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Dopamine Differentially Regulates Dynorphin, Substance P, and Enkephalin Expression in Striatal Neurons: *In situ* Hybridization Histochemical Analysis

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Dopamine regulation of the levels of dynorphin, enkephalin, and substance P messenger RNAs in rat striatal neurons was analyzed with *in situ* hybridization histochemistry (ISHH). Relative levels of peptide mRNA expression in the patch and matrix compartments of the dorsolateral striatum were compared among control rats, rats treated for 10 d with apomorphine, rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopaminergic system, and rats with nigrostriatal dopaminergic lesions followed 2 weeks later by 10 d of apomorphine treatment. Image analysis of ISHH labeling demonstrated that the number of neurons expressing each peptide mRNA remained constant, whereas the relative level of peptide mRNA per neuron changed significantly, depending on the experimental treatment. Dynorphin mRNA expression increased following chronic apomorphine treatment: striatal patch neurons increased to an average of 100% above control values, whereas striatal matrix neurons showed only a 25% increase. Dynorphin mRNA expression decreased following 6-OHDA lesions: patch neurons showed an average 75% reduction in expression, whereas matrix neurons showed no significant change. In animals with 6-OHDA lesions followed by apomorphine treatment, both patch and matrix neurons showed an average increase in dynorphin expression of 300% above control levels. Changes in dynorphin mRNA levels with these treatments were matched by qualitative changes in dynorphin immunoreactivity both in the striatum and in striatonigral terminals in the substantia nigra. Neither substance P nor enkephalin mRNA levels showed a significant difference between the striatal patch and matrix compartments in any experimental condition (in the dorsolateral striatum). Substance P mRNA expression was increased an average of 50% after 10 d of apomorphine treatment and showed an average decrease of 75% following 6-OHDA lesions of the mesostriatal system. There was no significant change in the expression of substance P mRNA in striatal neurons compared to control values in rats with combined 6-OHDA lesion and apomorphine treatment. Enkephalin mRNA expression was not significantly altered by chronic apomorphine treatment but showed an average increase per cell of some 130% above control levels following 6-OHDA-induced lesions of the mesostriatal system. In animals with a 6-OHDA lesion and apomorphine treatment, enkephalin mRNA was also elevated but not significantly above the levels produced by the lesions alone. These data show that the expression of dynorphin, enkephalin, and substance P is differentially regulated by the mesostriatal dopaminergic system and, further, suggests that the mechanisms by which this regulation occurs may be different for the 3 peptide families.

The major efferent neurons of the striatum are the medium spiny neurons (Grofova, 1975; Kitai et al., 1976; Wilson and Groves, 1980; Somogyi et al., 1981), which constitute 90–95% of the striatal cell population (Kemp and Powell, 1971). The rather homogeneous distribution of these neurons belies an underlying segregation of neuronal populations that may be characterized in at least 3 ways. First, medium spiny neurons are segregated into 2 mosaically organized striatal compartments, termed the patches and matrix (Gerfen, 1984, 1985; Kawaguchi et al., 1989). The patch compartment is marked by areas of dense μ-opiate receptor binding (Pert et al., 1976) and areas of low AChE staining (Graybiel and Ragsdale, 1978; Herkenham and Pert, 1982). The matrix compartment, which comprises over 80% of the striatum and is complementary to the patches, contains calbindin-immunoreactive neurons and a rich plexus of somatostatin-immunoreactive fibers (Gerfen et al., 1985). Neurons in the patches project to the location of dopaminergic cell bodies or their proximal dendrites in the substantia nigra, whereas neurons in the matrix project to the area of GABAergic neurons in the substantia nigra pars reticulata (Gerfen, 1984, 1985; Gerfen et al., 1985; Jiménez-Castellanos and Graybiel, 1989). Second, striatal projections to the globus pallidus and substantia nigra arise from separate populations of medium spiny neurons (Beckstead and Kersey, 1985; Loopuijt and van der Kooy, 1985; Kawaguchi et al., 1990). Pallidal- and nigral-projecting striatal neurons are intermingled and do not show a preferential distribution relative to the patch–matrix compartmental organization (Loopuijt and van der Kooy, 1985; Gerfen and Young, 1988). Third, striatal medium spiny neurons express a number of neuropeptides, including dynorphin (Vincent et al., 1982), enkephalin (Hökfelt et al., 1977; Sar et al., 1978; Pickel et al., 1980; DiFiglia et al., 1982) and substance P (Brownstein et al.,

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1977; Hong et al., 1977; Kanazawa et al., 1977; Bolam et al., 1983). Immunohistochemical (Penny et al., 1986) and *in situ* hybridization (Gerfen and Young, 1988) studies suggest that each peptide is expressed by some 50–60% of striatal neurons. As a result, there are overlapping populations of neurons that express combinations of more than one of these peptides. Although some immunohistochemical studies have shown patch–matrix heterogeneities in peptide-immunoreactive patterns, more recent studies have shown a more homogeneous distribution (Penny et al., 1986). *In situ* hybridization studies have shown that dynorphin, enkephalin, and substance P mRNAs are localized in neurons that are distributed relatively evenly between the patch and matrix compartments, though the relative expression appears to vary in patch and matrix compartments, in a regional manner (Gerfen and Young, 1988). Additionally, there is a segregation of peptide expression between pallidal- and nigral-projecting neurons in that the majority of the former express enkephalin, whereas the majority of the latter express dynorphin and substance P (Brownstein et al., 1977; Haber and Nauta, 1983; Beckstead and Kersey, 1985; Gerfen and Young, 1988).

Dopaminergic afferents from the midbrain cell groups in the ventral tegmental area, substantia nigra, and retrorubral area (the mesostriatal dopaminergic system) modulate the expression of striatal peptides. Numerous studies have shown that removal of dopamine input with 6-hydroxydopamine (6-OHDA)-induced lesions of the mesostriatal system (Young et al., 1986; Voorn et al., 1987; Normand et al., 1988) or chronic antagonism of dopamine receptors (Hong et al., 1978a; Tang et al., 1983; Chou et al., 1984; Mocchetti et al., 1985; Quirion et al., 1985; Bannon et al., 1986; Romano et al., 1987) results in increases in both enkephalin immunoreactivity and mRNA, whereas dynorphin and substance P immunoreactivity and mRNA decrease or are unaffected by the same treatments (Hanson et al., 1981a,b; Bannon et al., 1986; Young et al., 1986; Voorn et al., 1987). This suggests that dopamine normally stimulates expression of dynorphin and substance P in striatal neurons. Consistent with this conclusion are data showing that chronic treatment with the dopamine agonist apomorphine causes an increase in both dynorphin and substance P, but not enkephalin, immunoreactivity in striatal patches (Li et al., 1987, 1988). The latter finding is of interest given that there are separate dopamine input systems to the striatal patch and matrix compartments. Dopamine inputs to the matrix arise from a dorsal set of dopamine neurons that are located in the ventral tegmental area, dorsal tier of the substantia nigra pars compacta, and retrorubral area, whereas dopamine inputs to the patches arise from a ventral set of neurons in the ventral tier of the pars compacta and from islands of dopamine neurons in the substantia nigra pars reticulata (Gerfen et al., 1987; Jiménez-Castellanos and Graybiel, 1987).

In this paper, we examine dynorphin, enkephalin, and substance P mRNA expression in the striatum of control rats, rats treated chronically with apomorphine, and rats that received 6-OHDA injections into the ascending mesostriatal dopaminergic fiber system followed by repeated saline or apomorphine injections. Specific oligonucleotide probes complementary to the mRNAs for dynorphin, enkephalin, and substance P were used to label striatal neurons by *in situ* hybridization histochemistry (ISHH). A method is described that allows the relative levels of peptide mRNAs in striatal neurons to be quantified and compared among the experimental groups.

Materials and Methods

Experimental animals. Twenty-four adult male Sprague–Dawley rats (200–250 gm) were placed evenly into 4 groups as follows: Two groups were injected with 6-hydroxydopamine (4 μg in 2 μl 0.02% ascorbic acid), and 2 groups received ascorbate injections (2 μl 0.02%) into the left ascending mesostriatal bundle. Two weeks following the 6-OHDA or ascorbate injections, half of the group that had received lesions, and half of the ascorbate-injected group received subcutaneous injections of 5.0 mg/kg apomorphine twice daily for 10 d. Three hours after the last drug injection, all rats were deeply anesthetized with chloral hydrate and perfused transcardially with either 200 ml ice-cold saline for ISHH (5 rats from each group) or 50 ml saline followed by a phosphate-buffered (0.05 M sodium phosphate buffer and 0.9% saline) 4% paraformaldehyde solution for immunohistochemistry (1 rat from each group). The saline-perfused brains were removed and frozen in isopentane cooled on dry ice for 20 sec. These brains were then frozen at −80°C until cryostat sectioning for ISHH. The paraformaldehyde-perfused brains were post-fixed in the same solution for 3 hr and transferred to 15% sucrose overnight.

Immunohistochemistry tissue section preparation. From the brains that had been perfused with formaldehyde, 30-μm-thick frozen sections through the striatum and midbrain were collected into potassium phosphate-buffered saline (KPBS; 0.05 M; pH, 7.4). They were then incubated for 48 hr at 4°C in rabbit antisera directed against dynorphin A 1-17 (a gift from Dr. L. Terenius, Uppsala, Sweden; 1:2000 dilution) or tyrosine hydroxylase (TH; Eugentech; 1:1000 dilution) in KPBS to which had been added 0.5% Triton X-100 and 2% normal goat serum. Sections were then rinsed in KPBS twice for 15 min and reacted according to the avidin-biotin-peroxidase method with modifications described previously (Gerfen, 1985).

ISHH tissue section preparation. Serial 12-μm coronal sections through the striatum and midbrain from the brains that were perfused with saline alone were cut in a cryostat and adhered to glass slides that had been twice coated with gelatin. Sections were dried to the slide on a warm plate for 2 min and stored desiccated at −20°C. When processed further, the sections were first warmed to room temperature for 10 min, fixed in a 4% paraformaldehyde solution in 0.9% saline for 10 min, rinsed, and incubated in a fresh solution of 0.25% acetic anhydride in 0.1 M triethanolamine and 0.9% saline (pH, 8.0) for 10 min. Next, the slide-mounted sections were dehydrated in a series of ascending concentrations of ethanol and defatted for 2 × 5 min in chloroform, rehydrated, and air dried. These sections were then hybridized with ^{35}S-dAMP-tailed oligonucleotide probes.

Oligonucleotide probes. Tails of ^{35}S-dAMP (average tail length, 20 bases) were added to purified oligonucleotides (48-base cDNAs) complementary to the messenger RNAs for enkephalin, dynorphin, substance P, and tyrosine hydroxylase as described (Young et al., 1986) in the following reaction: One microliter of each probe (from a stock concentration of 5 μM) was reacted with ^{35}S-dATP (final concentration equal to 1 μM of 1350 Ci/mM ^{35}S-dATP) and 100 U/5 μl of terminal deoxynucleotidyl transferase (TdT; Boehringer Mannheim, Indianapolis, IN) for 5 min at 37°C. The reaction was terminated with the addition of 1 μl *t*RNA (25 μg/μl) and 400 μl 10 mM Tris HCl, pH 7.5/EDTA (TE), and the tailed probe was extracted with phenol/chloroform/isoamyl alcohol followed by chloroform/isoamyl alcohol. To the extracted aqueous phase was added 1/20 vol of 4 M NaCl and 1 ml ethanol, which was allowed to cool in dry ice for 30 min. The precipitate was pelleted at 14000 rpm for 30 min at 4°C, washed with ice-cold ethanol, and resuspended in 100 μl TE.

Hybridization. Tailed probes were added to the hybridization buffer (Young et al., 1986) to reach a concentration of 1 × 10^6 dpm per 25 μl of buffer. Twenty-five microliters of hybridization buffer containing labeled probe were added to each brain section. The sections were then coverslipped with parafilm and incubated at 37°C for 18 hr. After incubation, the parafilm coverslips were floated off in 1 × sodium citrate (SSC) and the slide-mounted sections were rinsed in 3 washes of 1 × SSC. The slides were then washed 4 × 15 min in 2 × SSC and 50% formamide at 42°C, followed by 2 × 30 min washes in 1 × SSC at room temperature and a brief water rinse before the sections were rapidly dried. The slides were then dipped in NTB3 emulsion (diluted 1:1 with water) and exposed for 4–6 weeks, after which time they were developed in Kodak D-19 developer for 2 min, fixed, rinsed, counterstained with thionin, dehydrated, and coverslipped out of xylene.

Naloxone binding in striatal patches. One series of sections from each

animal was processed to demonstrate ^{3}H-naloxone binding to the μ-opiate receptor-rich striatal patch compartment. The procedure described by Herkenham and Pert (1982) for the autoradiographic localization of receptor binding was followed. Slide-mounted sections were incubated in a solution containing 2.5 nM ^{3}H-naloxone (specific activity, 44.4 Ci/mmol; New England Nuclear) in 50 mM Tris buffer (pH, 7.4) and 100 mM NaCl at 4°C for 90 min, and then rinsed 3 × 1 min in Tris-buffered saline, air dried, fixed in formaldehyde vapors (80°C) for 90 min, dehydrated, defatted in chloroform/methanol, rehydrated, and air dried. Sections were then dipped in Kodak NTB-2 nuclear track emulsion, dried, and exposed for 3 months, after which time they were developed in Kodak D-19 developer (2 min at 17°C), stopped, fixed, and rinsed. After counterstaining with thionin, sections were coverslipped out of xylene.

Generation of ISHH standards. Standards were prepared using the enkephalin probe by running 2 "tailing" reactions simultaneously using the enkephalin oligonucleotide. In the first reaction, ^{35}S-dATP was used, and in the second reaction, nonradioactive dATP was used (Gerfen, 1989). Standards were then generated by combining the products of the 2 enkephalin tailing reactions in ratios that produced probes that contained 0, 25, 50, 75, or 100% radioactive tailed probe diluted with unlabeled probe to keep the total molar ratio of probe constant for each hybridization. These mixtures of equimolar concentrations of probes were added to the hybridization buffer and applied to sections that had been saved from experimental brains to serve as standards. Sections containing the standards and the experimental probes were processed concurrently. Each enkephalin probe standard, generated by diluting radioactive and nonradioactive tailed probes in ratios of 25:75, 50:50, 75:25, and 100:0, was applied to 4 sections of the striatum of 5 animals that had received unilateral 6-OHDA lesions. Sections hybridized with the enkephalin probes were exposed to photographic emulsion for 4 weeks. Sections hybridized with substance P and dynorphin probes were exposed for 7 weeks. This latter exposure time was used because it was determined from test slides to provide an equivalent average amount of label per cell in the control striatum for these peptide probes as for the enkephalin probe. Only the enkephalin probe was used to generate dilution standards. The adjustment in exposure time used for the dynorphin and substance P hybridization enabled the values derived for relative changes in enkaphalin mRNA levels to be applied to these other probes as well (see Results).

Quantitative image analysis. Quantitative analysis of ISHH labeling was performed by digitized image analysis using an image analysis system (Loats Associates, Inc., Westminister, MD). Sample areas from the dorsolateral striatum were digitized through a microscope at a magnification of 150× with a Dage 68 camera to generate a digitized image of 256 × 256 pixels. Each sample area was first digitized under bright-field illumination and then under dark-field illumination. For each illumination condition, the camera settings and lighting conditions were held constant for all sample areas that were digitized. The matched set of bright-field and dark-field images was then analyzed with a redirected analysis routine. With this routine, neurons in the dorsolateral striatum stained with thionin were selected from the bright-field image using a circular field of constant size (400 μm^2), and the average optical density (OD) of silver grains over neurons containing preprodynorphin, enkephalin, or tachykinin was measured in the corresponding area under dark-field optics. Figure 1 shows photomicrographs of thionin-stained neurons in a typical sample field in the dorsolateral striatum viewed with bright-field illumination (Fig. 1*A*) and enkephalin mRNA-labeled neurons in the same field as viewed with dark-field illumination (Fig. 1*B*). Redirected analysis used for measuring the inverse OD (OD_i) value for each neuron involved the selection of neurons from a bright-field-illuminated view and the measurement of the OD_i value in the corresponding area of the dark-field-illuminated view. The average optical density value obtained for each cell was subtracted from 255, which was the maximum optical density value, to provide an inverse optical density value, because the silver grains were visualized with dark-field illumination. The OD_i value was then used as the measure of label per cell, with higher values indicating greater labeling. Previous analysis had determined that the number of grains per cell was linearly related to OD_i using this image analysis system (Gerfen, 1989). In each sample field, all neurons were measured, with 100–150 neurons measured per field. Neurons were distinguished from glia, which are smaller and more intensely stained. All neurons, which could be identified by the intensity of the thionin stain, were sampled regardless of their size. Thus, because the section thickness of 12 μm is less than the average diameter of the average striatal neuron, which is 20–30 μm, many partial neurons were sampled. For each animal, 4 samples from each of 4 sections on each side were measured, which provided data on approximately 1000–1500 neurons per striatum per animal. The data from 5 animals per condition were averaged.

Results

Quantitative analysis of peptide mRNA expression standards

Enkephalin oligonucleotide probes, which were mixtures of ratios of radioactive and nonradioactive tailed probes reconstituted to equimolar concentrations of total probe (described in Materials and Methods), were used to generate standards for quantifying relative changes in peptide mRNA expression as measured by ISHH. Additionally, in order that values generated from the dilution standards covered the experimental range of mRNA expression, the standards were applied to sections from animals that had received unilateral 6-OHDA lesions.

Figure 2 shows graphs that were used to generate standard values. The OD_i values for each of 1000–1500 neurons per animal for each probe dilution standard were measured. These values were first expressed in a histogram of the number of neurons displaying OD_i values in fixed ranges (i.e., 0–5, 5–10, 10–15, etc.), as shown in Figure 2*A*. The histograms of distributions of cells measured showed a bimodal distribution of unlabeled and labeled cells, with the unlabeled cells' values distributed around 0 and the labeled cells' values distributed around 55 OD_i. In order to provide an estimate of the number of labeled cells whose OD_i values fall in the range where the unlabeled and labeled cell distributions overlap, the number of labeled cells was determined by extrapolating the numbers of cells from a theoretical normal distribution. Figure 2*A* shows the bimodal distribution of cells for 1 animal and the extrapolated distribution of labeled cells. Figure 2*B* shows a line plot of the distribution of labeled cells versus OD_i in the unlesioned striatum of 5 animals. The sections in this case were hybridized with enkephalin probe labeled with 100% radioactive dATP. Figure 2, *C* and *D*, shows the average distributions of cells displaying enkephalin labeling from 5 rats obtained for each dilution standard on the right, unlesioned control side (Fig. 2*C*) and left, lesioned side (Fig. 2*D*). Statistical analysis of these distributions showed a significant difference between each of the dilution standards ($p < 0.001$) using a 2-way ANOVA analysis. These normal distribution curves were used to calculate the average OD_i value per cell for each dilution standard, which is plotted in Figure 2*E*. This plot shows a linear relationship between the amount of radioactivity in the applied probe standards and the measured OD_i values. The slope of the calibration curve for the unlesioned side indicates that an average change of 9.85 $\pm$ 0.9 OD_i units per cell corresponds to an average of 25% change in mRNA expression. The slope of the line for the dilution probes from the lesioned side differs from that of the unlesioned side as a result of the increase in enkephalin mRNA levels that resulted from the lesion. Fitting the OD_i values for each dilution standard from the lesioned-side data into the formula of the line of the unlesioned-side data provides a means of estimating the relative increase in enkephalin mRNA expression on the lesioned side. This procedure indicates that on the lesioned side there is an average 130% higher level of enkephalin mRNA expression at 100% ^{35}S-dATP versus the unlesioned side. Using this value, the extrapolated relative levels of enkaphalin expression on the lesioned side fall on the line of the unlesioned-side standard values for the dilution standards containing 25, 50, and 75% labeled probe. The measured value for the 100%

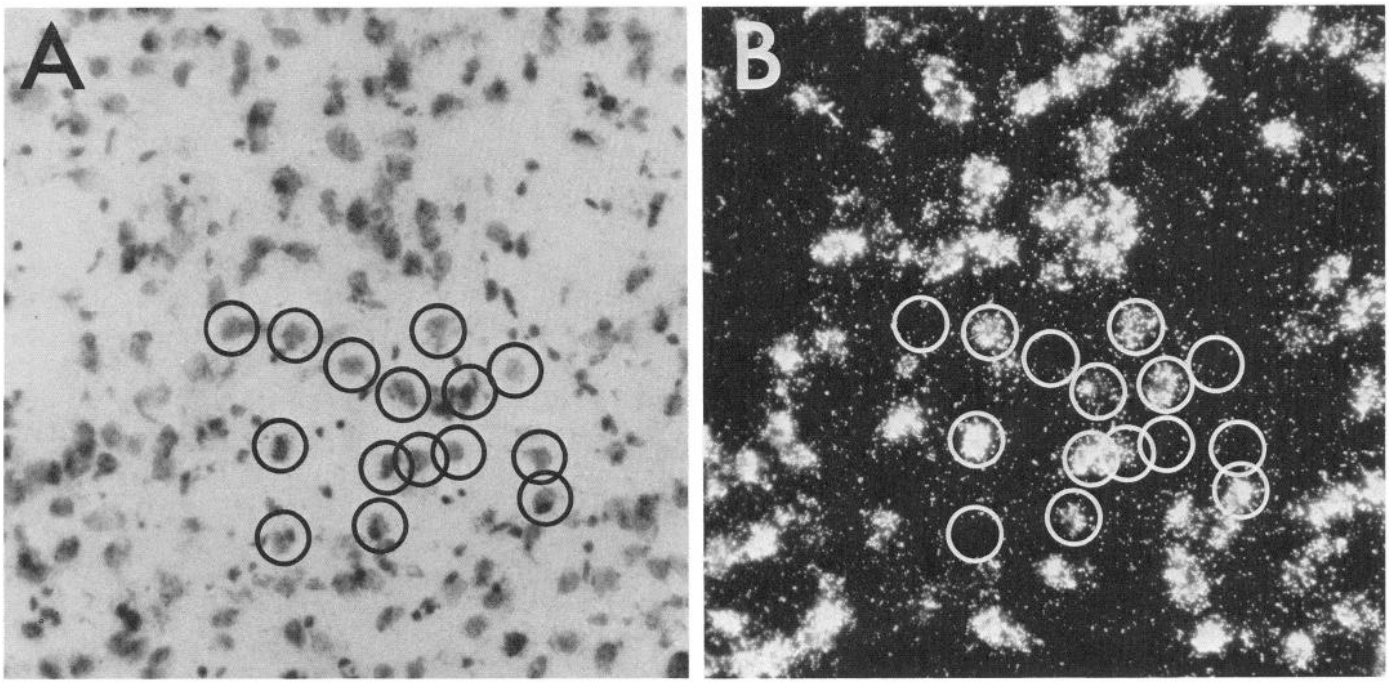

Figure 1. Photomicrographs of a field in the dorsolateral striatum viewed with bright-field (*A*) and dark-field illumination (*B*). Thionin-stained cells are identified under bright-field illumination (*A*), and cells labeled with the enkephalin probe are visualized by the accumulation of white grains under dark-field illumination (*B*). For quantitative measurements, matched sets of bright-field and dark-field images such as these were digitized with the image analysis system. Medium-sized neurons were selected with a circular field of constant size (400 μm^2) from the bright-field image, and the average OD_i was measured in the corresponding area of the dark-field image to measure the amount of labeling associated with the selected neuron. The area of measurement was approximately 40% larger than the aveage size of the neurons measured to assure that the majority of grains associated with an individual neuron were included in the measurement. Overlap between the areas measured for neighboring neurons was accepted as an unavoidable source of error in the measurements. During the selection routine, the dark-field image was hidden from view, and all medium-sized neurons from the field were selected. Examples of selected neurons (*circles* in *A*) and the corresponding area in the dark-field image (*circles* in *B*) are indicated.

labeled probe, 104.5 OD_i, is less than 3% below the value calculated assuming a linear relationship (Fig. 2*E*). This deviation reflects the saturation of measured OD_i values at the upper end of the range of values measured. While this deviation is within acceptable limits, it is assumed that the deviation due to saturation increases at higher OD_i values. Thus, OD_i values above those measured with the dilution standard procedures may be an underestimate of the actual relative expression of mRNA for a particular probe.

Quantitative analysis of dopamine-regulated striatal peptide mRNA changes. The effectiveness of the 6-OHDA lesions was judged by an examination of the number of TH-immunoreactive (see Fig. 6*H*) or mRNA-expressing neurons in the midbrain. Six animals per lesioned group (5 saline-perfused animals and 1 formaldehyde-perfused animal) satisfied the criterion of a greater than 90% reduction in the numbers of neurons expressing TH mRNA in the substantia nigra (data not shown) and were included in the experimental analysis.

Enkephalin mRNA expression. Figure 3 shows photomicrographs of enkephalin mRNA labeling in the dorsolateral striatum of a control animal (Fig. 3*A*), an animal treated chronically with apomorphine (Fig. 3*B*), an animal with a unilateral 6-OHDA lesion of the nigrostriatal pathway (Fig. 3*C*), and an animal that had first had a 6-OHDA lesion, followed 2 weeks later by chronic apomorphine treatment (Fig. 3*D*). In the region that was analyzed, the dorsolateral striatum, there was no significant difference between the patch and matrix compartments in the numbers of enkephalin-expressing neurons or in the amount of label per cell. Thus, for quantitative analysis, the neurons from both compartments are grouped together. Figure 3*E* shows a line plot in which the data from 5 animals for each experimental group were averaged. In the upper right of the line plot, the average values of OD_i per cell derived from these data are given. In control animals, 55% of dorsolateral striatal neurons express enkephalin mRNA, with an average level of expression of 47.8 OD_i. In animals chronically treated with apomorphine, there was no significant change in mRNA expression per cell (-2.8 OD_i; $<5\%$ change from control values). In animals that received a unilateral 6-OHDA lesion, there was an increase in the average expression of mRNA per cell ipsilateral to the lesion (average OD_i, 99.9 ± 6.9; $+130\%$ change from control values). Contralateral to the lesion, the values of expression per cell did not differ from control values (data not shown). In animals that received a unilateral 6-OHDA lesion followed by chronic apomorphine treatment, there was an increase in the average expression per cell (average OD_i, 107.5 ± 9.0; $+150\%$ change from control levels) ipsilateral to the lesion. Contralateral to the lesion, the values of enkephalin mRNA expression per cell were similar to control levels (-4.2 ± 1.2 OD_i change from control; $<10\%$ change from control values). In all experimental conditions, the percent of labeled cells on both sides was not significantly different from control values.

Substance P mRNA expression. Figure 4 shows photomicrographs of substance P mRNA labeling in the dorsolateral stria-

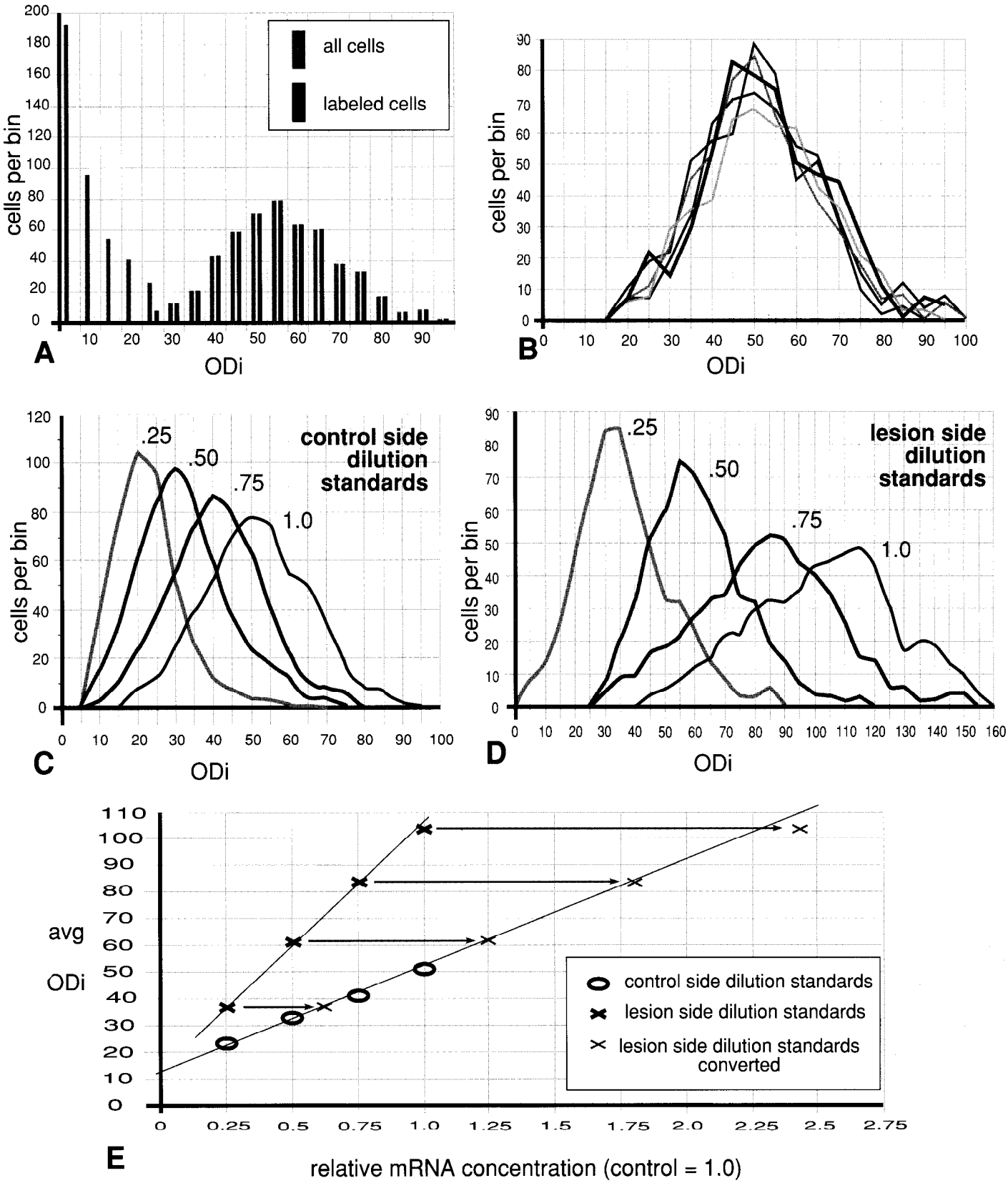

Figure 2. Calculation of relative dilution standards for the enkephalin oligonucleotide probe. *A*, Histogram of the number of cells displaying different levels of label measured as OD_i. This histogram represents the measurements from the unlesioned side of a 6-OHDA-lesioned animal of all selected neurons (1 sample area each of the striatum from 4 sections) from sections hybridized with an enkephalin probe labeled with 100% radioactive dATP. Sampled cells display a bimodal distribution (*shaded bars*) with unlabeled cells distributed around 0 OD_i and labeled cells distributed around 55 OD_i. The number of labeled cells (*solid bars*) at OD_i values where labeled and unlabeled cells overlap (in the range between 25 and 35 OD_i) is estimated by extrapolating a normal distribution from the population of labeled neurons. *B*, Distribution (charted as line plots)

tum of a control animal (Fig. 4A), an animal treated chronically with apomorphine (Fig. 4B), an animal with a unilateral 6-OHDA lesion of the nigrostriatal pathway (Fig. 4C), and an animal that had first had a 6-OHDA lesion, followed 2 weeks later by chronic apomorphine treatment (Fig. 4D). These sections were adjacent to those hybridized with enkephalin and dynorphin probes illustrated in Figures 3 and 5. Similar to the expression of enkephalin mRNA, the numbers of cells expressing substance P mRNA and the level of expression per cell did not differ significantly in the patch and matrix compartments in the dorsolateral striatum. Figure 4E shows a line plot of the distribution of neurons expressing substance P mRNA under the different experimental conditions. In control animals, an average of 52% of the striatal cells measured were labeled for substance P mRNA. The number of labeled neurons did not display a normal distribution, which suggests separate populations of substance P neurons that express different levels of mRNA. These 2 populations were distributed evenly between the patch and matrix compartments. The average OD_i value per labeled cell was 56.7 (±3.1). Chronic apomorphine treatment resulted in a significant increase in substance P mRNA levels per cell compared with controls (OD_i, 77.3 ± 7.9; a 50% increase over control). Conversely, 6-OHDA-induced dopaminergic deafferentation depressed substance P mRNA levels (average OD_i, 27.5 ± 4.1; a 75% decrease vs. control). Combined treatment with 6-OHDA followed by apomorphine did not significantly alter substance P mRNA levels per cell as compared with controls (average OD_i, 52.6 ± 3.8). In all experimental conditions, the percent of labeled cells on both sides was not significantly different from control values.

Dynorphin mRNA expression. Figure 5 shows photomicrographs of dynorphin mRNA labeling in the dorsolateral striatum of a control animal (Fig. 5A), an animal treated chronically with apomorphine (Fig. 5B), an animal with a unilateral 6-OHDA lesion of the nigrostriatal pathway (Fig. 5C), and an animal that first had a 6-OHDA lesion, followed 2 weeks later by chronic apomorphine treatment (Fig. 5D). Dynorphin mRNA is expressed more intensely in neurons in the patches than in the matrix in control rats. Adjacent sections marked with ^{3}H-naloxone autoradiography, which were aligned by identification of blood vessels or corticofugal fiber tracts, enabled the positive identification of the patch compartments in which the selective increase in dynorphin levels in neurons was observed (data not shown). The quantitative analysis from these animals is shown in Figure 5E. In control animals, dynorphin mRNA was expressed by an average of 53% of striatal patch neurons, with an average OD_i of 63.1 (±5.3) per cell, and 48% of matrix neurons, with an average OD_i of 32.5 (±2.8) per cell. None of the treatments altered the percentage of either patch or matrix neurons that express dynorphin mRNA. Chronic apomorphine treatment increased the level of dynorphin expression of patch neurons (average OD_i, 102.8 ± 8.9; a 100% increase over control patch neurons) more than that of matrix neurons (average OD_i, 44.1 ± 3.3; a 25% increase over control matrix neurons).

Analysis of compartmental differences in dynorphin expression following 6-OHDA lesions is complicated by the fact that the marker used to distinguish these compartmental differences, ^{3}H-naloxone binding in patches, is abolished with these lesions. For this reason, the data are not expressed in terms of localization in patch and matrix compartments after 6-OHDA lesions.

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of neurons that were labeled with the 100% radioactive enkephalin probe from 5 animals. These distributions were then averaged and plotted in C as the average distribution for the dilution standard that contains the ratio of 1.0:0.0 radioactive to nonradioactive enkephalin probe. Dilution probe standards were made by reconstituting mixtures of radioactive and nonradioactive labeled oligonucleotide probes in ratios of 25:75, 50:50, 75:25, and 100:0 to produce equimolar concentrations of probes that were applied to brain sections for ISHH. C, Average distribution of labeled striatal neurons in the unlesioned (control) side from 5 animals labeled with enkephalin probes containing 0.25, 0.50, 0.75, and 1.0 fractions of radioactive probes diluted with nonradioactive probes. D, Average distribution of labeled striatal neurons in the lesioned side from 5 animals labeled with enkephalin probes containing 0.25, 0.50, 0.75, and 1.0 fractions of radioactive probes diluted with nonradioactive probes. E, The average OD per cell for each dilution standard was calculated from the histograms in C and D and plotted (*Os* for the control-side standards and *dark Xs* for the lesioned-side standards) against the relative amount of radioactively labeled probe that was included in each dilution standard. There is a linear relationship between the calculated average OD_i values and the relative amount of radioactively labeled probe in the standards for both the control and the lesioned side. The line function for the control side is used to calculate a value that relates a difference in OD_i measurements per cell to the relative difference in the amount of labeled mRNA (10 OD_i units equals a 25% difference in relative mRNA concentration). Converting the values derived from the lesioned-side dilution standards into the function derived for the control side (marked as *light Xs*) allows an estimation of the relative change in enkephalin mRNA expression induced by the lesion (+130%). Additionally, this conversion shows that there is a nearly linear relationship between average OD_i values per cell and relative mRNA concentrations in the range between 25% and 225% of control levels of enkephalin mRNA labeling, with a less than 5% deviation at the highest level.

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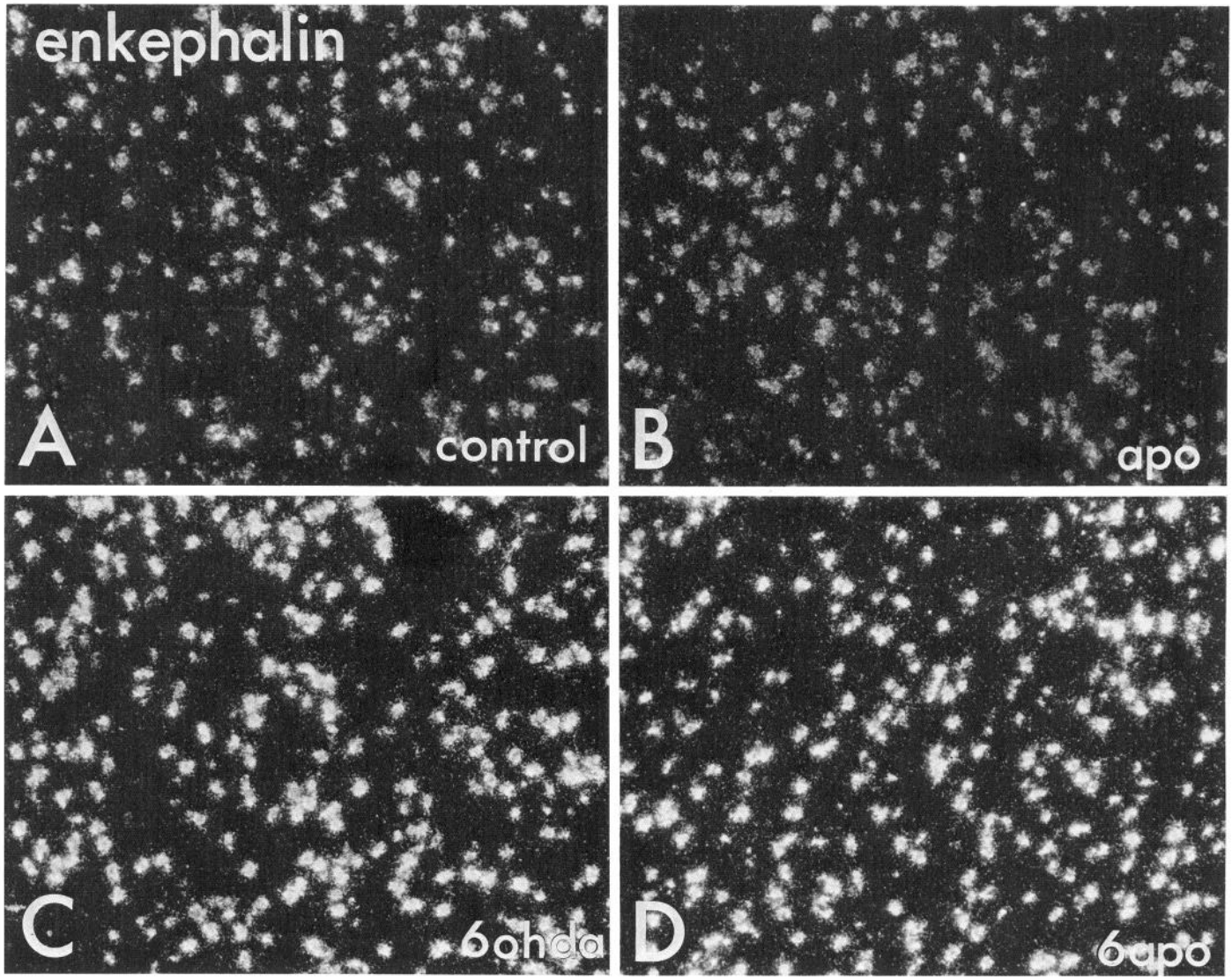
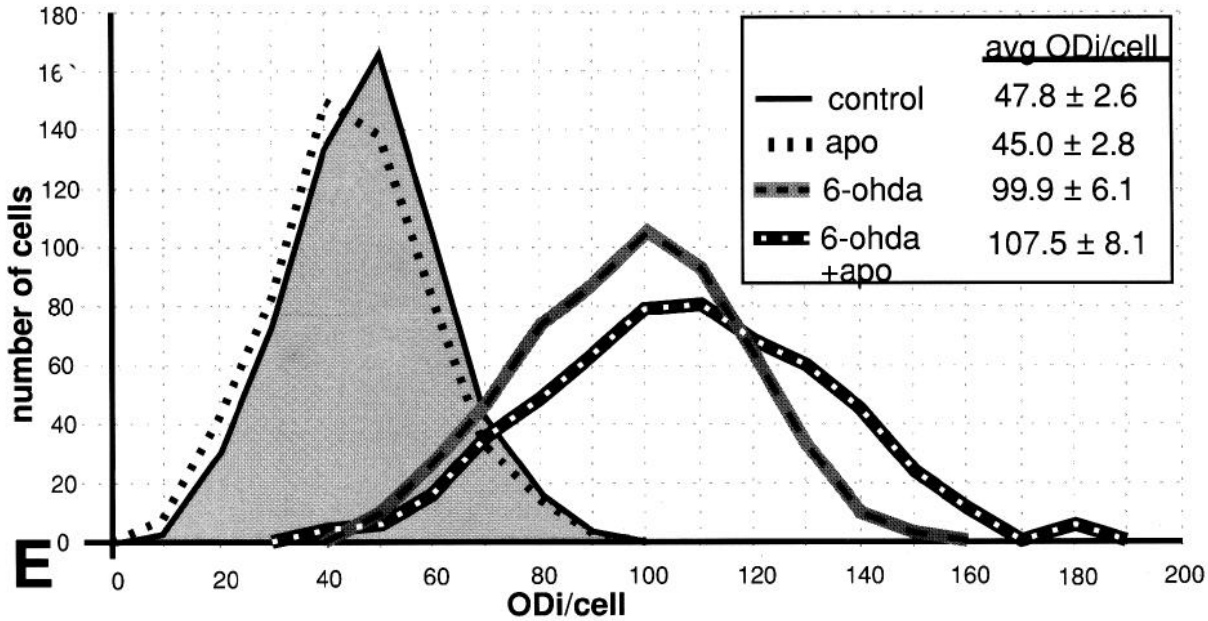
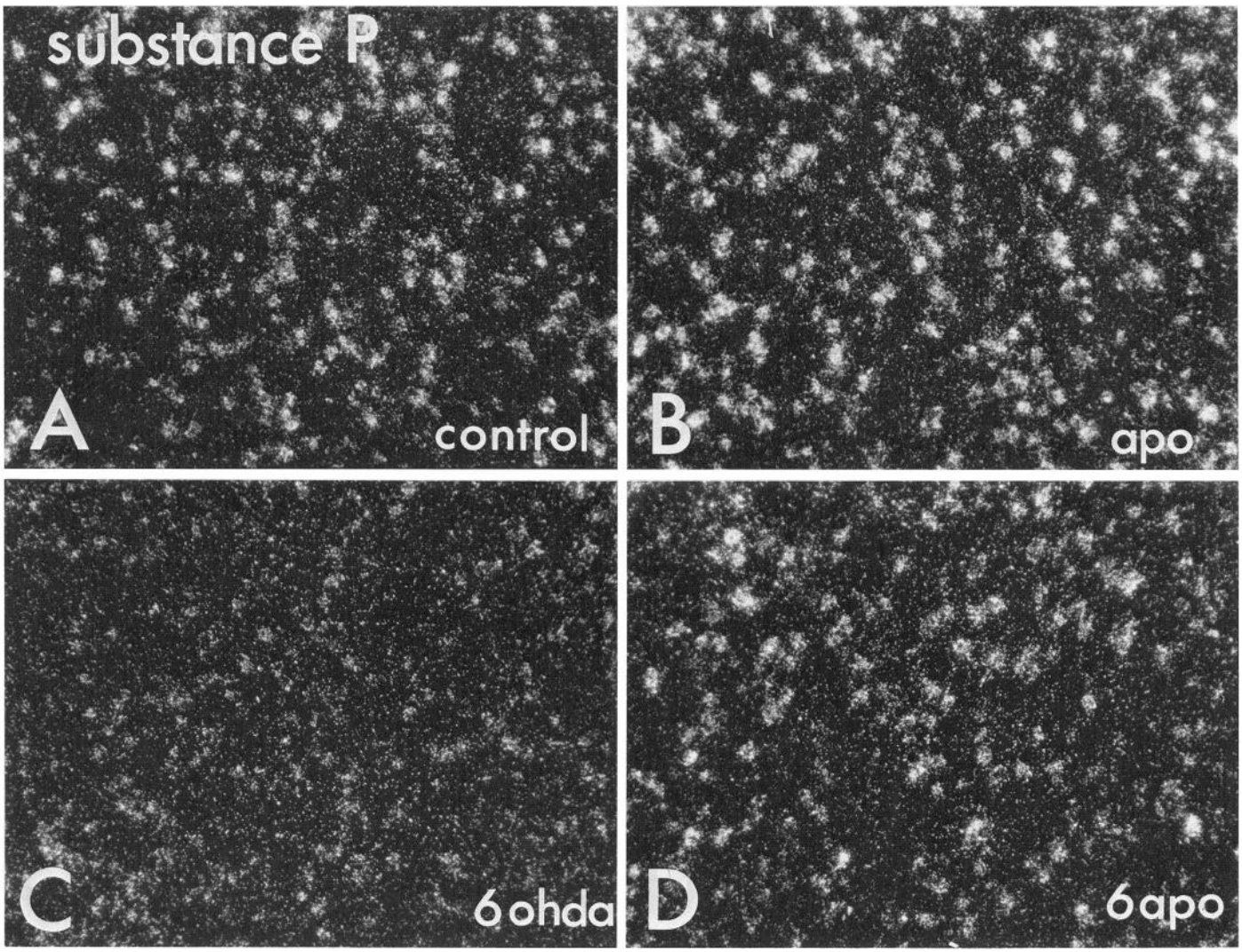
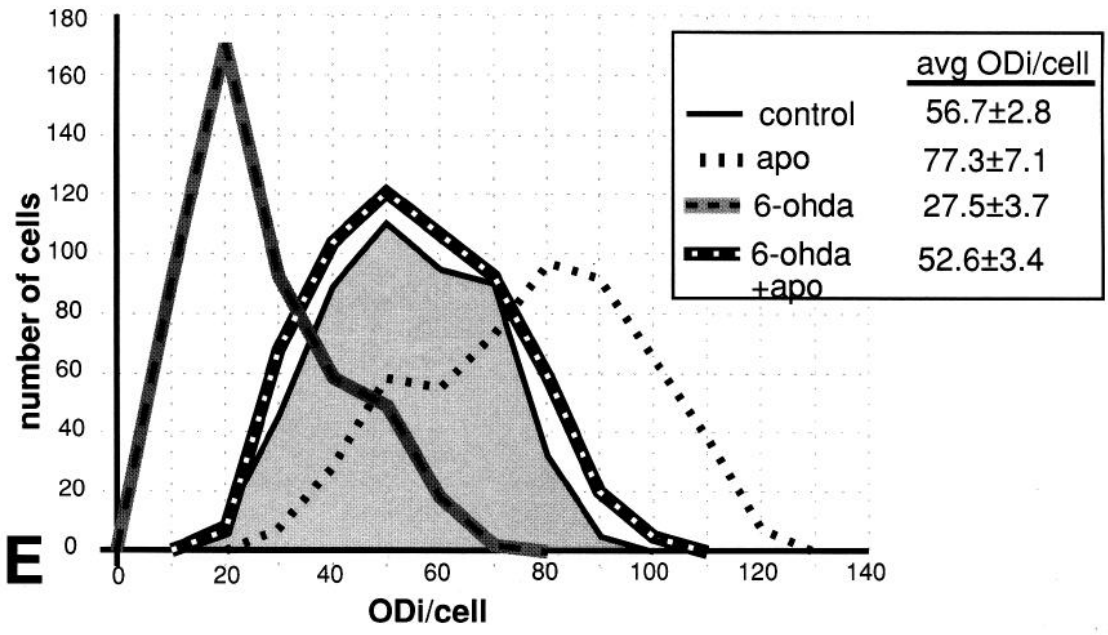
Figure 3. Dark-field photomicrographs of autoradiographically generated grains (seen as *white dots*) produced by ISHH labeling of striatal sections using an oligonucleotide probe complementary to enkephalin mRNA (*A–D*). Sample areas in the dorsolateral striatum are shown from a control animal (*A*), from an animal that received 10 d of twice-daily injections of 5 mg/kg apomorphine (*apo*; *B*), from the lesioned side of an animal that had a 6-OHDA lesion of the nigrostriatal pathway (*C*), and from the lesioned side of an animal that received a 6-OHDA lesion followed 2 weeks later by 10 d of twice-daily apomorphine treatments (*D*). *E*, Average OD_i per cell showing the distribution of cells labeled with the enkephalin probe from the 4 experimental groups (≈500 labeled cells per animal, 5 animals per group). These plots show that, relative to the control levels, apomorphine treatments caused no change in labeling, and 6-OHDA lesions resulted in a significant increase in labeling that was unaffected by subsequent apomorphine treatment.

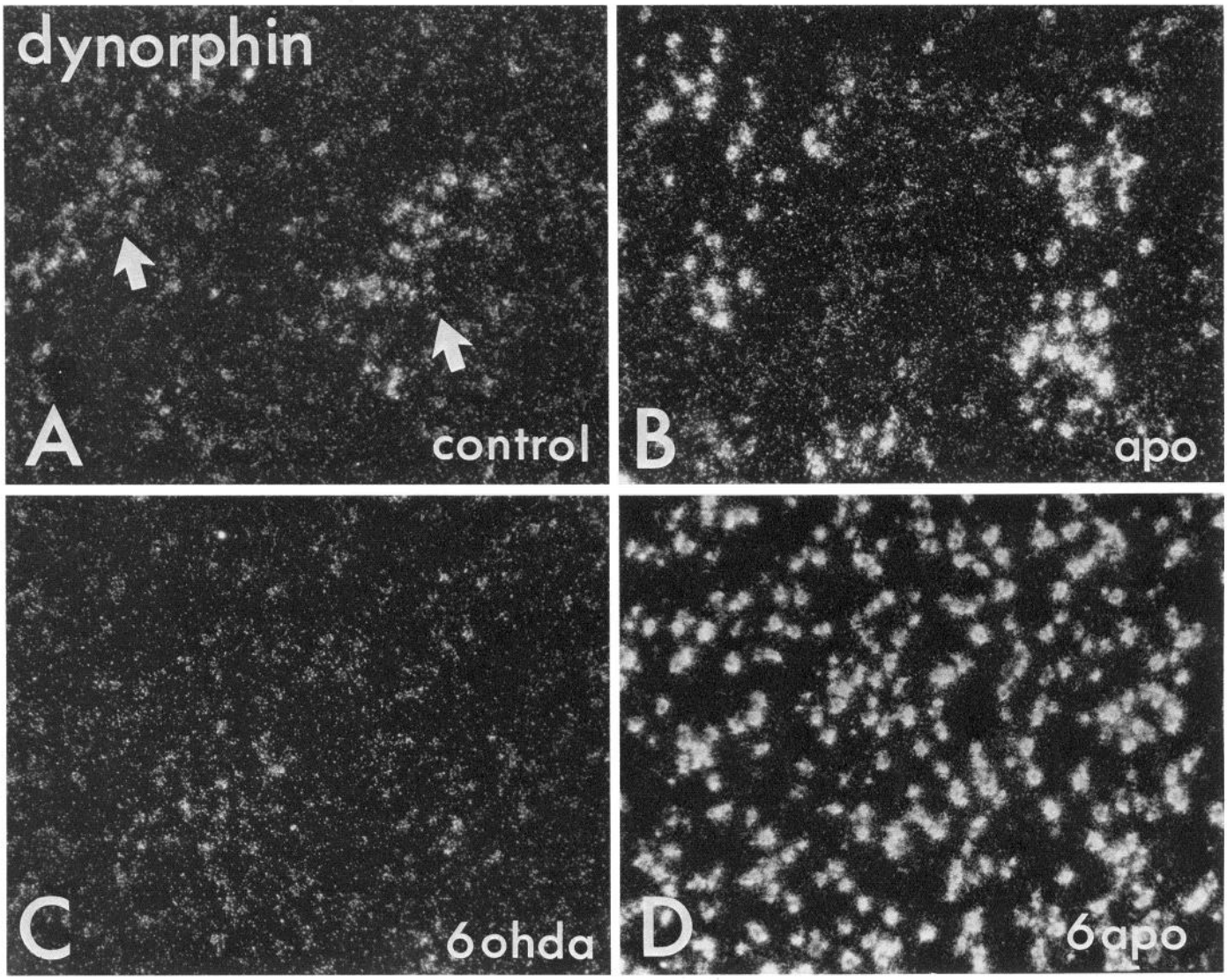
Figure 4. Dark-field photomicrographs of autoradiographically generated grains (seen as *white dots*) produced by ISHH labeling of striatal sections using an oligonucleotide probe complementary to substance P mRNA (*A–D*). Sample areas in the dorsolateral striatum are shown from a control animal (*A*), from an animal that received 10 d of twice-daily injections of 5 mg/kg apomorphine (*apo*; *B*), from the lesioned side of an animal that had a 6-OHDA lesion of the nigrostriatal pathway (*C*), and from the lesioned side of an animal that received a 6-OHDA lesion followed 2 weeks later by 10 d of twice-daily apomorphine treatments (*D*). *E*, Average OD_i per cell showing the distribution of cells labeled with the substance P probe from the 4 experimental groups (≈500 labeled cells per animal, 5 animals per group). These plots show that, relative to the control levels, apomorphine treatments caused an increase in labeling, 6-OHDA lesions resulted in a significant decrease in labeling, and 6-OHDA lesions followed by apomorphine treatments showed no difference compared to controls.

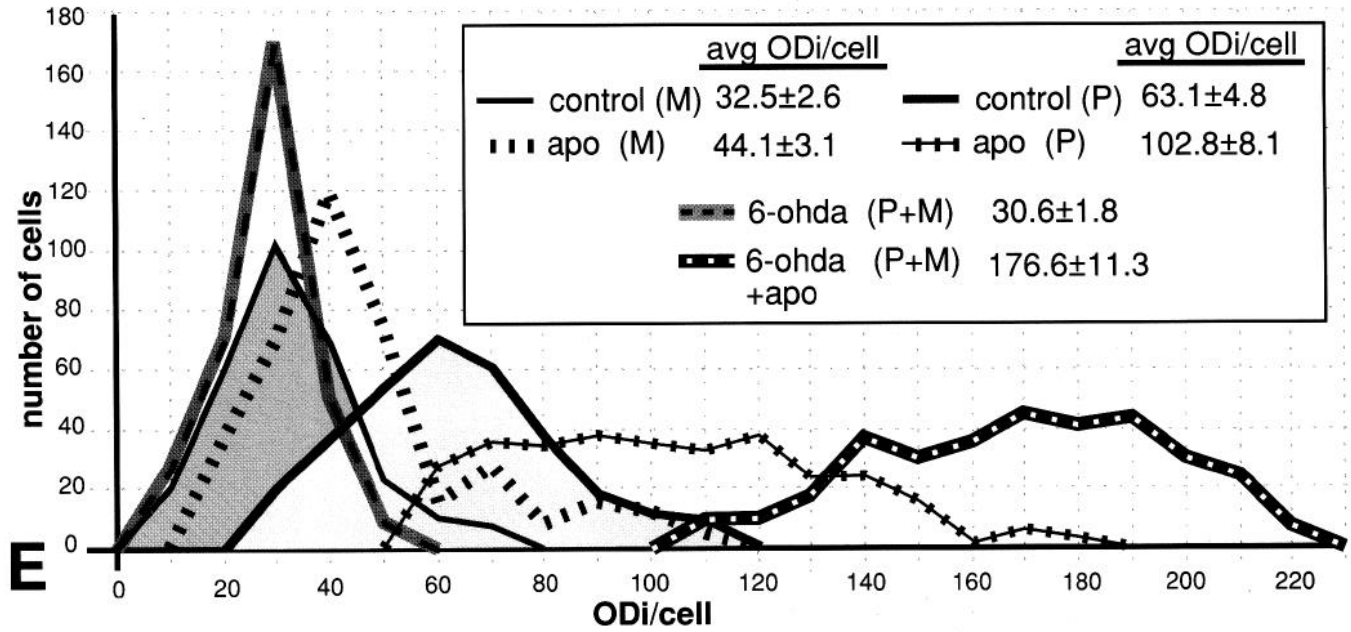

enkephalin
A
control
B
apo
C
6ohda
D
6apo


E
number of cells
ODi/cell
0
20
40
60
80
100
120
140
160
180
200
avg ODi/cell
control 47.8 ± 2.6
apo 45.0 ± 2.8
6-ohda 99.9 ± 6.1
6-ohda +apo 107.5 ± 8.1


substance P
A
control
B
apo
C
6ohda
D
6apo


E
number of cells
ODi/cell
0
20
40
60
80
100
120
140
160
180
avg ODi/cell
control 56.7±2.8
apo 77.3±7.1
6-ohda 27.5±3.7
6-ohda +apo 52.6±3.4


dynorphin
A
control
B
apo
C
6ohda
D
6apo


E
number of cells
ODi/cell
0
20
40
60
80
100
120
140
160
180
200
220
avg ODi/cell
control (M) 32.5±2.6
apo (M) 44.1±3.1
avg ODi/cell
control (P) 63.1±4.8
apo (P) 102.8±8.1
6-ohda (P+M) 30.6±1.8
6-ohda (P+M) +apo 176.6±11.3

Data for the striatum contralateral to the lesion were not significantly different from control values described above (data not shown). Following unilateral lesions, there was no change in the percent of labeled cells with the dynorphin probe when compared to that of the combined patch and matrix areas in the control animals. The distribution of labeled cells ipsilateral to a 6-OHDA lesion showed no distinction in the relative levels of dynorphin labeling such as characterized patch and matrix dynorphin-labeled cells in the control condition. The average level of expression (30.6 ± 2.1 OD_i) is approximately equal to the control value for matrix neurons and 75% below that of the control value for patch neurons. In animals that received chronic apomorphine treatment following 6-OHDA lesions, there was a marked elevation of expression of dynorphin mRNA per cell compared to the levels for control patch and matrix neurons (average OD_i, 176.6 ± 12.5). There was no obvious difference in the distribution of neurons that reflected a greater increase in one compartment than the other. This represents an increase of over 300% above the average control value for dynorphin expression in each compartment. There was no significant change in the percent of labeled neurons in these animals compared with the values of the combined control cases.

Comparison of dynorphin immunohistochemical and ISHH localization. Immunohistochemistry and ISHH confirmed that, relative to untreated animals, apomorphine treatment resulted in a prominent increase in dynorphin mRNA and immunoreactive levels in striatal patch neurons (Fig. 6*A,C*, respectively). The distribution of dynorphin-immunoreactive terminals in the substantia nigra in this brain after apomorphine treatment is shown in Figure 6*E* and compared with the normal distribution of TH-immunoreactive neurons in the adjacent section (Fig. 6*G*). 6-OHDA lesions led to no detectable change in dynorphin immunoreactivity compared to controls but did cause a decrease in ipsilateral dynorphin mRNA levels in striatal neurons 2 weeks after lesioning the mesostriatal pathway (data not shown). Combined 6-OHDA lesions and apomorphine treatment resulted in a profound increase in dynorphin mRNA and immunoreactivity in striatal patch and matrix neurons (Fig. 6*B,D*). The increase in dynorphin immunoreactivity in the striatum after 6-OHDA and apomorphine treatment was matched by a profound increase in the immunoreactive labeling of dynorphin terminals in the substantia nigra pars reticulata (Fig. 6*F*). Figure 6*H* documents the near-total depletion of TH-immunoreactive neurons in this brain after 6-OHDA lesions.

Discussion

Quantification of ISHH. To determine relative differences in mRNA expression using ISHH, labeling standards were generated with dilutions of radioactive and nonradioactive tailed cDNA oligonucleotide probes. Oligonucleotide mixtures containing known ratios of radioactive to nonradioactive probes were applied to sections, and the labeling generated per cell, measured in OD_i units, was compared. Because the total molar ratio of probe applied to each section was equal, it is assumed that the average amount of probe that hybridized per cell was also equal. Differences in the amount of observed label per cell, as measured by the OD_i of dark-field-illuminated silver grains, was attributable to the relative amount of radioactivity of the probe. Because this relative amount of radioactivity was known, it was possible to calculate a value that related a measured difference in average label per cell, in OD_i units, to a percentage difference in labeled mRNA per cell. This value was calculated to be approximately 10 OD_i units per 25% difference in mRNA concentration.

The enkephalin probe was used for the dilution standards because cells expressing this peptide mRNA are a more homogeneous population than those expressing dynorphin and substance P mRNA. In order to use the dilution standards for the enkephalin probe to calculate the relative change in expression of the other 2 probes, the time of exposure to autoradiographic emulsion was lengthened for both dynorphin and substance P so that the average OD_i per cell for the control level of expression matched that for the control level of expression for enkephalin. The rationale for this adjustment is based on the assumption that the number of autoradiographically generated grains over a cell is dependent on the concentration of labeled probe hybridized to mRNA in that cell multiplied by the decay constant for the radionuclide multiplied by the exposure time to the photographic emulsion. As described above for the case of the lesioned and unlesioned striatum, the difference in average OD_i per cell that corresponds to a difference in the relative amount of hybridized mRNA is dependent on the control levels of mRNA in the cells. In Figure 2*E*, note the difference in the slope of the lines for the 2 sides of the brain. To compare the lesioned and unlesioned side, on sections for which the exposure times were kept the same, this difference may be used to calculate the relative change in expression between the 2 sides. Analogously, in the case of a lower control level of expression of a peptide mRNA, such as for dynorphin and substance P, utilizing the same exposure time as for enkephalin would generate a dilution standard curve with a shallower slope than enkephalin's curve. In order to compensate for this difference in standard curve slope, a longer exposure time was used for dynorphin and substance P hybridizations. A simple calculation, using the formula (number of grains produced) = (concentration of hybridized probe) × (decay constant) ×

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Figure 5. Dark-field photomicrographs of autoradiographically generated grains (seen as *white dots*) produced by ISHH labeling of striatal sections using an oligonucleotide probe complementary to dynorphin mRNA (*A–D*). Sample areas in the dorsolateral striatum are shown from a control animal (*A*), from an animal that received 10 d of twice-daily injections of 5 mg/kg apomorphine (*apo*; *B*), from the lesioned side of an animal that had a 6-OHDA lesion of the nigrostriatal pathway (*C*), and from the lesioned side of an animal that received a 6-OHDA lesion followed 2 weeks later by 10 d of twice-daily apomorphine treatments (*D*). Note that, in the controls, neurons in the patches (indicated with *arrows* and identified in adjacent sections with ^{3}H-naloxone binding, not shown) show a greater average OD_i than do cells in the matrix. The average percentage of dynorphin-labeled cells is approximately equal in the 2 compartments. *E*, Line graphs of the average OD_i per cell showing the distribution of cells labeled with the dynorphin probe from the 4 experimental groups (≈500 labeled cells per animal, 5 animals per group). These plots show that, relative to the control levels, apomorphine treatments caused an increase in labeling in both patch (*P*) and matrix (*M*) neurons, though the relative increase is significantly larger in the patch neurons. In animals that had 6-OHDA lesions followed by apomorphine treatment, there is a substantial increase in expression of dynorphin levels throughout the dorsolateral striatum that does not show any pattern suggestive of a differential expression between patch and matrix compartments.

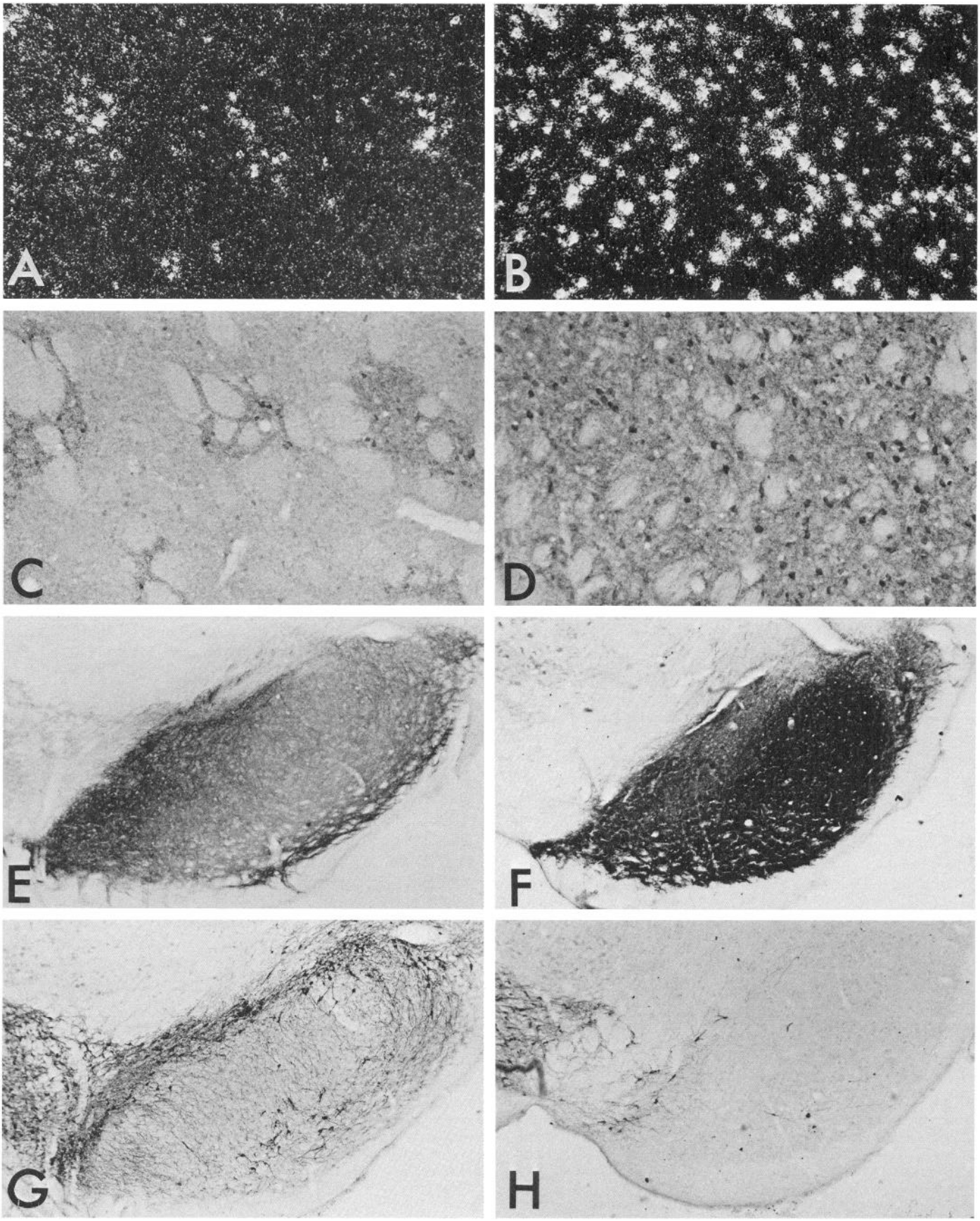
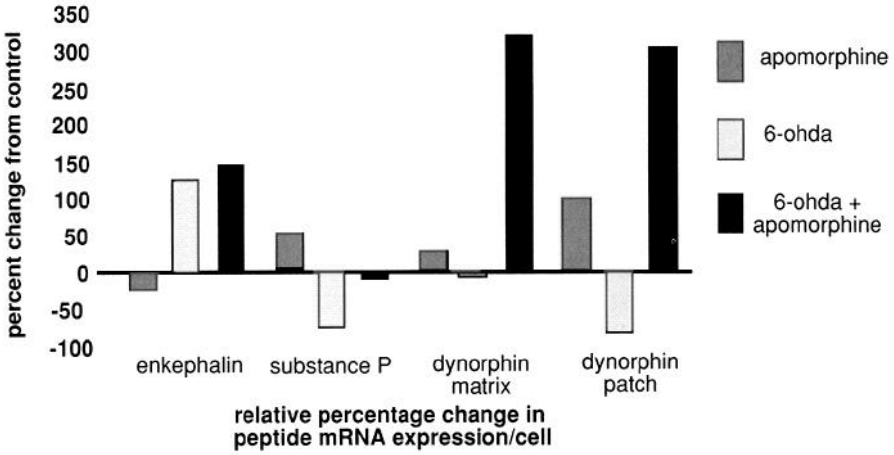


Figure 6. Photomicrographs comparing labeling in an animal receiving apomorphine treatment (*A, C, E, G*) and an animal that had a 6-OHDA lesion followed by apomorphine treatment (*B, D, F, H*). In a section from the apomorphine-treated animal, the same area of the dorsolateral



	control	apomorphine	6-ohda	6-ohda + apomorphine
enkephalin				
percent cells	54.8 ± 3.9	52.9 ± 3.3	52.5± 4.7	51.9 ± 4.1
ODi /cell	47.8 ± 2.6	45.0 ± 2.8	99.9 ± 6.1 **	107.5 ± 8.1 **
percent change		-7 %	+130 % **	+150 % **
substance P				
percent cells	52.5 ± 3.2	53.6 ± 4.8	49.1 ± 2.4	56.3 ± 5.1
ODi /cell	56.7 ± 2.8	77.3 ± 7.1 **	27.5 ± 3.7 **	52.6 ± 3.4
percent change		+ 50 % **	- 75 % **	-10 %
dyn (matrix)				
percent cells	47.5 ± 4.3	51.1 ± 5.4	48.8 ± 3.6	53.6 ± 5.3
ODi /cell	32.5 ± 2.6	44.1 ± 3.1 **	30.6 ± 1.8	176.6 ± 11.3 **
percent change		+ 29 % **	- 5 %	> +300 % **
dyn (patch)				
percent cells	52.6 ± 3.9	53.1 ± 4.4	combined with matrix	
ODi /cell	63.1 ± 4.8	102.8 ± 8.1 **		
percent change		+ 100 % **	- 82 % **	> +300 % **

Figure 7. *Top,* Bar graph of the relative percent change versus controls in enkephalin, substance P, and dynorphin mRNA levels in the dorsolateral striatum in animals that received apomorphine treatment (5 mg/kg twice daily for 10 d), unilateral 6-OHDA lesions, and unilateral 6-OHDA lesions followed by apomorphine treatment (2 weeks postlesion, 5 mg/kg twice daily for 10 d). Values for the 6-OHDA and 6-OHDA plus apomorphine groups are for the lesioned striatum compared to the control striatum. Values for enkephalin and substance P are for all striatal neurons in the dorsolateral striatum. The values for the apomorphine-induced changes in dynorphin mRNA levels reflect differences in patch and matrix neurons, whereas values for 6-OHDA- and 6-OHDA plus apomorphine-induced changes in dynorphin mRNA levels are the average of all dorsolateral striatal neurons compared with the control patch and matrix values. *Bottom,* Table of values from which the bar graph was calculated. Shown is the average percentage of cells expressing each peptide mRNA for each experimental condition. There was no significant difference between the experimental groups. Also shown are the average OD_i values per cell for each experimental condition. Differences in average OD_i were converted to relative percent change in mRNA expression per cell using the conversion factor of 9.85 OD_i units per 25% change in mRNA expression calculated from the standards shown in Figure 2. Values are given mean ± SEM; **, significantly different ($p < 0.001$).

(exposure time), verifies that lengthening the exposure time enables the use of the enkephalin probe standards to calculate the relative change in other peptide mRNAs.

Dopaminergic regulation of striatal peptide mRNA expression. The present data are consistent with previous studies employing a variety of techniques that have examined dopaminergic regulation of striatal peptide expression. Repeated administration of apomorphine (Li et al., 1986, 1987, 1988) or amphetamine (Peterson and Robertson, 1984; Hanson et al., 1987) increases dynorphin and substance P immunoreactivity and mRNA in the striatum with little or no effect on enkephalin immunoreactivity or mRNA. Conversely, dopamine deafferentation (Young et al., 1986; Normand et al., 1988) or dopamine receptor blockade (Hong et al., 1978b; Hanson et al., 1981; Tang et al., 1983; Chou et al., 1984; Mocchetti et al., 1985; Bannon et al., 1986; Romano et al., 1987) increases enkephalin synthesis, whereas these treatments decrease or fail to alter dynorphin and substance P synthesis.

The present data demonstrate that dopamine differentially regulates the relative levels of expression of the neuropeptides, enkephalin, dynorphin, and substance P at a cell-specific level in the striatum. Figure 7 provides a bar graph and table of the average changes in peptide mRNA levels observed for the different experimental conditions. These changes are expressed as the percent change in mRNA values versus control values. Chronic treatment with a dopaminergic agonist, apomorphine, markedly increased dynorphin mRNA and peptide immunoreactivity, whereas 6-OHDA lesions significantly depressed mRNA levels, in striatal patch neurons. 6-OHDA lesions followed by apomorphine treatment resulted in a dramatic ele-

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striatum was located in adjacent sections and shows that dynorphin ISHH labeling (*A*) and dynorphin immunoreactivity (*C*) is increased in cells in the patch compartment relative to control (not shown). In this animal, the pattern of dynorphin immunoreactivity in striatonigral terminals (*E*) is compared with the distribution of TH-immunoreactive neurons (*G*) and is seen to be densest over the ventral tier of the substantia nigra pars compacta, in the ventral medial and ventral lateral pars reticulata. In the animal that had combined 6-OHDA lesion and apomorphine treatment, there is an elevation of dynorphin ISHH labeling (*B*) and dynorphin immunoreactivity (*D*) in both patch and matrix neurons in the dorsolateral striatum. The increased expression of dynorphin in dorsolateral matrix neurons in these animals, compared with the apomorphine-treated animals, resulted in a substantial increase in dynorphin immunoreactivity in the striatonigral terminals in the substantia nigra pars reticulata (*F*). The adjacent section, stained for TH immunoreactivity (*H*), shows that the 6-OHDA lesion resulted in the loss of nearly all dopaminergic neurons in the substantia nigra pars compacta, while sparing the neurons in the ventral tegmental area.

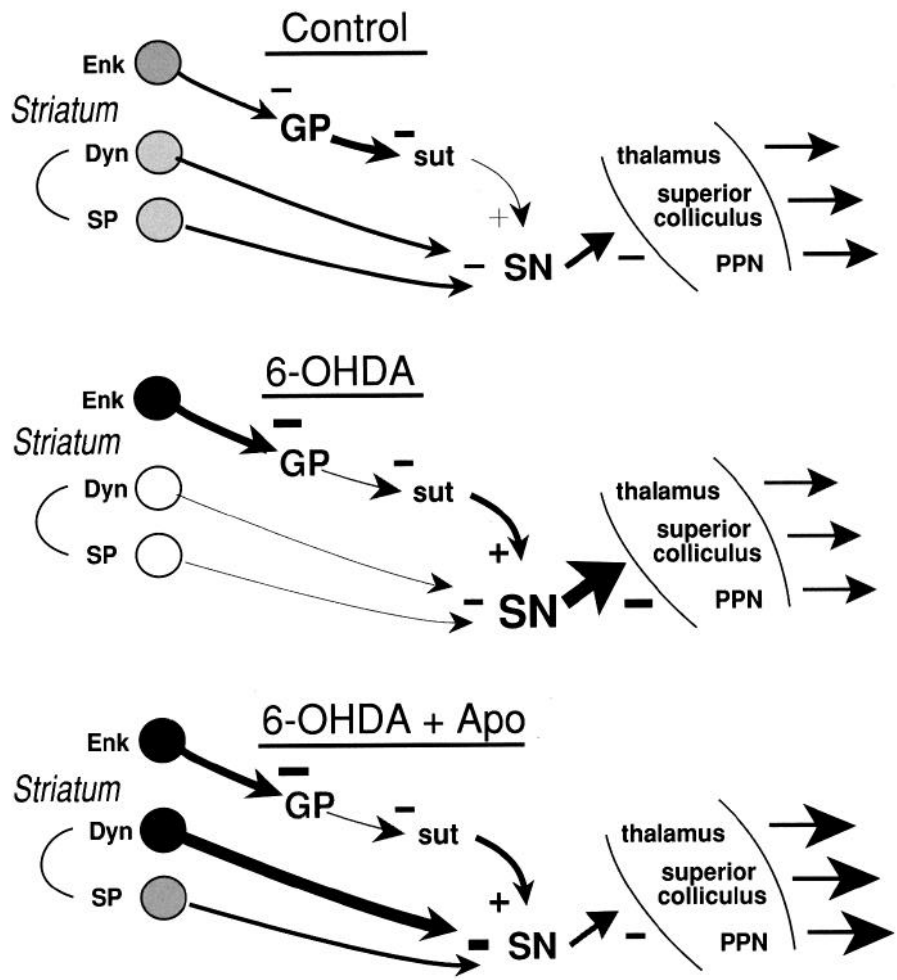

Figure 8. Diagrammatic representation of the major direct and indirect connections of the striatal output neurons and the probable functional consequences that result from the altered regulation of these pathways described in the present paper. The *gray level* of the cells denotes relative peptide mRNA levels, and the *thickness* of the *lines* indicates relative activity. *Control,* Striatal neurons that contain enkephalin (*Enk*) provide an inhibitory input to the globus pallidus (*GP*). Pallidal neurons provide an inhibitory input to the subthalamic nucleus (*sut*), which provides an excitatory input to the substantia nigra pars reticulata (*SN*). Striatal neurons that express dynorphin (*Dyn*) and substance P (*SP*) provide an inhibitory input to the substantia nigra (*SN*). Substantia nigra GABAergic neurons (*SN*) inhibit neurons in the thalamus, superior colliculus, and pedunculopontine nucleus (*PPN*). Normal behavioral activity (*arrows* at the extreme *right* of the diagram) is dependent on coordinated striatonigral and striatopallidal outputs that regulate substantia nigra output. *6-OHDA,* Dopamine lesions result in increased enkephalin and activity in striatopallidal neurons. This results in increased firing of substantia nigra GABAergic neurons and in diminished behavioral activity (*arrows* at *right* of diagram). *6-OHDA + Apo,* Apomorphine (*Apo*) treatment after 6-OHDA lesions does not alter the lesion-induced increase in enkephalin in the striatopallidal pathway but reverses the decrease in substance P and significantly increases dynorphin in striatonigral neurons. The increased activity in the striatonigral pathway overcomes the lesion-induced increases in nigral activity caused by increase subthalamic inputs. The behavioral consequence of these changes is increased behavioral activity that results from reduced inhibition by the nigrothalamic, nigrotectal, and nigropedunculopontine pathways.

vation of dynorphin immunoreactivity and mRNA in both striatal patch and matrix neurons to levels more than triple those produced by apomorphine treatment alone. Chronic apomorphine treatment resulted in an observable increase in substance P mRNA per cell, whereas dopaminergic deafferentation resulted in a marked decrease in substance P mRNA per cell. Combined 6-OHDA and chronic apomorphine treatment did not significantly alter substance P mRNA expression compared with controls. In contrast, chronic apomorphine treatment had little affect on enkephalin mRNA expression, whereas dopamine deafferentation markedly elevated enkephalin mRNA levels per cell. Deafferentation followed by apomorphine treatment elevated enkephalin mRNA expression to the same level obtained with the lesion alone. Changes in enkephalin and substance P mRNA were seen uniformly in the dorsal striatum, with no differences between the striatal patch and matrix compartments. These results suggest that increased striatal dopamine activity stimulates dynorphin and substance P expression, whereas a decrease in striatal dopamine stimulates the expression of enkephalin in striatal neurons. Dopamine receptor supersensitivity, as after 6-OHDA lesions, heightens the effect of apomorphine on dynorphin expression in both the patch and matrix compartments but does not alter the lack of effect of apomorphine on enkephalin expression and actually neutralizes the effect of apomorphine treatment on substance P expression. Thus, different mechanisms mediate the regulating affects of dopamine on the expression of these striatal peptides.

Functional considerations. The present data suggest that the

manner in which dopamine affects striatal peptide expression is different for each peptide and may be related to the segregation of separate populations of striatal medium spiny neurons according to their neuroanatomical connections and neurochemical phenotype. As outlined in the introductory remarks, striatal medium spiny neurons may be subdivided into distinct populations on the basis of at least 3 determinants: (1) distribution in either the patch or matrix striatal compartments, (2) projections to either the globus pallidus or substantia nigra, and (3) expression of the neuropeptides enkephalin, substance P, or dynorphin. Alterations in peptide mRNA expression induced by manipulation of the striatal dopamine system suggest that each of these determinants influences the patterns of change.

Patch–matrix compartments. Although it appears that the peptides examined in the present study are expressed in roughly equal numbers of neurons in the striatal patch and matrix compartments, the relative expression of these peptides per cell in these compartments varies in different regions of the striatum (Gerfen and Young, 1988). In order to focus this study, we chose to analyze only the dorsolateral quadrant of the striatum, and the data concerning dopamine regulation that are described are meant to apply only to this quadrant. In the dorsolateral quadrant, it appears that only dynorphin mRNA expression is differentially affected in the patch and matrix compartments by the manipulations of the dopamine system described. In the normal rat, dynorphin mRNA expression in patch neurons is significantly higher than that in dynorphin-expressing matrix neurons, whereas there appears to be no significant difference in the expression of enkephalin or substance P between the compartments. Removal of striatal dopamine with 6-OHDA-induced deafferentation or chronic treatment with the dopamine agonist apomorphine also has a greater effect on patch versus matrix dynorphin-expressing neurons, whereas these treatments do not differentially affect the compartmental expression of the other peptides. Several possible mechanisms for such differential dopamine action may be considered. First, it has been demonstrated that different sets of midbrain dopamine neurons provide inputs to the striatal patches and matrix (Gerfen et al., 1987; Jiménez-Castellanos and Graybiel, 1987). Thus, it is possible that the patch-specific elevation of dynorphin mRNA expression is mediated by a select drug action on the ventral set of substantia nigra dopamine neurons that provides inputs to the patches. However, this would not explain the elevation of substance P in both patch and matrix neurons that results from the same drug treatment. This does not preclude the possibility that other drug treatments that might differentially affect patch- and matrix-directed dopamine neurons may provide such differential action, but it appears that chronic apomorphine treatment does not. Second, the patch-specific dopamine effect on dynorphin expression as compared with the other peptides appears to be regionally specific within the striatum. In the ventral striatum, for instance, substance P mRNA is higher in patch than in matrix neurons, whereas there is a smaller difference between compartments for dynorphin expression in this region (Gerfen and Young, 1988). For example, following chronic apomorphine treatment, substance P has been described as elevated in the patches in the ventromedial striatum (Li et al., 1987). Such regional variations may be mediated through striatal interneurons, which show similar regional variations in distribution (Gerfen et al., 1985). In this regard, it is of interest that one of the striatal interneurons, the cholinergic neuron, has been implicated in dopaminergic regulation of peptide expression. Hong et al. (1980) reported that scopolamine, a muscarinic receptor antagonist, partially blocks the haloperidol-induced increase in striatal enkephalin expression. In contrast, stimulation of muscarinic receptors decreases, and muscarinic receptor blockade increases, apomorphine's effect on dynorphin in patch neurons (Daunais and McGinty, 1990).

Striatopallidal versus striatonigral systems. The differential effect that dopamine exerts on the regulation of enkephalin- versus dynorphin- and substance P-expressing striatal neurons is of interest in that the projections of these neurons have different targets. Striatal enkephalin-expressing neurons provide inputs to the globus pallidus, whereas both dynorphin- and substance P-expressing neurons project to the substantia nigra (Brownstein et al., 1977; Hong et al., 1977; Kanazawa et al., 1977; Sar et al., 1978; Finley et al., 1981; Vincent et al., 1982; Haber and Nauta, 1983; Zamir et al., 1984; Beckstead and Kersey, 1985; Gerfen and Young, 1988). These differential projections are not related to patch–matrix compartmental organization (Gerfen and Young, 1988). Furthermore, striatopallidal and striatonigral neurons have been shown to be intermingled in the striatum such that neighboring neurons may provide inputs to different targets (Loopuijt and van der Kooy, 1985). Thus, the demonstration that dopamine exerts opposite effects on pallidal-projecting/enkephalin-expressing neurons and nigral-projecting/substance P- and dynorphin-expressing neurons suggests that the differential effects of dopamine on these neurons may result in different effects on the targets of these neurons. It must be stressed that all of these peptide neurons use GABA as a transmitter (Kita and Kitai, 1988), and that the physiologic consequence of alteration in these pathways by dopamine may be mediated through GABA neurotransmission. The functional significance of the changes in peptide expression is at this point unknown.

The significance of the differential effects of dopamine on striatopallidal and striatonigral neurons is related to their regulation of the tonic activity of GABAergic neurons in the substantia nigra pars reticulata. The connections responsible for this regulation are diagrammed in Figure 8. The tonic activity of pars reticulata neurons is produced in part by excitatory inputs from the subthalamic nucleus (Kita and Kitai, 1987; Nakanishi et al., 1987). The activity of the subthalamic nucleus is regulated by the inhibitory GABAergic pathway from the globus pallidus (Kita et al., 1983), which is in turn regulated by the striatopallidal pathway. Thus, the effect of the inhibitory output of the striatopallidal pathway on the firing rates of the pars reticulata neurons is excitatory. On the other hand, the striatonigral pathway provides inhibition of the pars reticulata neurons such that activity in this pathway is directly responsible for the generation of movements. Activity of both striatonigral and striatopallidal neurons is produced by excitatory inputs from the cortex and thalamus (Kitai et al., 1976; Kawaguchi et al., 1989), but it appears that dopaminergic inputs regulate the levels of such activity. The removal of dopamine, as with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- or 6-OHDA-induced lesions, results in an increase in striatopallidal activity and a decrease in striatonigral activity as measured with 2-deoxyglucose (Trugman and Wooten, 1987; Mitchell et al., 1989). Increased striatopallidal activity reduces the inhibition on the subthalamic nucleus, which in turn results in increased tonic activity of pars reticulata neurons. This increased activity of pars reticulata neurons, coupled with a decrease in the output of the striatonigral pathway, makes the generation of move-

ments more difficult. Such a condition is thought to be responsible for the bradykinesia of Parkinson's disease (Albin et al., 1989).

The presently reported changes in peptide mRNA levels that result from 6-OHDA lesion and from lesions followed by apomorphine treatment are diagrammed in Figure 8 (6-OHDA and 6-OHDA + Apo, respectively). The changes in peptide levels correspond to changes in the activity in these pathways as measured with 2-deoxyglucose activity (Kozlowski and Marshall, 1980; Wooten and Collins, 1983). Dopamine deafferentation results in an increase in enkephalin mRNA expression and metabolic activity in neurons contributing to the striatopallidal pathway. Dopamine deafferentation followed by twice-daily injections of apomorphine for 10 d does not alter the lesion-induced changes in the striatopallidal pathway but results in significant increase in both dynorphin and substance P mRNA expression and metabolic activity (Kozlowski and Marshall, 1980; Wooten and Collins, 1983) in the striatonigral pathway. Thus, it is proposed that the relative changes in peptide mRNA expression by neurons contributing to the striatopallidal and striatonigral pathways that result from pharmacologic manipulation of striatal dopamine may be used as an assay of the alterations in the regulation of the functional activity of the neurons contributing to these pathways.

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