intact.

The Cholinergic and NOS Interneurons of the Striatum Function in the Control of Movement. As an initial test of this interneuron ablation model, we analyzed the effects of the intrastriatal SP-PE35 injections on behavioral responses to dopamine agonist treatments. Such agonist treatments typically elicit abnormal rotational behavior after a variety of interventions affecting the striatum unilaterally, and they induce contralateral turning in transgenic mice after unilateral immunotoxin-mediated ablation of cholinergic interneurons (30). Opposing effects of dopamine and acetylcholine on striatal projection neurons are thought to underlie such behavioral asymmetries (30, 31). We found contralateral turning in response to dopaminergic challenge a week or more after unilateral SP-PE35 injections, but at earlier times found ipsiversive turning. This initial ipsilateral turning could have resulted from the initial phase of increased NK-1 expression within the SP-PE35 injection sites, at a time when many cholinergic and NOS neurons still survived. Increased activation of such receptors could have led to increased release of acetylcholine from still-surviving cholinergic interneurons (32, 33).

Cholinergic and/or NOS Interneurons Can Influence Differential Responses of the Striosome and Matrix Compartments of the Striatum.

With early-gene expression assays, we found that SP-PE35 treatment led to two clear changes in the responses of striatal neurons to systemic dopaminergic treatments. First, the striosome-predominant pattern of Fos activation typical of responses to combined D1-D2 agonist challenges was greatly diminished in the SP-PE35-injected striatum, and this difference was the result of a greater activation of projection neurons in the matrix rather than decreased expression in striosomes. There was no change in the phenotype of the responding projection neurons: they were dynorphin-immunoreactive. This finding suggests that the cholinergic and/or NOS interneurons influence the dopamine-related responses of the striatal projection neurons that give rise to the direct, movement-enhancing pathway of the basal ganglia. Second, we found an increased Fos response of the parvalbumin-containing striatal interneurons in response to dopamine agonist stimulation in the animals exposed to chronic intermittent agonist treatment and withdrawal, but not the animals given single acute agonist treatments. This result suggests that a generalized change in local network activity occurs as a result of targeting the cholinergic and NOS neurons of the striatum and suggests further that this change occurs under conditions eliciting striatal neuroplasticity.

The decline in striosomal patterning of the early-gene distributions in the SP-PE35-injected striatum raises the possibility that striatal interneurons are critical to the so-called D1-D2 synergisms that characterize the early-gene responses. In normal animals, such striosome-predominant response patterns occur only with combined agonist stimulation of D1-class and D2-class receptors (34–36). Combined D1-D2 agonist treatment is also required to elicit a Fos response in the cholinergic interneurons themselves (11). In the chronically treated animals studied here, the striosome-predominant pattern was very pronounced in the control animals. This pattern, although diminished, was still detectable in the SP-PE35-treated animals, despite the greater

activation of the matrix within the zones of NK-1 depletion. Other network changes normally associated with dopaminergic stimulation may have partly compensated for the loss of the interneurons in the animals given chronic intermittent treatments, such as increased dynorphin and decreased SP (37), or lack of up-regulated activation of NOS interneurons (missing after SP-PE35 treatment). Compensation could also have reflected recruitment of parvalbumin-containing interneurons, which normally produce widespread inhibition of striatal projection neurons and are regulated by cholinergic interneurons (missing after SP-PE35 treatment) (27).

The stereotypies induced by such combined D1-class and D2-class agonist treatments are a central feature of the behavioral sensitization that develops as a result of repeated exposure to dopamine receptor agonists (8, 38). Because the SP-PE35 injections decreased the striosome-predominant neural response that in normal animals is highly correlated with the development of stereotypies, we predicted that the stereotypies in bilaterally SP-PE35-injected animals would also be reduced. They were not. We did, however, confirm the correlation in uninjected controls. This result raises the testable possibility that the loss of cholinergic and NOS interneurons may have led to a breakdown in the correlation between the neural and behavioral responses. Whether or not such a breakdown occurs, our findings establish that when the cholinergic and NOS neurons of the striatum are selectively ablated dopamine-receptor agonist treatments lead to abnormal patterns of activation in the striosome and matrix compartments of the striatum.

We thank Ms. Patricia Harlan for her insightful help with the immunohistochemistry, Mr. H. F. Hall, who is responsible for the photography, Dr. R. Bravo and Dr. S. Watson for their generous gifts of antisera, and Dr. J. M. Tepper for commenting on the manuscript. The work was funded by National Institutes of Health/National Institute of Mental Health Grant MH60379, Udall Center National Institutes of Health/National Institute of Mental Health Grant NS38372, and The Scientific and Technical Research Council of Turkey.

- Graybiel, A. M. (1990) *Trends Neurosci.* **13**, 244–254.
- Holt, D. J., Graybiel, A. M. & Saper, C. B. (1997) *J. Comp. Neurol.* **384**, 1–25.
- Donoghue, J. P. & Herkenham, M. (1986) *Brain Res.* **365**, 397–403.
- Gerfen, C. R. (1992) *Trends Neurosci.* **15**, 133–139.
- Flaherty, A. W. & Graybiel, A. M. (1994) *J. Neurosci.* **14**, 599–610.
- Eblen, F. & Graybiel, A. M. (1995) *J. Neurosci.* **15**, 5999–6013.
- White, N. M. & Hiroi, N. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 6486–6491.
- Canales, J. J. & Graybiel, A. M. (2000) *Nat. Neurosci.* **3**, 377–383.
- Brown, L. L., Feldman, S. M., Smith, D. M., Cavanaugh, J. R., Ackerman, R. F. & Graybiel, A. M. (2002) *J. Neurosci.* **22**, 305–314.
- Kawaguchi, Y., Wilson, C. J., Augood, S. J. & Emson, P. C. (1995) *Trends Neurosci.* **18**, 527–535.
- Svenningsson, P., Fredholm, B. B., Bloch, B. & Le Moine, C. (2000) *Neuroscience* **98**, 749–757.
- Cicchetti, F., Prensa, L., Wu, Y. & Parent, A. (2000) *Brain Res. Brain Res. Rev.* **34**, 80–101.
- Graybiel, A. M., Aosaki, T., Flaherty, A. W. & Kimura, M. (1994) *Science* **265**, 1826–1831.
- Centonze, D., Gubellini, P., Bernardi, G. & Calabresi, P. (1999) *Brain Res. Brain Res. Rev.* **31**, 1–5.
- Sandell, J. H., Graybiel, A. M. & Chesselet, M.-F. (1986) *J. Comp. Neurol.* **243**, 326–334.
- Graybiel, A. M., Baughman, R. W. & Eckenstein, F. (1986) *Nature (London)* **323**, 625–627.
- Kubota, Y. & Kawaguchi, Y. (1993) *J. Comp. Neurol.* **332**, 499–513.
- Li, J. L., Wang, D., Kaneko, T., Shigemoto, R., Nomura, S. & Mizuno, N. (2000) *J. Comp. Neurol.* **418**, 156–163.
- Gray, P. A., Janczewski, W. A., Mellen, N., McCrimmon, D. R. & Feldman, J. L. (2001) *Nat. Neurosci.* **4**, 927–930.
- Martin, J. L. & Sloviter, R. S. (2001) *J. Comp. Neurol.* **436**, 127–152.
- Moratalla, R., Elibol, B., Vallejo, M. & Graybiel, A. M. (1996) *Neuron* **17**, 147–156.
- Bolam, J. P. & Izzo, P. N. (1988) *Exp. Brain Res.* **70**, 361–377.
- Galarraga, E., Hernandez-Lopez, S., Tapia, D., Reyes, A. & Bargas, J. (1999) *Synapse* **33**, 26–35.
- Reiner, A. & Anderson, K. D. (1990) *Brain Res. Brain Res. Rev.* **15**, 251–265.
- Graybiel, A. M., Moratalla, R. & Robertson, H. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6912–6916.
- Tan, A., Moratalla, R., Lyford, G. L., Worley, P. & Graybiel, A. M. (2000) *J. Neurochem.* **74**, 2074–2078.
- Koós, T. & Tepper, J. M. (2002) *J. Neurosci.* **22**, 529–535.
- Saka, E., Elibol, B., Erdem, S. & Dalkara, T. (1999) *Brain Res.* **825**, 104–114.
- Cenci, M. A., Tranberg, A., Andersson, M. & Hilbertson, A. (1999) *Neuroscience* **94**, 515–527.
- Kaneko, S., Hikida, T., Watanabe, D., Ichinose, H., Nagatsu, T., Kreitman, R. J., Pastan, I. & Nakanishi, S. (2000) *Science* **289**, 633–637.
- di Chiara, G. & Morelli, M. (1994) in *The Basal Ganglia IV*, eds. Percheron, G., McKenzie, J. S. & Féger, J. (Plenum, New York), pp. 491–505.
- Arenas, E., Alberch, J., Perez-Navarro, E., Solsona, C. & Marsal, J. (1991) *J. Neurosci.* **11**, 2332–2338.
- Anderson, J. J., Chase, T. N. & Engber, T. M. (1993) *Brain Res.* **623**, 189–194.
- Paul, M. L., Graybiel, A. M., David, J.-C. & Robertson, H. A. (1992) *J. Neurosci.* **12**, 3729–3742.
- LaHoste, G. J. & Marshall, J. F. (1993) *Brain Res.* **611**, 108–116.
- Wirtshafter, D. & Asin, K. E. (1994) *Brain Res. Bull.* **35**, 85–91.
- Steiner, H. & Gerfen, C. R. (1998) *Exp. Brain Res.* **123**, 60–76.
- Capper-Loup, C., Canales, J. J., Kadaba, N. & Graybiel, A. M. (2002) *J. Comp. Neurol.*, in press.