Cocaine-induced *c-fos* Messenger RNA Is Inversely Related to Dynorphin Expression in Striatum

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The effects of the indirect dopamine receptor agonist cocaine in the striatum on levels of mRNAs of the immediate-early gene *c-fos* and the neuropeptides dynorphin, substance P, and enkephalin were analyzed with quantitative *in situ* hybridization histochemistry. Both single (acute) and repeated (twice a day for 4 d) systemic injections of cocaine (3.75–30 mg/kg) to rats resulted in dose-dependent, regionally specific elevations of mRNA expression in striatal neurons. A single drug treatment elevated *c-fos* mRNA expression, whereas repeated treatments resulted in little *c-fos* expression but elevated dynorphin mRNA levels. Both the regional and temporal patterns of gene expression revealed an inverse relationship between dynorphin and *c-fos* expression. This relationship was examined in a time course experiment in which cocaine (30 mg/kg) was administered for 1, 2, 3 or 4 d. Basal levels of dynorphin expression were relatively high in the ventral striatum, including the nucleus accumbens, a ventrolateral region, and an area along the medial bank of the striatum. A single injection of cocaine induced *c-fos* mRNA in striatal areas with low basal expression of dynorphin. Thus, *c-fos* mRNA induction was highest in the dorsal central striatum, where basal dynorphin mRNA levels were lowest. In this region, dynorphin mRNA expression increased on subsequent treatment days parallel to diminished *c-fos* mRNA induction. Changes in substance P mRNA levels appeared to match directly both the temporal and regional patterns of *c-fos* induction. Enkephalin mRNA expression was altered, but only slightly, by these cocaine treatments.

Statistical analysis of the regional patterns of basal and altered mRNA levels shows a unique inverse relationship between basal dynorphin expression and *c-fos* induction by cocaine. Further evidence of this relationship is provided by the dose-dependent blockade of cocaine-induced *c-fos* expression by spiradoline, a dynorphin agonist. Together, these results suggest that the restricted regional pattern of cocaine-induced *c-fos* expression is related, in part, to the basal level of dynorphin expression, and that cocaine treatment elevates dynorphin expression in striatal regions with

a strong *c-fos* response, thereby limiting subsequent *c-fos* induction by cocaine. These findings lead to the hypothesis that dynorphin acts to regulate the responsiveness of striatal neurons to dopamine stimulation.

[Key words: *c-fos*, dynorphin, substance P, enkephalin, cocaine, striatum, dopamine, κ -receptor]

Experimental manipulations of dopamine neurotransmission alter gene regulation in connectionally and neurochemically defined populations of neurons in the striatum (Gerfen, 1992). The direct action of dopamine has opposite effects on gene regulation in neurons giving rise to the two major striatal output pathways. These neurons—medium spiny neurons, which make up 95% of the striatal neuron population (Kemp and Powell, 1971)—can be classified into two main subtypes based on their axonal projection targets (Kawaguchi et al., 1990). One subtype, striatopallidal neurons, projects to the globus pallidus. The other subtype, striatonigral neurons, provides a small axon collateral to the globus pallidus, and more extensive axon projections to the entopeduncular nucleus and/or the substantia nigra. Striatopallidal and striatonigral neurons are approximately equal in number and are intermingled throughout the striatum (Beckstead and Kersey, 1985; Gerfen and Young, 1988). Both types of neurons use GABA as their transmitter (Kita and Kitai, 1988), but express different neuropeptides; most striatopallidal neurons express enkephalin, whereas most striatonigral neurons express both dynorphin and substance P (Brownstein et al., 1977; Vincent et al., 1982; Beckstead and Kersey, 1985; Gerfen and Young, 1988). It has been shown that enkephalin mRNA and peptide levels in striatopallidal neurons are elevated by dopamine depletion or by neuroleptic treatment (Hong et al., 1978; Young et al., 1986; Voorn et al., 1987; Gerfen et al., 1990, 1991; Li et al., 1990). In contrast, substance P and dynorphin mRNA and peptide levels in striatonigral neurons are reduced by striatal dopamine depletion and elevated by dopamine agonist treatments (Young et al., 1986; Bannon et al., 1987; Hanson et al., 1987, 1988; Voorn et al., 1987; Li et al., 1988, 1990; Gerfen et al., 1990, 1991). These opposite effects of dopamine manipulations on peptide expression appear to be mediated by the selective expression of the D₁ and D₂ dopamine receptor subtypes in striatonigral and striatopallidal neurons, respectively (Gerfen et al., 1990; Le Moine et al., 1990). Thus, in the dopamine-depleted striatum, D₂-selective agonist treatment reverses the elevated enkephalin mRNA levels in striatopallidal neurons (Gerfen et al., 1990). Conversely, D₁-selective agonist treatment elevates peptide mRNA levels specifically in striatonigral neurons (Gerfen et al., 1990). Consistent with these results are studies demonstrating the induction of the immediate-early gene *c-fos*, which encodes the transcription factor

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Fos, by acute pharmacological treatments. In rats with nigrostriatal lesions, *c-fos* is induced selectively in striatonigral neurons by a single injection of the D₁-selective agonist SKF-38393 (Robertson et al., 1990, 1992). Conversely, in unlesioned animals, *c-fos* is induced selectively in striatopallidal neurons by a single treatment with the D₂ antagonist haloperidol (Dragunow et al., 1990; Robertson et al., 1992). Together, these studies suggest that dopamine exerts a direct and opposite effect on gene regulation in striatonigral and striatopallidal neurons through D₁ and D₂ receptors, respectively.

Direct effects of dopamine on striatonigral and striatopallidal neurons are demonstrated in studies employing the model of the dopamine-deafferented striatum, where it is possible to analyze effects of activation of one dopamine receptor subtype. Experimental paradigms in which dopamine neurotransmission is altered in the presence of an intact nigrostriatal dopamine system provide additional information that is relevant to the normal functional organization of the striatum. Cocaine, which blocks dopamine reuptake (Heikkilä et al., 1975; Taylor and Ho, 1978; Missale et al., 1985) and thereby prolongs its action, alters gene regulation in striatal neurons. Acute cocaine treatment induces *c-fos* in striatonigral neurons (Cenci et al., 1992), primarily through a D₁ receptor mechanism (Graybiel et al., 1990; Young et al., 1991). Moreover, repeated treatment with cocaine elevates dynorphin and substance P (Sivam, 1989; Smiley et al., 1990; Hurd et al., 1992) in striatonigral neurons. One striking finding from these studies is the distinct regional pattern in neuronal gene regulation in response to cocaine treatment, with the most pronounced effects occurring in the dorsal striatum (Graybiel et al., 1990; Young et al., 1991). Additionally, whereas the *c-fos* response has been reported after acute treatment, the elevation of dynorphin has been demonstrated after repeated treatments. In order to analyze possible relationships between cocaine-induced changes in various neuronal gene products, the present study examined regional variations and the time course of changes in the expression of the immediately early gene *c-fos* and the neuropeptides dynorphin, substance P, and enkephalin, which serve as markers for the distinct sets of striatal projection neurons.

Materials and Methods

Subjects and drugs. Male Sprague-Dawley rats (200–300 gm) were used in these experiments. They were housed in groups of five under standard laboratory conditions. In experiments 1 and 2, rats were injected intraperitoneally with one of the following five doses of cocaine ($n = 5$ each): 0 (vehicle), 3.75, 7.5, 15, or 30 mg/kg (cocaine hydrochloride; Sigma; in 0.02% ascorbic acid, 1 ml/kg). Animals in experiment 1 ("acute cocaine") received a single injection. Rats in experiment 2 ("chronic cocaine") were injected twice daily (at 10 A.M. and 5 P.M.) for 3 d and received the final injection (same dose) on day 4 (20–22 hr after the last injection). In experiment 3 ("time course"), rats were injected with cocaine (30 mg/kg, i.p.) twice daily for 0–3 d ($n = 5$ each) and received the final injection (30 mg/kg) on drug days 1 (1 d group), 2 (2 d group), 3 (3 d group), or 4 (4 d group). A control group was injected with vehicle (0 d group). The number of injections was equalized with vehicle injections. That is, all groups received a total of seven injections over 4 d. In experiment 4 ("dynorphin agonist"), rats received one of five doses of the κ -receptor agonist spiradoline [U-62066, Research Biochemicals; 0 (vehicle), 0.5, 1, 5, 10 mg/kg, i.p.; $n = 4$ or 5] 15 min prior to a single injection of 30 mg/kg cocaine. These animals had been habituated to the injection procedure by administration of six vehicle injections over the 2 d preceding the experiment. In all of these experiments, drug effects on behavior were assessed by observing the animals in their home cage.

Tissue section preparation. All rats were decapitated 30 min after the last injection. The brains were quickly removed, frozen for 15 sec in

isopentane cooled on dry ice, and stored at -30°C until cryostat sectioning. Coronal sections (12 μm) were adhered to glass slides twice coated with gelatin, and then dried to the slide on a warm plate and stored at -20°C . For further processing, the slides were first warmed to room temperature, and then fixed in a 4% paraformaldehyde solution (in 0.9% saline) for 10 min 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. The slide-mounted sections were then dehydrated in a series of ascending concentrations of ethanol, defatted for 2×5 min in chloroform, rehydrated, and air dried. The sections were stored at -80°C until hybridization.

Oligonucleotide probes. Tails of ^{35}S -dAMP (average tail length, 20 bases) were added to purified oligonucleotides (48-base cDNAs) complementary to the mRNAs for *c-fos* (experiments 1–4), dynorphin (experiments 1–3), substance P and enkephalin (experiment 3) (Young et al., 1986) in the following reaction: 1 μl 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/mmol ^{35}S -dATP) and 80 U/4 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 of tRNA (25 $\mu\text{g}/\mu\text{l}$) and 450 μl of 10 mM Tris HCl (pH 7.5)/EDTA (TE), and the labeled 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 of ethanol, which was allowed to cool on dry ice for 20 min. The precipitate was pelleted at 14000 rpm for 12 min, washed with ice-cold ethanol, and resuspended in 50 μl of TE.

Hybridization. Labeled probes were added to hybridization buffer (Young et al., 1986) to reach a concentration of $0.4\text{--}1 \times 10^6$ dpm per 25 μl of buffer. Twenty-five microliters of hybridization buffer containing labeled probe were added to each brain section. The sections were coverslipped with parafilm and incubated overnight at 37°C . After incubation, the coverslips were floated off in $1 \times$ saline-sodium citrate (SSC) ($1 \times$ SSC contains 150 mM sodium chloride and 15 mM sodium citrate), and the slide-mounted sections were rinsed in four washes of $1 \times$ SSC. The slides were then washed 4×15 min in $2 \times$ SSC/50% formamide at 41°C , followed by 2×30 min washes in $1 \times$ SSC at room temperature and a brief water rinse, before the sections were air dried. The slides were then apposed to x-ray film (X-Omat, Kodak) for 2–5 weeks. After the films were developed, the slides were dipped in NTB3 autoradiography emulsion (Kodak) (diluted 1:1 with water containing 0.1% Drefit) and exposed for 8–12 weeks. After developing, they were counterstained with thionin, dehydrated, and coverslipped out of xylene.

Analysis of autoradiograms. Sections from four rostrocaudal levels were examined (Fig. 1): a frontal (about at 11.5 mm rostral to the interaural line; Paxinos and Watson, 1982), a rostral striatal (10.5 mm), a mid-striatal (9.5 mm), and a caudal striatal level (8.0 mm). In experiments 1 and 2, striatal mRNA levels were assessed in dorsomedial, dorsolateral, ventrolateral, and ventromedial quadrants or ventral parts (Fig. 1B–D, left side). These arbitrary regions were sufficient for the purpose of these experiments, in which parameters of drug dose and delivery were determined. However, for the more detailed analysis performed in experiment 3, the striatum was subdivided on the basis of its more complex regional heterogeneity. Thus, the following striatal areas were chosen (Fig. 1B–D, right side): a dorsomedial periventricular zone with high basal dynorphin expression; a dorsal central zone, which shows maximal *c-fos* induction by cocaine; an area with high basal expression of substance P, situated dorsolaterally on the rostral striatal section, laterally at the middle, and more ventrolaterally at the caudal striatal level; and a ventrolateral or ventral area also rich in basal dynorphin mRNA. Additionally, the nucleus accumbens was dissected into a core and a shell region (Zaborszky et al., 1985), based on differential basal expression of substance P in these regions. In the first three experiments, *c-fos* expression was also analyzed in the claustrum and the medial frontal cortex (frontal level), and in the cingulate cortex (rostral through caudal striatal levels) (Fig. 1). In experiment 4, *c-fos* expression was assessed in the dorsal central region of the striatum and in the cingulate cortex, at rostral, middle, and caudal levels.

Labeling was measured from film with a Macintosh-based image analysis system (IMAGE, Wayne Rasband, NIMH). For each region, mean optical density (OD) values were assessed in both hemispheres and averaged. OD values given are corrected for background by subtraction of the OD value of the corpus callosum. For the analysis of regional differences in changes in neuropeptide expression (experiment 3), the maximal increase in mRNA levels (peak induction) was determined as

Figure 1. Schematic representation of the forebrain areas in which changes in gene expression were analyzed. OD values were measured in regions at a frontal (A), rostral (B), middle (C), and caudal striatal (D) level. The regions examined in the dose-response experiments (experiments 1 and 2) are shown on the left side of each diagram: dorsomedial (DM), dorsolateral (DL), ventrolateral (VL), and ventromedial (VM) quadrants and ventral (V) parts of the striatum, and medial frontal cortex (MF), claustrum (CL), and cingulate cortex (CG). The striatal regions depicted on the right side of the diagrams were designed for the time course experiment (experiment 3): dorsomedial (DM), ventrolateral (VL) and ventral (V) areas with high basal dynorphin expression, dorsal (D) central areas with high c-fos induction by cocaine, dorsolateral (DL) and lateral (L) regions with high basal substance P expression, and nucleus accumbens (NAC) core and shell regions.

follows: for dynorphin and enkephalin, signal in the 2 d group divided by signal in the 0 d group; for substance P, signal in the 1 d group divided by that in the 0 d group; for c-fos, signal in the 1 d group minus that in the 0 d group (this measure was chosen for c-fos due to the very low basal levels of this mRNA).

Statistics. Drug effects were determined with one-factor analysis of variance (ANOVA). In experiments 1, 2, and 4, Dunnett's test was used post hoc to assess the effects of individual doses of cocaine, or spiradoline, compared to vehicle, or cocaine controls, respectively. In experiment 3, the Tukey-Kramer test was applied to compare the different groups. In addition, the expression (basal expression and peak induction) of the four mRNAs in the 14 striatal regions was compared by a correlation analysis (Pearson's correlation). All *p* values presented are two tailed.

Results

Experiment 1: acute cocaine

Behavior. Vehicle-injected rats did not exhibit much activity, but spent most of the postinjection period piled up in a corner of the cage. After the injection of the two lower doses of cocaine (3.75 and 7.5 mg/kg), rats were more activated, showing repetitive sniffing, rearing, and some locomotor activity for several minutes. The higher doses (15 and 30 mg/kg) produced more intense repetitive behavior, including sniffing, rearing, locomotion, and some side-to-side head movements (head bobbing). These two doses mainly differed in the duration of activation. Whereas rats that received 15 mg/kg were relatively inactive by the time of decapitation (30 min after the injection), those injected with the highest dose were still activated.

c-fos mRNA expression. Acute injection of cocaine resulted in a dose-dependent induction of c-fos mRNA in striatal neu-

Table 1. Expression of c-fos and dynorphin mRNA in striatal regions following a single (acute) or repeated (chronic) administration of various doses of cocaine

Striatum		c-fos expression					Dynorphin expression				
		0 mg/kg	3.75 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg	0 mg/kg	3.75 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg
Acute cocaine											
Ros-tral	DM	4.1 (0.8)	4.0 (0.2)	9.9 (0.8)	19.5 (1.7)**	26.8 (2.4)**	26.2 (1.4)	25.8 (1.1)	28.7 (2.5)	26.7 (1.5)	28.1 (1.3)
	DL	2.8 (0.9)	1.8 (0.5)	6.6 (1.5)	16.5 (1.3)**	20.4 (2.5)**	25.3 (1.2)	25.4 (0.8)	27.7 (1.5)	25.3 (1.5)	27.7 (1.1)
	V	3.6 (0.7)	2.0 (0.4)	3.0 (2.1)	5.7 (0.7)	8.6 (0.8)**	40.7 (1.0)	38.0 (1.6)	42.4 (1.4)	39.6 (1.6)	42.5 (1.9)
Mid-dle	DM	3.8 (1.2)	5.0 (0.9)	9.5 (2.5)	20.3 (1.5)**	23.5 (2.5)**	22.6 (0.7)	21.3 (0.6)	25.6 (0.9)	23.6 (1.0)	24.1 (0.6)
	DL	1.9 (1.0)	2.3 (0.7)	5.6 (2.1)	14.6 (1.8)**	19.3 (1.9)**	18.5 (0.5)	17.2 (0.9)	22.1 (1.4)	19.1 (1.0)	19.7 (0.8)
	VL	3.3 (1.9)	1.3 (0.6)	5.3 (2.4)	9.3 (0.9)*	14.5 (1.9)**	30.6 (0.4)	28.0 (1.3)	33.3 (2.2)	29.3 (0.7)	30.1 (0.8)
Cau-dal	VM	2.0 (1.5)	3.5 (0.6)	7.2 (2.9)*	10.2 (0.6)**	14.4 (0.7)**	25.7 (0.7)	25.1 (1.2)	30.0 (1.2)	26.1 (1.4)	27.2 (0.6)
	DM	6.6 (1.0)	8.2 (1.3)	9.9 (1.3)	24.2 (1.1)**	22.5 (1.2)**	24.9 (1.0)	26.0 (0.6)	28.2 (1.1)	27.6 (0.9)	28.6 (1.0)*
	DL	2.1 (1.0)	1.8 (1.4)	2.6 (0.9)	14.1 (1.0)**	16.0 (1.6)**	20.1 (1.2)	19.9 (1.2)	22.4 (0.6)	20.9 (0.6)	21.5 (1.4)
	V	1.3 (1.5)	-0.4 (1.3)	-1.4 (0.5)	3.2 (0.5)	3.1 (0.6)	27.1 (1.4)	29.0 (1.2)	29.5 (0.5)	29.2 (1.1)	30.1 (1.8)
Chronic cocaine											
Ros-tral	DM	2.3 (0.7)	1.1 (0.4)	4.2 (0.4)	3.9 (1.3)	2.3 (0.7)	42.3 (1.2)	40.1 (1.7)	40.8 (1.9)	46.3 (1.6)	43.9 (1.7)
	DL	1.3 (0.6)	-1.3 (0.6)	1.6 (0.6)	1.8 (1.1)	2.1 (0.7)	47.1 (0.7)	45.6 (2.1)	43.0 (1.7)	46.9 (2.3)	50.2 (1.5)
	V	0.5 (1.4)	-2.6 (1.5)	-0.4 (2.3)	1.8 (0.7)	-1.4 (1.2)	66.4 (1.8)	66.2 (2.5)	61.1 (3.9)	66.2 (1.4)	65.2 (2.5)
Mid-dle	DM	4.9 (0.9)	5.1 (1.1)	5.9 (0.6)	8.7 (1.9)	9.7 (0.8)*	29.9 (1.0)	32.7 (0.7)	34.4 (1.0)	38.5 (2.4)**	44.6 (1.7)**
	DL	3.4 (1.1)	4.2 (1.2)	2.6 (0.4)	3.6 (1.1)	6.6 (0.4)*	28.1 (0.6)	28.3 (0.6)	30.2 (0.7)	29.5 (0.8)	36.5 (1.6)**
	VL	4.2 (1.3)	3.9 (2.0)	3.6 (0.8)	3.2 (0.8)	4.5 (1.5)	51.8 (1.6)	48.3 (1.4)	49.7 (2.5)	47.3 (1.6)	51.1 (3.7)
Cau-dal	VM	5.3 (1.2)	4.1 (2.0)	5.7 (1.2)	5.9 (1.0)	5.5 (2.1)	42.5 (1.3)	44.8 (1.8)	43.5 (2.1)	44.1 (1.1)	45.5 (1.9)
	DM	5.0 (0.8)	4.9 (0.7)	4.8 (1.0)	7.5 (1.3)	6.1 (0.8)	43.8 (1.5)	42.7 (2.0)	47.6 (1.8)	54.2 (4.0)*	53.7 (3.0)*
	DL	4.5 (0.6)	3.0 (1.0)	2.5 (1.5)	4.3 (1.3)	4.9 (1.6)	35.0 (1.4)	32.6 (2.1)	33.8 (1.8)	37.3 (2.5)	41.0 (3.0)
	V	1.6 (0.6)	-1.3 (1.1)	-0.8 (2.8)	0.6 (1.8)	-1.7 (1.9)	54.9 (2.5)	49.8 (3.0)	45.4 (2.3)	48.7 (1.3)	48.8 (2.3)

Animals were killed 30 min after the single injection, or 30 min after the last injection of the 4 d chronic treatment. Data represent mean (SEM) OD values measured in dorsomedial (DM), dorsolateral (DL), ventrolateral (VL), or ventromedial (VM) quadrants, or ventral parts (V), at rostral, middle, and caudal striatal levels. Data were analyzed with ANOVA and Dunnett's tests: **, *p* < 0.01; *, *p* < 0.05; compared to vehicle (0 mg/kg) groups.

Figure 2. Dose-dependent changes in striatal *c-fos* and dynorphin mRNA expression following acute and chronic cocaine treatments. OD values (mean + SEM) in dorsomedial (DM), dorsolateral (DL), ventrolateral (VL), and ventromedial (VM) quadrants at the middle striatal level are given for animals killed 30 min after the single injection (acute cocaine, left), or 30 min after the last injection of the 4 d repeated treatment (chronic cocaine, right) with 0–30 mg/kg of cocaine. Acute cocaine produces a dose-dependent increase in *c-fos* expression (top), whereas the *c-fos* response is much reduced or absent following the chronic treatment. In contrast to *c-fos* expression, repeated cocaine treatment results in a dose-dependent increase in dynorphin expression (bottom) in areas that show a relatively low basal expression of dynorphin and a strong *c-fos* response to acute cocaine. **, $p < 0.01$; *, $p < 0.05$; compared to vehicle (0 mg/kg) groups (ANOVA, Dunnett's test).

rons, with distinct regional variations in amount (Table 1, Fig. 2). The two higher (15 and 30 mg/kg), but not the two lower, doses produced a significant increase in *c-fos* labeling (compared to vehicle controls) in dorsomedial and dorsolateral quadrants at all three striatal levels. In contrast, in ventral striatal parts, the *c-fos* response was generally weaker and less uniform. No influence of cocaine on *c-fos* expression was seen in the cortical areas examined (Fig. 3).

Dynorphin mRNA expression. Thirty minutes after the acute cocaine injection, there was minimal influence on striatal dynorphin expression (Table 1, Fig. 2).

Experiment 2: chronic cocaine

Behavior. After the first drug injection, the rats displayed behavioral patterns similar to those described above. The lower

doses induced some sniffing and rearing, with little locomotion, for 5–15 min. The higher doses produced, in addition to more intense sniffing and rearing, more locomotor activity and some head bobbing. Repeated cocaine administration seemed to produce more activation. However, especially with 30 mg/kg, a shift in behavioral patterns was observed, rather than simply an increase in intensity or duration. For example, such rats exhibited more intense head bobbing, sometimes accompanied by highly repetitive scanning of restricted parts of the wall. In such situations, locomotion decreased considerably.

***c-fos* mRNA expression.** In contrast to the effects seen after a single cocaine injection, repeated cocaine treatment did not produce a comparable *c-fos* response in any of the striatal regions (Table 1, Fig. 2). Only in the dorsal quadrants of the middle striatum after a 30 mg/kg injection was there an increase in *c-fos*

Figure 3. Influence of acute and chronic cocaine treatments on *c-fos* expression in cortical areas. OD values (mean + SEM) in claustrum (CL), medial frontal cortex (MF), and cingulate cortex at rostral (R/CG), middle (M/CG), and caudal (C/CG) striatal levels are depicted for animals killed 30 min after the single injection (acute cocaine, left), or 30 min after the last injection of the 4 d repeated treatment (chronic cocaine, right) with 0–30 mg/kg of cocaine. No statistically significant effects of either treatment are seen in these cortical areas.

labeling. Also, repeated cocaine did not induce changes in *c-fos* expression in cortical areas (Fig. 3). However, in these cortical regions, the basal levels of *c-fos* expression (0 mg/kg) appeared to be lower after repeated injections than in the acute experiment, while basal levels in striatum were similar in these two conditions. There are several possible reasons for this difference, some of which may be related to differences in the handling procedures.

The exception was a rat from the 15 mg/kg group that developed seizures after the last cocaine injection, and whose data were excluded from the statistical analysis. This rat showed an increased *c-fos* signal in many brain areas, including the cortical areas examined and all parts of the striatum. Under these conditions, the *c-fos* value in, for example, the rostral ventral part was about 20–30 times higher than that in similarly treated animals without seizures or in vehicle controls.

Dynorphin mRNA expression. Repeated cocaine treatment affected dynorphin expression in dorsal parts of the striatum at middle and caudal levels (Table 1, Fig. 2). In the dorsomedial quadrant of the middle striatal level, injections of both 15 and 30 mg/kg cocaine increased the dynorphin signal, whereas in the dorsolateral quadrant only the latter dose did. A drug effect was also seen in the dorsomedial part at the caudal level, with both higher doses increasing dynorphin labeling. The striatal dynorphin mRNA levels of the rat that developed seizures were in the range of the values of the other animals in that group.

Experiment 3: time course

Behavior. The final cocaine injection induced the most locomotor activity in 2 d rats. Three rats of the 1 d group were activated by the drug, but to a lesser degree, showing sniffing, rearing, and some locomotion. Two animals of this group were not activated by the injection and were excluded from the study, based on our experience that rats lacking a behavioral response to such cocaine doses fail to show effects on gene regulation. Rats of the 3 and 4 d groups, in contrast, exhibited less locomotor activity than the 2 d group, but more intense head bobbing, in addition to sniffing and rearing. Some of these animals stayed for longer periods in a given sector of the cage, showing only sniffing and the highly repetitive side-to-side head movements. The 0 d group, by comparison, spent most of the survival period relatively inactive in a corner of the cage.

Basal expression of striatal *c-fos*, dynorphin, substance P, and enkephalin mRNA. In contrast to the first two experiments in which the striatum was divided into quadrants, in experiment 3 mRNA levels were examined in regions defined on the basis of basal dynorphin and substance P expression, as well as *c-fos* induction by cocaine. Regional differences in basal expression of the four mRNAs in the 0 d group are reported in Tables 2 and 3. At all three striatal levels, basal *c-fos* expression was generally very low and did not differ between the regions examined. Dynorphin was expressed in all striatal regions, but mRNA levels varied considerably between areas (Table 3; Figs. 4A, 5). The area with the highest level of basal dynorphin expression was the ventrolateral region throughout the rostrocaudal extent of the striatum (Table 3). The nucleus accumbens displayed the next highest level, with no difference between core and shell regions (Fig. 6), followed by the dorsomedial zone bordering the lateral ventricle. The areas with the lowest levels of basal dynorphin expression were the dorsal central and lateral regions. In these areas, patches displayed greater dynorphin expression than matrix (Gerfen et al., 1991) (see Fig. 4A). Basal

substance P expression displayed a distinct medial-to-lateral gradient, with highest expression laterally (Table 3, Fig. 7A). Along the rostral-to-caudal axis, the center of highest labeling shifted from dorsolateral (rostral level) to more ventrolateral. Comparing levels of dynorphin and substance P expression shows that in most sections the zones with the highest substance P mRNA levels displayed the lowest dynorphin mRNA levels, and vice versa. The nucleus accumbens displayed varying levels of substance P mRNA in shell and core (Fig. 6). Striatal enkephalin expression was, overall, more evenly distributed than the expression of the other neuropeptides (Table 2). However, there was more enkephalin expression in the ventrolateral region, which also contained the highest dynorphin mRNA levels.

Cocaine-induced *c-fos* mRNA expression. Similar to the results of the acute experiment, *c-fos* mRNA induction in the 1 d group was greatest in dorsal central and dorsolateral regions (Table 2, Figs. 4C, 5, 6, 8). The 2 d group showed an increase similar to that of the 1 d group. In contrast, in the 3 and 4 d groups, *c-fos* expression was not or only minimally enhanced over that of vehicle controls. Statistical analysis of regional differences in the maximal induction of *c-fos* expression by cocaine shows that *c-fos* induction was significantly higher in the dorsal central zone than in all other zones, at all three striatal levels (Table 3). Rostrally, the increase in *c-fos* labeling was also significantly higher in the dorsolateral, substance P mRNA-rich area than in the medial or ventral areas, and higher in the ventrolateral zone than in the nucleus accumbens regions.

Cocaine-induced dynorphin mRNA expression. The influence of cocaine treatment on striatal dynorphin expression was strongest in dorsal central and dorsolateral parts at all three striatal levels (Table 2, Figs. 4B, 6, 8). The increase in dynorphin labeling was maximal in the 2 d group, and could also be seen in both the 3 and 4 d groups, but was absent in the 1 d group. In almost all regions affected by the treatment, dynorphin mRNA levels were significantly higher in the 2 d group than after the first drug treatment. The regional analysis confirms that the peak induction of dynorphin during chronic cocaine treatment was significantly greater in dorsal central and dorsolateral areas than in the other striatal areas examined (Table 3).

Cocaine-induced substance P mRNA expression. Cocaine affected the levels of substance P mRNA in dorsal and lateral parts of the striatum (Table 2, Figs. 6, 7B, 8). In contrast to dynorphin, the time course of changes in substance P expression matched that of changes in *c-fos* expression. That is, the increase in substance P expression also peaked on drug day 1 and was reduced thereafter, resulting in no or only a slight increase on days 3 and 4. The regional analysis of peak induction showed significantly greater substance P responses in dorsal central and dorsomedial zones than in ventral parts, especially at mid- and caudal striatal levels (Table 3).

Cocaine-induced enkephalin mRNA expression. Changes in striatal enkephalin mRNA levels due to chronic cocaine treatment followed, overall, a time course similar to that of dynorphin expression, with increased enkephalin labeling in 2 and 3 d groups, again mainly in dorsal central and dorsolateral parts of the striatum (Table 2, Figs. 6, 8). However, these changes were of a smaller magnitude than those in expression of the other neuropeptides.

Regional correlations between the expression of *c-fos*, dynorphin, substance P, and enkephalin. Basal expression and peak induction by cocaine of these mRNAs in different striatal areas were also compared with correlation analysis (Table 4, Fig. 9).

Table 2. Expression of *c-fos*, dynorphin (DYN), substance P (SP) and enkephalin (ENK) mRNA in striatal regions during chronic cocaine treatment

		0 d	1 d	2 d	3 d	4 d
<i>c-fos</i>						
Rostral	DM	9.7 (1.1)	16.8 (1.5)	15.9 (2.3)	9.3 (0.9) ²	9.7 (1.4)
	D	9.4 (0.7)	51.9 (1.8)**	40.5 (4.9)**	13.4 (0.7) ^{2,22}	14.4 (1.8)
	DL	7.2 (0.3)	29.6 (2.0)**	30.2 (3.3)**	8.4 (0.8) ^{2,22}	9.6 (0.5)
	VL	9.1 (1.0)	19.7 (1.1)**	17.2 (1.1)**	10.6 (1.3) ^{2,22}	10.3 (0.8)
Middle	DM	8.9 (0.8)	20.0 (2.4)**	15.0 (1.9)*	10.8 (0.8) ¹¹	11.1 (0.9)
	D	6.8 (1.3)	49.7 (0.5)**	47.4 (2.3)**	19.9 (1.4)** ^{2,22}	16.7 (3.7)*
	L	6.6 (1.2)	20.0 (2.2)**	24.6 (1.4)**	6.6 (0.6) ^{2,22}	6.6 (1.3)
	VL	7.5 (0.7)	18.0 (2.2)**	11.5 (1.1) ¹	9.2 (1.3) ¹¹	6.8 (0.9)
Caudal	DM	5.9 (1.1)	22.9 (4.3)**	17.6 (1.6)**	10.5 (1.5) ¹¹	12.7 (1.3)
	D	7.4 (1.5)	57.5 (2.3)**	53.3 (4.3)**	18.8 (1.8) ^{2,22}	24.1 (4.0)**
	L	4.2 (0.9)	19.6 (2.4)**	21.1 (2.5)**	5.5 (1.2) ^{2,22}	5.6 (1.1)
	V	5.1 (0.9)	6.3 (1.8)	6.6 (1.2)	5.4 (1.2)	6.8 (0.5)
SP						
Rostral	DM	39.6 (2.5)	51.7 (3.5)*	52.9 (2.4)**	50.1 (2.4)*	45.9 (2.1)
	D	65.2 (4.0)	112.8 (7.2)**	98.6 (4.8)**	77.2 (2.2) ^{11,2}	80.2 (5.1)
	DL	83.7 (3.2)	119.0 (9.0)**	110.8 (9.3)*	85.0 (3.2) ²	94.9 (3.1)
	VL	58.2 (6.1)	74.8 (7.3)	68.3 (4.0)	60.6 (2.8)	58.3 (5.6)
Middle	DM	26.3 (1.9)	49.6 (1.1)**	37.2 (1.5)* ¹	37.2 (3.6)*	40.4 (2.4)**
	D	58.7 (1.1)	109.0 (2.8)**	92.2 (5.5)**	70.7 (3.3) ^{11,2}	74.0 (9.2)
	L	86.1 (3.8)	120.2 (2.8)**	115.5 (5.6)**	92.9 (2.4) ²	99.5 (7.0)
	VL	38.9 (2.2)	37.5 (2.5)	41.0 (2.4)	43.5 (1.9)	39.2 (5.5)
Caudal	DM	23.7 (3.3)	40.9 (2.7)**	27.4 (2.2) ¹	25.8 (3.2)	33.2 (2.0)
	D	40.8 (2.4)	93.7 (8.1)**	74.1 (7.0)**	58.1 (4.9) ¹¹	66.3 (5.9)*
	L	78.0 (4.9)	114.8 (9.9)	102.5 (12.5)	80.8 (4.5)	101.4 (7.4)
	V	44.1 (2.0)	29.5 (3.4)	46.4 (4.3)	50.8 (1.7) ¹	44.7 (5.9)
DYN						
Rostral	DM	51.2 (3.6)	57.8 (1.4)	57.6 (1.9)	54.8 (0.9)	57.0 (1.7)
	D	41.2 (1.6)	45.3 (0.7)	63.3 (4.3)** ¹¹	54.9 (2.8)*	53.1 (1.3)*
	DL	40.9 (1.7)	45.5 (2.0)	64.2 (4.6)** ¹¹	57.1 (1.9)**	54.8 (2.5)*
	VL	78.1 (2.3)	78.1 (0.3)	88.8 (2.1)	86.3 (3.3)	85.2 (3.4)
Middle	DM	51.2 (1.9)	57.3 (2.9)	59.1 (2.2)	58.4 (1.5)	60.7 (3.1)
	D	28.9 (0.9)	28.5 (0.4)	50.5 (5.9)** ¹¹	47.4 (3.4)**	43.2 (1.3)*
	L	34.5 (1.6)	34.4 (0.5)	50.9 (5.4)** ¹	44.1 (2.6)	43.5 (1.6)
	VL	65.8 (2.7)	62.5 (2.2)	75.1 (2.9)	69.5 (3.0)	68.8 (3.1)
Caudal	DM	61.5 (2.1)	74.2 (4.8)	77.7 (2.9)**	75.9 (1.7)*	72.3 (3.3)
	D	33.7 (1.6)	37.7 (0.5)	68.8 (7.4)** ¹¹	67.9 (2.2)**	57.9 (3.2)**
	L	40.2 (1.9)	42.1 (4.4)	52.1 (3.0)	44.7 (3.1)	43.9 (2.0)
	V	91.7 (3.5)	93.3 (4.4)	107.7 (3.2)** ¹	99.9 (2.0)	88.5 (1.6) ^{2,22}
ENK						
Rostral	DM	24.2 (2.2)	29.4 (1.8)	26.3 (1.5)	25.6 (1.0)	25.4 (0.7)
	D	38.3 (2.1)	45.9 (0.8)	49.1 (1.7)**	49.3 (1.8)**	42.3 (1.3)
	DL	35.2 (2.9)	38.1 (2.7)	46.8 (2.1)**	43.4 (1.8)	36.6 (1.4) ²
	VL	47.3 (2.5)	42.5 (1.0)	51.6 (2.0)	50.2 (0.6)	47.7 (2.3)
Middle	DM	29.5 (1.5)	33.6 (3.7)	34.9 (1.0)	36.7 (2.5)	31.9 (1.5)
	D	34.8 (0.9)	38.6 (1.1)	46.3 (3.3)**	47.3 (1.5)**	38.1 (1.6) ³
	L	34.5 (2.0)	34.9 (1.2)	40.6 (3.3)	38.4 (1.4)	31.7 (2.2)
	VL	51.7 (1.7)	55.3 (6.3)	58.7 (2.2)	56.8 (1.2)	50.9 (1.2)
Caudal	DM	40.3 (1.8)	39.4 (0.9)	46.8 (2.0)	48.0 (3.3)	47.0 (2.0)
	D	38.1 (2.1)	39.8 (3.5)	48.5 (3.9)	53.2 (1.6)**	48.0 (2.9)
	L	38.7 (1.8)	33.9 (1.3)	40.3 (1.8)	40.5 (1.4)	33.1 (1.9) ³
	V	60.0 (3.4)	56.5 (1.6)	65.1 (4.0)	65.6 (4.5)	56.8 (2.8)

Animals were injected with vehicle (0 d group), or with cocaine (30 mg/kg, i.p.) once (1 d group) or twice daily for 2, 3, or 4 d, and were killed 30 min after the last injection. Data represent mean (SEM) OD values measured in dorsomedial (DM), dorsal (D), dorsolateral (DL), lateral (L), ventrolateral (VL), and ventral (V) regions at rostral, middle, and caudal striatal levels. Data were analyzed with ANOVA followed by Tukey–Kramer tests. Indicated are all significant differences between cocaine-treated groups and vehicle controls, and in most cases, significant differences between consecutive cocaine treatment days only. *, versus 0 d group; 1, versus 1 d group; 2, versus 2 d group; 3, versus 3 d group. **, ^{11,22,33}, $p < 0.01$; *, ^{1,2,3}, $p < 0.05$.

Table 3. Regional variations in basal expression of substance P (SP) and dynorphin (DYN) mRNA and in peak induction by cocaine of *c-fos*, dynorphin, and substance P mRNA in striatum

Rostral						
SP (basal)	DL 83.7	>	D 65.2	=	VL 58.2	> DM 39.6
DYN (basal) ^a	DL 40.9	=	D 41.2	<	DM 51.2	<< VL 78.1
<i>c-fos</i> (peak)	D 42.5	>>	DL 22.4	>>	VL 10.6	= DM 7.1
DYN (peak)	DL 1.57	=	D 1.54	>	VL 1.14	= DM 1.13
SP (peak)	D 1.73	=	DL 1.42	=	DM 1.31	= VL 1.29
Middle						
SP (basal)	L 86.1	>>	D 58.7	>>	VL 38.9	> DM 26.3
DYN (basal) ^a	D 28.9	=	L 34.5	<<	DM 51.2	<< VL 65.8
<i>c-fos</i> (peak)	D 42.9	>>	L 13.4	=	DM 11.0	= VL 10.4
DYN (peak)	D 1.75	=	L 1.47	=	DM 1.15	= VL 1.14
SP (peak)	DM 1.89	=	D 1.86	>>	L 1.40	>> VL 0.96
Caudal						
SP (basal)	L 78.0	>>	V 44.1	=	D 40.8	> DM 23.7
DYN (basal) ^a	D 33.7	=	L 40.2	<<	DM 61.5	<< V 91.7
<i>c-fos</i> (peak)	D 50.1	>>	DM 17.0	=	L 15.4	> V 1.2
DYN (peak)	D 2.04	>>	L 1.30	=	DM 1.26	= V 1.17
SP (peak)	D 2.30	=	DM 1.73	=	L 1.47	> V 0.67

Basal expression (basal) was determined in animals that had received repeated vehicle injections. Peak induction (peak) was defined as follows: for dynorphin, as expression in the 2 d group divided by basal expression; for substance P, as expression in the 1 d group divided by basal expression; for *c-fos*, as expression in the 1 d group minus basal expression (see Materials and Methods). Data represent (basal expression), or are based on (peak induction), mean OD values measured in dorsomedial (DM), dorsal (D), dorsolateral (DL), lateral (L), ventrolateral (VL), and ventral (V) striatal areas, at rostral, middle and caudal levels. Data were analyzed with ANOVA followed by Tukey-Kramer tests. >, $p < 0.01$; >, $p < 0.05$; =, not significantly different.

^a Note the inverse rank order for dynorphin basal expression.

Basal levels of dynorphin, substance P, and enkephalin expression were not correlated in the striatal regions examined. The peak induction of *c-fos* was negatively correlated with the basal expression of dynorphin, but was unrelated to the basal expression of substance P or enkephalin. That is, the higher the basal dynorphin expression in a given area, the smaller the *c-fos* response to the first drug application in that area. Similar negative correlations were seen between basal dynorphin expression and peak induction of substance P and dynorphin. On the other hand, a highly significant positive correlation existed between the peak induction of *c-fos* and the peak induction of dynorphin, indicating that a region with a large increase in *c-fos* expression on drug day 1 showed a large increase in dynorphin expression on drug day 2. A positive correlation was also seen between the peak induction of *c-fos* and that of substance P, indicating a regional covariation in *c-fos* and substance P response to the

first cocaine injection, as well as between the peak induction of *c-fos* and that of enkephalin.

Experiment 4: blockade of *c-fos* induction by dynorphin agonist

Cocaine-induced *c-fos* mRNA expression. In the three experiments presented above, we found an inverse relationship between striatal dynorphin and *c-fos* expression induced by cocaine, based on correlative analyses. The fourth experiment reported here describes the first test of a possible causal relationship between dynorphin and *c-fos* expression. We examined the influence of a dynorphin agonist on *c-fos* induction by a single cocaine injection in the region with the strongest *c-fos* response, the dorsal central striatal region, and the cingulate cortex. Figure 10 depicts pooled data from rostral, middle, and caudal striatal levels. The dynorphin agonist spiradoline, a spe-

Figure 4. Basal and cocaine-induced dynorphin and *c-fos* mRNA expression. Dark-field photomicrographs (labeling seen as white dots) of mid-striatal sections show basal dynorphin (*DYN*) expression (day 1; note that dynorphin expression in 1 d group corresponds to basal expression; see Results) (*A*), dynorphin peak expression (after 30 mg/kg cocaine on drug day 2) (*B*), and *c-fos* peak expression [after the first injection of 30 mg/kg cocaine (day 1)] (*C*). Comparison of basal dynorphin (*A*) and *c-fos* mRNA labeling after cocaine (*C*) on adjacent sections reveals the complementary patterns in the distribution of these two mRNAs (arrows indicate the edge of the lateral ventricle). In *D*, high-power dark-field photomicrograph of a region in the dorsal central striatum (from the section in *C*) shows silver grains (white dots) over *c-fos*-labeled cells (arrows).

Figure 5. Regional patterns of basal dynorphin mRNA expression (blue) and *c-fos* mRNA induction by cocaine (red) in striatum. Areas of overlap are pink. The illustration is a composite of adjacent sections at the rostral (A), middle (B), and caudal striatal level (C), from a rat killed 30 min after a single injection of 30 mg/kg cocaine (1 d group; note that dynorphin mRNA levels at this time point reflect basal expression; see Results). The composite image was constructed by thresholding the separate images of dynorphin and *c-fos* labeling at an 80% level, and then combining them. *ac*, anterior commissure.

Figure 6. Influence of cocaine treatment on *c-fos*, dynorphin, substance P, and enkephalin mRNA expression in nucleus accumbens (NAC). OD values (mean + SEM) in nucleus accumbens shell and core regions are given for animals injected with vehicle (0 d group) or with cocaine (30 mg/kg, i.p.) either once (1 d group) or twice daily for 2–4 d, and killed 30 min after the last injection.

cific κ -receptor agonist, blocked cocaine-induced *c-fos* expression in a dose-dependent manner. Administration of 5 and 10 mg/kg, but not the two lower doses, of spiradoline 15 min before the cocaine injection (30 mg/kg) significantly reduced striatal

c-fos induction. In the cingulate cortex, *c-fos* labeling was reduced by 1–10 mg/kg of spiradoline. Spiradoline, in doses that blocked cocaine-induced *c-fos* expression in striatum (5–10 mg/kg), also prevented the behavioral activation (locomotion, rearing, sniffing) produced by the dopamine agonist. While such animals typically maintained a flat body position when left undisturbed, especially with the highest dose, they did orient to sensory stimuli.

Discussion

In the present experiments, the regulation by dopamine of striatal output pathways was analyzed in terms of the effects of the indirect dopamine receptor agonist cocaine on gene expression of *c-fos*, which serves as a marker of neuronal activation, and the neuropeptides dynorphin, substance P, and enkephalin, which serve as markers for striatonigral and striatopallidal neurons. The most important results are the following. (1) Cocaine produces a dose-dependent induction of *c-fos* following acute treatment, and elevation of dynorphin expression following repeated treatment. These changes display distinct regional patterns within the striatum. (2) An inverse relationship exists between dynorphin and cocaine-induced *c-fos* expression, as indicated by (i) the complementary regional patterns of basal dynorphin expression and *c-fos* induction after the first cocaine injection (i.e., striatal regions with low basal dynorphin expression show high levels of *c-fos* induction, and vice versa); (ii) the inverse time course of changes in expression of these two mRNAs during subsequent cocaine treatments (i.e., in areas of maximal initial *c-fos* induction, dynorphin expression increases in parallel to decreasing *c-fos* induction); and (iii) the ability of the dynorphin agonist spiradoline to block, in a dose-dependent manner, acute cocaine-induced *c-fos* expression. (3) Cocaine treatment also

Figure 7. Basal and cocaine-induced substance P mRNA expression. Dark-field photomicrographs (labeling seen as white dots) of mid-striatal sections depict basal substance P (SP) expression (day 0) (A) and substance P peak expression [after the first injection of 30 mg/kg cocaine (day 1)] (B).

Figure 8. Time course of changes in striatal *c-fos*, dynorphin, substance P, and enkephalin mRNA expression during repeated cocaine treatment. OD values (mean + SEM) in dorsomedial (DM), dorsal (D), lateral (L), and ventrolateral (VL) striatal regions at the middle level are shown for animals injected with cocaine (30 mg/kg, i.p.) either once (1 d group) or twice daily for 2–4 d, or repeatedly with vehicle (0 d group), and killed 30 min after the last injection. Indicated are all significant differences between cocaine-treated groups and vehicle controls, and, in most cases, significant differences between consecutive cocaine treatment days only. Changes in expression of both *c-fos* and substance P mRNA display similar regional and temporal patterns: a maximal induction in the dorsal central region following the first drug application and significantly reduced induction from treatment days 2 or 3 on. In contrast, changes in dynorphin expression follow an inverse time course. That is, the expression is maximally increased on treatment day 2 and remains elevated through day 4 in the region with the strongest response (i.e., in the dorsal central region). The increase in dynorphin expression is limited to the areas with relatively low basal dynorphin expression and the strongest *c-fos* response on treatment day 1. The enkephalin expression displays a minor and transient increase in the dorsal central region. **, $p < 0.01$; *, $p < 0.05$ (ANOVA, Tukey–Kramer test).

produces a marked increase in striatal substance P expression that, in both its regional patterns and time course of induction, is very similar to that of *c-fos* induction. Minor, but statistically significant, increases in enkephalin mRNA levels are also seen, which are similar regionally, but not temporally, to the *c-fos* response. The specific inverse relationship between dynorphin and *c-fos* expression in response to cocaine treatment suggests that dynorphin may be involved in regulating the response of striatonigral neurons to dopamine receptor stimulation.

Altered striatal gene regulation in response to cocaine

Pharmacologic treatment with dopamine agonists provides information about dopamine receptor-mediated regulation of the two main populations of striatal projection neurons, striatonigral and striatopallidal neurons, by the specific effects on levels

of mRNAs they express. For the most part, striatonigral neurons contain dynorphin, substance P and the D_1 dopamine receptor, whereas striatopallidal neurons contain enkephalin and the D_2 dopamine receptor. In the 6-hydroxydopamine-lesioned striatum, dynorphin and substance P (Gerfen et al., 1990), and *c-fos* (Robertson et al., 1990) are regulated in striatonigral neurons by D_1 agonist treatment, whereas enkephalin is regulated by D_2 agonist treatment (Gerfen et al., 1990). The patterns of altered gene expression observed in the present study are consistent with a predominant effect of cocaine on striatonigral neurons through a D_1 receptor-mediated process. First, as reported here and elsewhere (Sivam, 1989; Smiley et al., 1990; Hurd et al., 1992), cocaine produces an elevation of both dynorphin and substance P, which are contained in striatonigral neurons. Second, previous studies have demonstrated that cocaine admin-

Figure 9. Regional correlations between basal expression and peak induction by cocaine of *c-fos*, dynorphin, and substance P mRNA in striatum. Scatter plots depict correlations (or lack of) between basal substance P (SP) and basal dynorphin (DYN) expression (A), and between *c-fos* peak induction and basal dynorphin expression (B), dynorphin peak induction (C), basal substance P expression (D), and substance P peak induction (E) in the following 14 striatal areas. Rostral level: dorsomedial (1), dorsal central (2), dorsolateral (3), ventrolateral (4), nucleus accumbens shell (5) and core (6). Middle level: dorsomedial (7), dorsal central (8), lateral (9), ventrolateral (10). Caudal level: dorsomedial (11), dorsal central (12), lateral (13), ventral (14). Data represent, for basal expression, mean OD values from vehicle-treated animals (0 d group), and for peak induction, expression on day 1 minus basal expression (*c-fos*), expression on day 2 divided by basal expression (dynorphin), or expression on day 1 divided by basal expression (substance P). Values are indicated by the position of the area's number on the scatter plot. This analysis shows the *negative correlation* between basal dynorphin expression and *c-fos* peak induction, the *positive correlations* between *c-fos* peak induction and dynorphin peak induction, and substance P peak induction, as well as *no regional correlation* between basal substance P expression and basal dynorphin expression, and *c-fos* peak induction. *r*, Pearson's correlation coefficient. **, $p < 0.01$; *, $p < 0.05$ (*t* test).

Table 4. Correlations between basal expression and peak induction by cocaine of *c-fos*, dynorphin, substance P, and enkephalin mRNA in the 14 striatal regions

	Basal			Peak			
	Dynorphin	Substance P	Enkephalin	<i>c-fos</i>	Dynorphin	Substance P	Enkephalin
Basal							
Dynorphin	—						
Substance P	-0.42	—					
Enkephalin	0.53	-0.03	—				
Peak							
<i>c-fos</i>	-0.70*	0.20	-0.03	—			
Dynorphin	-0.72*	0.32	-0.07	0.91**	—		
Substance P	-0.73*	-0.04	-0.33	0.81**	0.72*	—	
Enkephalin	-0.64	0.19	-0.15	0.77*	0.77*	0.60	—

The correlation analysis was performed between basal expression of each neuropeptide (basal) and peak induction of these neuropeptides (peak) in each of the 14 striatal regions examined. Basal expression was defined as expression in vehicle controls. Peak induction was defined as follows: for dynorphin, as expression in the 2 d group divided by basal expression; for substance P, as expression in the 1 d group divided by basal expression; for *c-fos*, as expression in the 1 d group minus basal expression. Values are presented as *r*, Pearson's correlation coefficient. **, $p < 0.01$; *, $p < 0.05$ (*t* test).

Figure 10. Dose-dependent blockade of cocaine-induced *c-fos* mRNA expression by the dynorphin agonist spiradoline. OD values (mean + SEM) in the dorsal central striatum and in the cingulate cortex are depicted for groups of rats that had received an injection of 0–10 mg/kg of spiradoline (U-62066) 15 min prior to cocaine administration (30 mg/kg). The values presented are means from rostral, middle, and caudal striatal sections. The injection of the two higher doses (5 and 10 mg/kg) of spiradoline blocks completely the behavioral activation induced by cocaine (locomotion, sniffing, rearing) and reduces significantly striatal *c-fos* induction by this drug. The *c-fos* signal in the cingulate cortex is also decreased by the dynorphin agonist. **, $p < 0.01$, compared to controls [0 mg/kg (vehicle) + cocaine] (ANOVA, Dunnett's test).

istration induces Fos protein principally in striatal neurons retrogradely labeled from the substantia nigra (Cenci et al., 1992). Third, several studies have shown that *c-fos* expression produced by cocaine or other dopamine reuptake inhibitors is blocked by D_1 antagonist treatment (Graybiel et al., 1990; Young et al., 1991; Morelli et al., 1992). Finally, in the present study, the time course and regional patterns of *c-fos* induction parallel those of altered substance P mRNA expression. While these data all point to a predominant effect of cocaine on gene regulation in striatonigral neurons, the small, yet significant, elevation of enkephalin expression reported here suggests some influence on striatopallidal neurons as well. As pointed out, the changes in gene regulation after cocaine treatment are consistent with a principal action of this drug on dopamine transmission (see also Sivam, 1989). However, cocaine also affects reuptake of other monoamines, and it is therefore possible that a non-dopaminergic effect contributes to the changes in gene expression observed here.

As discussed, it is reasonable to conclude that cocaine treatment activates D_1 dopamine receptors on striatonigral neurons to elevate *c-fos*, substance P, and dynorphin mRNA levels in these neurons. However, the present data suggest that these genes are differentially regulated. For example, distinct regional patterns in the basal expression of substance P and dynorphin exist. In addition, whereas both *c-fos* and substance P mRNA levels are elevated within 30 min after a single injection of cocaine, the elevation of dynorphin mRNA levels is delayed. Such a dissociation is also observed at later time points during repeated cocaine treatment, in that *c-fos* and substance P mRNA levels parallel one another in declining, while dynorphin mRNA levels remain elevated. Thus, in individual striatonigral neurons the two neuropeptide genes encoding substance P and dynorphin appear to be under different regulatory control.

Inverse relationship between cocaine-induced c-fos and dynorphin expression

In the present study, the regional patterns of *c-fos* induction are shown to be inversely related to basal levels of dynorphin mRNA expression. Rank ordering striatal regions by their basal dynorphin expression shows that ventrolateral and dorsomedial regions express significantly higher levels of dynorphin mRNA than dorsal central and lateral regions. This pattern is complementary to the rank ordering of levels of *c-fos* induction in response to a single cocaine treatment. Thus, *c-fos* induction is greatest in dorsal and dorsolateral regions and significantly lower in the ventrolateral and dorsomedial striatum. Mapping the distribution of the striatal regions showing the highest basal levels of dynorphin expression with the regions in which *c-fos* is induced by a single cocaine injection illustrates these complementary patterns (Fig. 5). The specificity of this relationship is provided by the lack of a similar relationship between basal substance P mRNA levels and *c-fos* induction patterns. Further evidence for the inverse relationship between *c-fos* induction and dynorphin expression is provided by the temporal patterns of changes in these mRNAs following repeated injections of cocaine. Thus, with successive daily injections of this drug, dynorphin mRNA levels increase, and *c-fos* mRNA levels decrease in the dorsal and lateral striatal regions, regions in which basal dynorphin expression is lowest and *c-fos* induction is highest after a single cocaine treatment. On the other hand, levels of substance P mRNA appear to parallel both regionally and temporally the initial increase and subsequent decrease in *c-fos* induction.

These data suggest that dynorphin acts to control the response of striatal neurons, as indicated by the induction of *c-fos* mRNA, to dopamine stimulation. According to such a hypothesis, neurons in regions with high basal expression of dynorphin would show a limited response to dopamine stimulation. Neurons in regions with relatively low basal dynorphin expression would show a greater response. This greater response would trigger increased expression and release of dynorphin, which, in turn, would downregulate the response to future dopamine stimulation. Presently, it is not certain that chronic cocaine treatment produces changes in striatal dynorphin release. However, it has been shown that increased dynorphin mRNA levels are accompanied by increased dynorphin peptide levels in striatum after treatment with cocaine or other dopamine agonists (Hanson et al., 1988; Li et al., 1988; Sivam, 1989; Gerfen et al., 1991). In addition, our finding of a blockade of cocaine-induced *c-fos* expression by systemic application of the dynorphin agonist spiradoline—while inconclusive in terms of site of action—is consistent with this proposed role of dynorphin. Moreover, recent results by Engber et al. (1991), which showed that spiradoline inhibits D_1 (but not D_2) agonist-induced rotational behavior after a nigrostriatal lesion, further support this hypothesis.

There are several possible ways by which dynorphin could control the responsiveness of striatal neurons. (1) Dynorphin may exert an inhibitory effect on dopamine release through presynaptic mechanisms. Dynorphin is a κ -opioid receptor agonist (Chavkin et al., 1982; Corbett et al., 1982), and κ -receptor binding is present throughout the striatum (Mansour et al., 1987; Tempel and Zukin, 1987). *In vivo* and *in vitro* studies have reported dynorphin- or κ -agonist-induced reduction of dopamine release within the striatum, suggesting a presynaptic localization of κ -receptors on nigrostriatal afferent terminals

(Mulder et al., 1984; Broderick, 1987; Di Chiara and Imperato, 1988; Werling et al., 1988). However, the minimal reduction of striatal κ -receptor binding that occurs after destruction of nigrostriatal dopamine terminals (Eghbali et al., 1987) argues against such presynaptic receptor localization. (2) Gauchy et al. (1991) have reported that dynorphin and κ -agonists inhibit ACh-mediated dopamine release within the striatum, which raises the possibility of cholinergic involvement. (3) Another presynaptic mechanism might involve striatal dynorphin acting to reduce corticostriatal input. Findings from studies of the hippocampus indicate that dynorphin may be released by dentate granule cells to presynaptically reduce glutamate input from the cortex to the dentate gyrus (Wagner et al., 1992). A similar role for dynorphin in the striatum is conceivable. Since striatal neurons are driven by cortical input (and dopamine is thought to modulate this input), presynaptic inhibition of cortical input would provide a very powerful mechanism to control striatal excitability. However, such an interaction remains to be demonstrated. (4) Alternatively, dynorphin could influence the response of striatal neurons by postsynaptic mechanisms, such as the control of ion channels and/or adenylate cyclase activity. Opioid receptors are coupled through G-proteins to second messenger systems and ion channels (West and Miller, 1983; Childers, 1991). Dynorphin and κ -agonists have been shown to inhibit Ca^{2+} influx (e.g., Gross and Macdonald, 1987; Attali et al., 1989a) and reduce adenylate cyclase activity in various neuronal tissues including striatal neurons (Gentleman et al., 1983; Attali et al., 1989b; Eriksson et al., 1990; but see Schoffelemeier et al., 1988). Since Ca^{2+} and cAMP levels are involved in the regulation of, for example, *c-fos* induction (see below), activation of postsynaptic κ -receptors could thus also provide a direct mechanism for dynorphin to control gene expression.

Therefore, there are several potential mechanisms by which dynorphin could downregulate the responsiveness of striatal neurons. Consistent with this hypothesis, chronic amphetamine treatment, which also produces increased striatal dynorphin expression (Hanson et al., 1987, 1988; Li et al., 1988), has been found to render striatal neurons subsensitive to dopaminergic input (Kamata and Rebec, 1985).

Functional significance

Studies of altered gene regulation in striatonigral neurons following a variety of pharmacologic manipulations have consistently shown that stimulation of D_1 receptors on these neurons increases levels of mRNAs encoding Fos and the neuropeptides dynorphin and substance P. In order to relate these changes to function, it is necessary to consider what these specific alterations in mRNA levels reflect in the context of the pharmacologic manipulations employed.

The induction of *c-fos* and other immediate-early genes in neurons is thought to reflect increased neuronal activity. This is based on findings that a number of experimental manipulations that activate neuronal systems, including drug application, electrical stimulation, and sensory stimulation, lead to a rapid and transient induction of *c-fos*, as well as on findings that depolarization and elevated intracellular levels of cAMP and Ca^{2+} increase *c-fos* expression in many brain systems (for reviews, see Morgan and Curran, 1989, 1991; Sheng and Greenberg, 1990). Therefore, it has been suggested that *c-fos* mRNA or the Fos protein could serve as a marker for mapping neuronal activity and tracing functional pathways (Sagar et al., 1988; Dragunow and Faull, 1989).

In the dopamine-depleted striatum, D_1 receptor activation produces *c-fos* induction in striatonigral neurons (Robertson et al., 1990, 1992). In contrast, *c-fos* induction in striatopallidal neurons results from D_2 receptor blockade in the normally innervated striatum (Dragunow et al., 1990; Robertson et al., 1992), probably due to release from the presumed tonic inhibition dopamine exerts through the D_2 receptor on these neurons. Thus, the induction of *c-fos* appears to reflect an excitatory response of striatal neurons. After a single high dose of cocaine, the temporal pattern of striatal *c-fos* mRNA induction parallels closely the increased extracellular dopamine levels in the striatum (e.g., Kuczenski and Segal, 1992) as well as behavioral activation (e.g., Kalivas and Duffy, 1993), with *c-fos* mRNA, dopamine levels, and behavioral activation reaching peak levels by 20–40 min and returning to basal levels by 90 min to 2 hr after the injection (H. Steiner and C. R. Gerfen, unpublished observations). Since *c-fos* levels are very low in the normal striatum, the induction of *c-fos* by cocaine treatment therefore presumably reflects the increase in dopamine activation of D_1 receptors on striatonigral neurons.

On the other hand, the present results demonstrate that other mRNAs, specifically dynorphin, display a temporal pattern of elevation that is not tightly correlated with *c-fos* mRNA levels and therefore not with the level of activation of striatonigral neurons. It is suggested that such changes in gene regulation, that appear with a delay and persist beyond the period of direct drug action, reflect a compensatory response—in the case of dynorphin in striatonigral neurons, to regulate the responsiveness of those neurons to subsequent dopamine stimulation. This is supported by the reduction in cocaine-induced *c-fos* as dynorphin levels increase over days and by the ability of the dynorphin agonist to block acute cocaine-induced *c-fos* expression.

The regional variations in the basal and drug-induced levels of mRNAs expressed by striatal neurons provide further information about the functional organization of the striatum. First, as shown here, there are significant differences between striatal regions in the basal levels of dynorphin and substance P expression. Since the levels of dynorphin and substance P mRNA are regulated, at least in part, by D_1 dopamine receptor-mediated mechanisms (Gerfen et al., 1990), regional differences in basal levels may reflect normal differences in dopamine neurotransmission and/or interactions with other transmitter systems.

Second, the present results—by demonstrating different basal levels of dynorphin mRNA and a differential influence of cocaine treatment on gene regulation—especially suggest functional differences between dorsal and ventral striatal regions. The hypothesis presented here, that dynorphin levels reflect a compensatory mechanism to limit the response of neurons to dopamine neurotransmission, might lead to the prediction of greater basal dopamine release in areas with higher basal dynorphin expression. While this might be the case in some striatal regions (e.g., patch vs. matrix in the dorsal striatum), the situation is more complex with respect to dorsal–ventral differences. In fact, a comparison of extracellular dopamine levels between the dorsal central striatum, which displays low basal dynorphin expression, and the ventral striatum/nucleus accumbens, with high basal dynorphin expression, shows results opposite to the prediction: higher basal dopamine levels in the dorsal striatum than in the nucleus accumbens (Kuczenski and Segal, 1992). However, these regions also differ in the distribution of the dopamine transporter, which functions to remove dopamine

from the extracellular space. Recent studies have shown that the density of the dopamine transporter is higher in the dorsal than in the ventral striatum (Richfield, 1991; Boja et al., 1992). Thus, although there may be more dopamine released in the dorsal striatum, the dopamine transporter could conceivably limit its action in this region, whereas in the ventral striatum, lower levels of dopamine release could have a greater (prolonged) action, owing to the relatively lower levels of the transporter. This could result in higher levels of basal dynorphin expression in the ventral striatum. Cocaine is thought to block the dopamine transporter (Heikkilä et al., 1975; Taylor and Ho, 1978; Missale et al., 1985), and the dorsally restricted c-fos induction after cocaine treatment may reflect, in part, the distribution pattern of the transporter. However, cocaine is not without effect on dopamine levels in the ventral striatum. In fact, the relative increase in dopamine levels produced by cocaine is even greater in the ventral striatum (Di Chiara and Imperato, 1988; Kuczenski and Segal, 1992), due to the lower basal level in this region (Kuczenski and Segal, 1992). Based on the present data, it would be suggested that the higher basal dynorphin levels in the ventral striatum blunt the neuronal response to cocaine in this region, which would lead to a reduced effect on gene regulation.

Conclusions

Previous analysis of pharmacologically altered gene regulation has shown that the induction of c-fos or the elevation of neuropeptide mRNA levels in striatonigral neurons occurs through the activation of D₁ dopamine receptors expressed by these neurons. The present results suggest that changes in the expression of c-fos and substance P are an immediate response to receptor-mediated activation, and that changes in dynorphin expression are a compensatory response that regulates the responsiveness to dopamine in striatonigral neurons.

References

- Attali B, Saya D, Nah S-Y, Vogel Z (1989a) κ opiate agonists inhibit Ca²⁺ influx in rat spinal cord-dorsal root ganglion cocultures. *J Biol Chem* 264:347–353.
- Attali B, Saya D, Vogel Z (1989b) κ -opiate agonists inhibit adenylate cyclase and produce heterologous desensitization in rat spinal cord. *J Neurochem* 52:360–369.
- Bannon MJ, Elliott PJ, Bunney EB (1987) Striatal tachykinin biosynthesis: regulation of mRNA and peptide levels by dopamine agonists and antagonists. *Mol Brain Res* 3:31–37.
- Beckstead RM, Kersey KS (1985) Immunohistochemical demonstration of differential substance P-, met-enkephalin-, and glutamic acid decarboxylase-containing cell and axon distributions in the corpus striatum of the cat. *J Comp Neurol* 232:481–498.
- Boja JW, Mitchell WM, Patel A, Kopajtic TA, Carroll FI, Lewin AH, Abraham P, Kuhar MJ (1992) High-affinity binding of [¹²⁵I]RTI-55 to dopamine and serotonin transporters in rat brain. *Synapse* 12:27–36.
- Broderick PA (1987) Striatal neurochemistry of dynorphin-(1-13): *in vivo* electrochemical semidifferential analyses. *Neuropeptides* 10:369–386.
- Brownstein MJ, Mroz EA, Tappaz ML, Leeman SE (1977) On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. *Brain Res* 135:315–323.
- Cenci MA, Campbell K, Wictorin K, Björklund A (1992) Striatal c-fos induction by cocaine or apomorphine occurs preferentially in output neurons projecting to the substantia nigra in the rat. *Eur J Neurosci* 4:376–380.
- Chavkin C, James IF, Goldstein A (1982) Dynorphin is a specific endogenous ligand of the kappa opioid receptor. *Science* 215:413–415.
- Childers SR (1991) Opioid receptor-coupled second messenger systems. *Life Sci* 48:1991–2003.
- Corbett AD, Paterson SJ, McKnight AT, Magnan J, Kosterlitz HW (1982) Dynorphin₁₋₈ and dynorphin₁₋₉ are ligands for the κ -subtype of opiate receptor. *Nature* 299:79–81.
- Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278.
- Dragunow M, Faull R (1989) The use of c-fos as a metabolic marker in neuronal pathway tracing. *J Neurosci Methods* 29:261–265.
- Dragunow M, Robertson GS, Faull RLM, Robertson HA, Jansen K (1990) D₂ dopamine receptor antagonists induce Fos and related proteins in rat striatal neurons. *Neuroscience* 37:287–294.
- Eghbali M, Santoro C, Paredes W, Gardner EL, Zukin RS (1987) Visualization of multiple opioid-receptor types in rat striatum after specific mesencephalic lesions. *Proc Natl Acad Sci USA* 84:6582–6586.
- Engber TM, Boldry RC, Chase TN (1991) The κ -opioid receptor agonist spiradoline differentially alters the rotational response to dopamine D₁ and D₂ agonists. *Eur J Pharmacol* 200:171–173.
- Eriksson PS, Hansson E, Ronnback L (1990) Opiate receptors in neuronal primary cultures. *Neuropharmacology* 29:799–804.
- Gauchy C, Desban M, Krebs MO, Glowinski J, Keme ML (1991) Role of dynorphin-containing neurons in the presynaptic inhibitory control of the acetylcholine-evoked release of dopamine in the striosomes and the matrix of the cat caudate nucleus. *Neuroscience* 41:449–458.
- Gentleman S, Parenti M, Neff NH, Pert CB (1983) Inhibition of dopamine-activated adenylate cyclase and dopamine binding by opiate receptors in rat striatum. *Cell Mol Neurobiol* 3:17–26.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. *Annu Rev Neurosci* 15:285–320.
- Gerfen CR, Young WS III (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an *in situ* hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res* 460:161–167.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D₁ and D₂ dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250:1429–1432.
- Gerfen CR, McGinty JF, Young WS III (1991) Dopamine differentially regulates dynorphin, substance P and enkephalin expression in striatal neurons: *in situ* hybridization histochemical analysis. *J Neurosci* 11:1016–1031.
- Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc Natl Acad Sci USA* 87:6912–6916.
- Gross RA, Macdonald RL (1987) Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. *Proc Natl Acad Sci USA* 84:5469–5473.
- Hanson GR, Merchant KM, Letter AA, Bush L, Gibb JW (1987) Methamphetamine-induced changes in the striatal-nigral dynorphin system: role of D-1 and D-2 receptors. *Eur J Pharmacol* 144:245–246.
- Hanson GR, Merchant KM, Letter AA, Bush L, Gibb JW (1988) Characterization of methamphetamine effects on the striatal-nigral dynorphin system. *Eur J Pharmacol* 155:11–18.
- Heikkilä RE, Orlansky HO, Cohen G (1975) Studies on the distinction between uptake inhibition and release of [³H]dopamine in rat brain tissue slices. *Biochem Pharmacol* 24:847–852.
- Hong JS, Yang H-YT, Fratta W, Costa E (1978) Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and noncataleptogenic drugs. *J Pharmacol Exp Ther* 205:141–147.
- Hurd YL, Brown EE, Finlay JM, Fibiger HC, Gerfen CR (1992) Cocaine self-administration differentially alters mRNA expression of striatal peptides. *Mol Brain Res* 13:165–170.
- Kalivas PW, Duffy P (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. *J Neurosci* 13:266–275.
- Kamata K, Rebec GV (1985) Iontophoretic evidence for subsensitivity of postsynaptic dopamine receptors following long-term amphetamine administration. *Eur J Pharmacol* 106:393–397.
- Kawaguchi Y, Wilson CJ, Emson PC (1990) Projection subtypes of

- rat neostriatal matrix cells revealed by intracellular injection of biocytin. *J Neurosci* 10:3421-3438.
- Kemp JM, Powell TPS (1971) The structure of the caudate nucleus of the cat: light and electron microscopy. *Philos Trans R Soc Lond [Biol]* 262:383-401.
- Kita H, Kitai ST (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. *Brain Res* 447:346-352.
- Kuczenski R, Segal DS (1992) Differential effects of amphetamine and dopamine uptake blockers (cocaine, nomifensine) on caudate and accumbens dialysate dopamine and 3-methoxytyramine. *J Pharmacol Exp Ther* 262:1085-1094.
- Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990) Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proc Natl Acad Sci USA* 87:230-234.
- Li SJ, Sivam SP, McGinty JF, Jiang HK, Douglass J, Calavetta L, Hong JS (1988) Regulation of the metabolism of striatal dynorphin by the dopaminergic system. *J Pharmacol Exp Ther* 246:403-408.
- Li SJ, Jiang HK, Stachowiak MS, Hudson PM, Owyang V, Nanry K, Tilson HA, Hong JS (1990) Influence of nigrostriatal dopaminergic tone on the biosynthesis of dynorphin and enkephalin in rat striatum. *Mol Brain Res* 8:219-225.
- Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ (1987) Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. *J Neurosci* 7:2445-2464.
- Missale C, Castelletti L, Govoni S, Spano PF, Trabucchi M, Hanbauer I (1985) Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens. *J Neurochem* 45:51-56.
- Morelli M, Carboni E, Cozzolino A, Tanda GL, Pinna A, Di Chiara G (1992) Combined microdialysis and Fos immunohistochemistry for the estimation of dopamine neurotransmission in the rat caudate-putamen. *J Neurochem* 59:1158-1160.
- Morgan JI, Curran T (1989) Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. *Trends Neurosci* 12:459-462.
- Morgan JI, Curran T (1991) Proto-oncogene transcription factors and epilepsy. *Trends Pharmacol Sci* 12:343-349.
- Mulder AH, Wardeh G, Hogenboom F, Frankhuysen AL (1984) κ - and δ -opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. *Nature* 308:278-280.
- Paxinos G, Watson C (1982) The rat brain in stereotaxic coordinates. New York: Academic.
- Richfield EK (1991) Quantitative autoradiography of the dopamine uptake complex in rat brain using [3 H]GBR 12935: binding characteristics. *Brain Res* 540:1-13.
- Robertson GS, Vincent SR, Fibiger HC (1990) Striatonigral projection neurons contain D, dopamine receptor-activated *c-fos*. *Brain Res* 523:288-290.
- Robertson GS, Vincent SR, Fibiger HC (1992) D₁ and D₂ dopamine receptors differentially regulate *c-fos* expression in striatonigral and striatopallidal neurons. *Neuroscience* 49:285-296.
- Sagar SM, Sharp FR, Curran T (1988) Expression of *c-fos* protein in brain: metabolic mapping at the cellular level. *Science* 240:1328-1331.
- Schoffelmeer ANM, Rice KC, Jacobson AE, Van Gelderen JG, Hogenboom F, Heijna MH, Mulder AH (1988) μ -, δ - and κ -opioid receptor-mediated inhibition of neurotransmitter release and adenylate cyclase activity in rat brain slices: studies with fentanyl isothiocyanate. *Eur J Pharmacol* 154:169-178.
- Sheng M, Greenberg ME (1990) The regulation and function of *c-fos* and other immediate early genes in the nervous system. *Neuron* 4:477-485.
- Sivam SP (1989) Cocaine selectively increases striatonigral dynorphin levels by a dopaminergic mechanism. *J Pharmacol Exp Ther* 250:818-824.
- Smiley PL, Johnson M, Bush L, Gibb JW, Hanson GR (1990) Effects of cocaine on extrapyramidal and limbic dynorphin systems. *J Pharmacol Exp Ther* 253:938-943.
- Taylor D, Ho BT (1978) Comparison of the inhibition of monoamine uptake by cocaine, methylphenidate and amphetamine. *Res Commun Chem Pathol Pharmacol* 21:67-75.
- Tempel A, Zukin RS (1987) Neuroanatomical patterns of the μ , δ , and κ opioid receptors of rat brain as determined by quantitative *in vitro* autoradiography. *Proc Natl Acad Sci USA* 84:4308-4312.
- Vincent SR, Hökfelt T, Christensson I, Terenius L (1982) Immunohistochemical evidence for a dynorphin immunoreactive striatonigral pathway. *Eur J Pharmacol* 85:251-252.
- Voorn P, Roest G, Groenewegen HJ (1987) Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions. *Brain Res* 412:391-396.
- Wagner JJ, Caudle RM, Chavkin C (1992) κ -opioids decrease excitatory transmission in the dentate gyrus of the guinea pig hippocampus. *J Neurosci* 12:132-141.
- Werling LL, Frattali A, Portoghesi PS, Takemori AE, Cox BM (1988) Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs. *J Pharmacol Exp Ther* 246:282-286.
- West RE, Miller RJ (1983) Opiates, second messengers and cell response. *Br Med Bull* 39:53-58.
- Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal *c-fos*-immunoreactive proteins via dopaminergic D₁ receptors. *Proc Natl Acad Sci USA* 88:1291-1295.
- Young WS III, Bonner TI, Brann MR (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. *Proc Natl Acad Sci USA* 83:9827-9831.
- Zaborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M (1985) Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. *Neuroscience* 14:427-453.