

Topography of Methylphenidate (Ritalin)-Induced Gene Regulation in the Striatum: Differential Effects on c-Fos, Substance P and Opioid Peptides

Motoyo Yano¹ and Heinz Steiner^{*,1}

¹Department of Cellular and Molecular Pharmacology, Rosalind Franklin University of Medicine and Science/The Chicago Medical School, North Chicago, IL, USA

Dopamine action alters gene regulation in striatal neurons. Methylphenidate increases extracellular levels of dopamine. We investigated the effects of acute methylphenidate treatment on gene expression in the striatum of adult rats. Molecular changes were mapped in 23 striatal sectors mostly defined by their predominant cortical inputs in order to determine the functional domains affected. Acute administration of 5 and 10 mg/kg (i.p.) of methylphenidate produced robust increases in the expression of the transcription factor c-fos and the neuropeptide substance P. Borderline effects were found with 2 mg/kg, but not with 0.5 mg/kg. For 5 mg/kg, c-fos mRNA levels peaked at 40 min and returned to baseline by 3 h after injection, while substance P mRNA levels peaked at 40–60 min and were back near control levels by 24 h. These molecular changes occurred in most sectors of the caudate-putamen, but were maximal in dorsal sectors that receive sensorimotor and medial agranular cortical inputs, on middle to caudal levels. In rostral and ventral striatal sectors, changes in c-fos and substance P expression were weaker or absent. No effects were seen in the nucleus accumbens, with the exception of c-fos induction in the lateral part of the shell. In contrast to c-fos and substance P, acute methylphenidate treatment had minimal effects on the opioid peptides dynorphin and enkephalin. These results demonstrate that acute methylphenidate alters the expression of c-fos and substance P preferentially in the sensorimotor striatum. These molecular changes are similar, but not identical, to those produced by other psychostimulants.

Neuropsychopharmacology (2005) **30**, 901–915, advance online publication, 22 December 2004; doi:10.1038/sj.npp.1300613

Keywords: immediate-early genes; dopamine; methylphenidate; neuropeptide; psychostimulant; Ritalin; striatum

INTRODUCTION

Psychostimulants such as cocaine and amphetamine produce changes in gene regulation in striatal projection neurons (Harlan and Garcia, 1998; Torres and Horowitz, 1999). These molecular alterations are thought to result in functional changes in the affected basal ganglia-cortical circuits and are implicated in drug-induced behavioral changes (Berke and Hyman, 2000; Graybiel *et al*, 2000). Psychostimulant-induced gene regulation in the striatum is related to excessive stimulation of dopamine receptors (Hyman and Malenka, 2001; Nestler, 2001). Methylphenidate (Ritalin) is a psychostimulant that is widely used in the

treatment of attention-deficit hyperactivity disorder in adolescents and adults, and is also diverted for recreational purposes (Kollins *et al*, 2001; Swanson and Volkow, 2003). Methylphenidate binds and inhibits the dopamine transporter (Schwartz *et al*, 1985; Gattley *et al*, 1996) and produces dopamine overflow in the striatum (Hurd and Ungerstedt, 1989; Butcher *et al*, 1991; Kuczenski and Segal, 1997; Gerasimov *et al*, 2000; Volkow *et al*, 2001). In addition, this psychostimulant inhibits norepinephrine reuptake (Kuczenski and Segal, 1997) and affects vesicular monoamine transport (Sandoval *et al*, 2002). Although relatively little is known regarding methylphenidate's propensity to induce neuronal alterations, a number of studies demonstrated that repeated methylphenidate treatment in animal models causes behavioral changes similar to other psychostimulants. These include increased psychomotor activation by a subsequent psychostimulant challenge (behavioral sensitization) (eg Shuster *et al*, 1982; Gaytan *et al*, 1997; Crawford *et al*, 1998; McDougall *et al*, 1999; Brandon *et al*, 2001), place preference conditioning (Martin-Iverson *et al*, 1985; Meririnne *et al*, 2001; Andersen *et al*, 2002), enhanced cocaine self-administration (Brandon *et al*, 2001; Schenk

*Correspondence: Dr H Steiner, Department of Cellular and Molecular Pharmacology, Rosalind Franklin University of Medicine and Science/The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064, USA, Tel: +1 847 578 8679; Fax: +1 847 578 3268, E-mail: Heinz.Steiner@rosalindfranklin.edu
Received 15 June 2004; revised 9 September 2004; accepted 5 October 2004
Online publication: 10 October 2004 at <http://www.acnp.org/citations/Npp101104040279/default.pdf>

and Izenwasser, 2002), as well as several other behavioral changes (Bolanos *et al*, 2003; Carlezon *et al*, 2003). These behavioral effects indicate that methylphenidate can produce enduring neuronal changes.

In a recent study, we investigated whether a methylphenidate treatment regimen that resulted in psychomotor sensitization and enhanced cocaine self-administration in adolescent rats (Brandon *et al*, 2001) would alter gene regulation in striatal neurons. Our results showed that repeated treatment with 10 mg/kg once daily for 7 days produced increased expression of the neuropeptide dynorphin and attenuated the inducibility of immediate-early genes (*c-fos*, *zif 268*) and substance P in the striatum (Brandon and Steiner, 2003). These effects were principally similar to those of repeated cocaine and amphetamine treatments (see Brandon and Steiner, 2003; Willuhn *et al*, 2003, for discussion). Substance P and dynorphin are contained in neurons of the direct striatal output pathway (striatonigral neurons) (Gerfen and Young, 1988; Graybiel, 1990; Gerfen and Wilson, 1996), and our results thus suggested that repeated methylphenidate treatment produces molecular alterations in the direct pathway.

In the present study, we further characterized molecular changes in the striatum produced by methylphenidate. We investigated effects of acute methylphenidate treatment in adult rats for comparison with the wealth of findings on acute molecular effects of psychostimulants such as cocaine and amphetamine (Harlan and Garcia, 1998; Torres and Horowitz, 1999). Acute effects are often predictive of long-term molecular changes in terms of location and magnitude (eg Brandon and Steiner, 2003; Willuhn *et al*, 2003). Of the few studies that have examined effects of acute methylphenidate on striatal gene expression, most focused on *c-fos* induction (Lin *et al*, 1996; Acheson *et al*, 2001; Penner *et al*, 2002; Brandon and Steiner, 2003; Chase *et al*, 2003). Acute effects on the expression of neuropeptides in the striatum have so far not been investigated. We determined and compared dose-response relationships and time courses for changes in the expression of *c-fos* and the neuropeptides substance P and dynorphin (direct pathway), and enkephalin (indirect pathway) after acute methylphenidate administration. Moreover, in order to assess whether such molecular alterations are associated with particular cortico-basal ganglia-cortical circuits (functional domains), we mapped the topography of these effects by using 23 sampling areas (sectors) defined mostly by their predominant cortical inputs (Willuhn *et al*, 2003). These results were compared with those of our recent study on the topography of cocaine-induced gene regulation in the striatum (Willuhn *et al*, 2003). Preliminary findings of this work were presented in abstract form (Yano and Steiner, 2003).

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats (210–300 g) were housed two per cage under standard laboratory conditions (12 h light:12 h dark cycle; lights on at 0700). Food and water were available *ad libitum*. The rats were allowed 1 week to acclimate, during which they were handled repeatedly. All procedures met the NIH guidelines for the care and use of laboratory

animals and were approved by the Rosalind Franklin University Animal Care and Use Committee.

Drugs and Injection Procedures

Drug treatments were performed between 1300 and 1700. Rats were kept in their home cage between drug injection and killing. In the dose-response study, rats received an intraperitoneal injection of 0 (vehicle), 0.5, 2, 5, or 10 mg/kg of methylphenidate hydrochloride (generously provided by the National Institute on Drug Abuse; in 0.02% ascorbic acid, 1 ml/kg; $n=6$ each) and were killed 1 h later. In the time-course study, 5 mg/kg of methylphenidate was administered, and the rats were killed either immediately (0 min group), or 20, 40 min, 1, 3, or 24 h after the injection ($n=5-6$ each).

Tissue Preparation and *In Situ* Hybridization Histochemistry

The rats were killed with CO₂. The brain was rapidly removed and frozen in isopentane cooled on dry ice. Brains were stored at -20°C until they were sectioned on a cryostat. Coronal sections (12 μm) were thaw-mounted onto glass slides (Superfrost/Plus, Daigger, Wheeling, IL), dried on a slide warmer, and stored at -20°C . Tissue sections were fixed in 4% paraformaldehyde/0.9% saline for 10 min at room temperature, incubated in a fresh solution of 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% saline (pH 8.0) for 10 min, dehydrated, defatted for 2×5 min in chloroform, rehydrated, and air-dried. The slides were then stored at -30°C until hybridization.

Oligonucleotide probes (48-mers; Life Technologies, Rockville, MD) were labeled with [³⁵S]dATP (Steiner and Gerfen, 1993). The probes had the following sequence: *c-fos*, complementary to bases 207–254, GenBank Accession number X06769; dynorphin, bases 862–909, M10088; enkephalin, bases 436–483, M28263; substance P, bases 128–175, X56306. Labeled probe ($\sim 3 \times 10^6$ c.p.m.) in 100 μl of hybridization buffer was added to each slide (Steiner and Kitai, 2000). The sections were coverslipped and incubated at 37°C overnight. After incubation, the slides were first rinsed in four washes of $1 \times$ saline citrate (150 mM sodium chloride, 15 mM sodium citrate). They were then washed 3×20 min each in $2 \times$ saline citrate/50% formamide at 40°C , followed by two washes of 30 min each in $1 \times$ saline citrate at room temperature. After a brief water rinse, the sections were air-dried and then apposed to X-ray film (BioMax MR-2, Kodak). The film exposure times were on average 4 (enkephalin, substance P), 9 (dynorphin), and 14 days (*c-fos*).

Analysis of Autoradiograms

Gene expression in the striatum was assessed on three rostrocaudal levels (one section each) (Figure 1): 'Rostral' corresponds to approximately +1.6 mm relative to bregma (Paxinos and Watson, 1998), 'middle' to +0.4 mm, and 'caudal' to -0.8 mm. The rostral striatum was divided into 10, the middle into six, and the caudal into seven sectors, for a total of 18 sectors representing the caudate-putamen and five the nucleus accumbens (Figure 1). These sectors

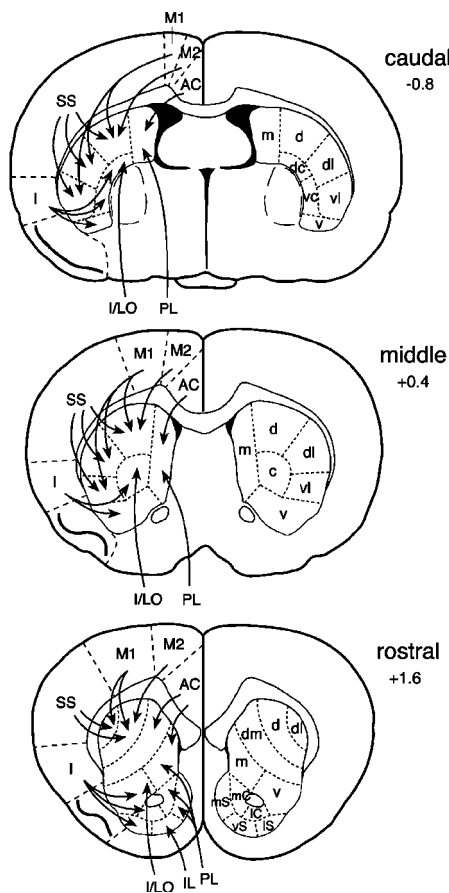


Figure 1 Schematic illustration of the 23 striatal sectors used for sampling gene expression. Gene regulation was assessed in coronal sections from three rostrocaudal levels ('rostral', approximately at +1.6 mm relative to bregma (Paxinos and Watson, 1998); 'middle', +0.4; and 'caudal', -0.8) in a total of 18 sectors representing the caudate-putamen and five sectors representing the nucleus accumbens. These sectors were mostly defined by their predominant cortical inputs, as determined in tract tracing and functional mapping studies (see Materials and methods). These inputs are indicated by arrows. Caudate-putamen: m, medial; dm, dorsomedial; d, dorsal; dl, dorsolateral; vl, ventrolateral; v, ventral; c, central; vc, ventral central; dc, dorsal central; nucleus accumbens: mC, medial core; IC, lateral core; mS, medial shell; vS, ventral shell; IS, lateral shell; cortex: AC, anterior cingulate; M2, medial agranular; M1, motor; SS, somatosensory; I, insular; LO, lateral orbital; IL, infralimbic; PL, prelimbic.

are mostly defined by their predominant cortical inputs (see Willuhn *et al.*, 2003, for a more detailed discussion). The design of these sectors is based on findings of numerous tract tracing (eg Beckstead, 1979; Donoghue and Herkenham, 1986; McGeorge and Faull, 1987, 1989; Reep *et al.*, 1987; Berendse *et al.*, 1992; Brog *et al.*, 1993; Kincaid and Wilson, 1996; Wright *et al.*, 1999; Reep and Corwin, 1999; Hoffer and Alloway, 2001; Reep *et al.*, 2003) and functional mapping studies (eg Carelli and West, 1991; Brown and Sharp, 1995; Brown *et al.*, 1998). However, their outlines are simplified in many ways in order to facilitate reliable sampling, and thus do not describe exact or exclusive target areas of the connections depicted in Figure 1. Moreover, in Figure 1, some corticostriatal connections are omitted for simplicity, including visual cortical input to the dorsal striatum (eg Reep *et al.*, 2003), projections from the piriform and entorhinal cortex to the ventral and medial striatum (eg

McGeorge and Faull, 1989; Brog *et al.*, 1993), converging corticostriatal projections from functionally related cortical areas on different rostrocaudal levels, as well as all interhemispheric projections (eg McGeorge and Faull, 1987, 1989; Wright *et al.*, 2001).

Gene expression was measured by densitometry on film autoradiograms using a Macintosh-based image analysis system (NIH Image, Wayne Rasband, NIMH). The films were captured using a light table (Northern Light, Imaging Research, St. Catharines, Ontario, Canada) and a Sony CCD camera (Imaging Research). The 'mean density' value of a sector was measured by placing a template over the captured image. The mean densities of corresponding sectors in the two hemispheres were averaged after correcting for background by subtracting mean density measured over white matter (corpus callosum). Treatment effects were determined by one-factor ANOVA, followed by two-tailed Dunnett's *post hoc* tests (Superanova software). For correlation analyses and illustrations of topographies, the increase in gene expression in a given sector was expressed as the percentage of the maximal increase (% of max.) in the 23 striatal sectors, for a particular probe. The illustrations of film autoradiograms are computer-generated images, and are contrast-enhanced when necessary. Maximal hybridization signal is black.

RESULTS

Dose-Dependent Induction of *c-fos* and Substance P by Acute Methylphenidate

Acute injection of methylphenidate altered striatal gene expression in a dose-dependent and regionally selective manner (Figures 2 and 3, Table 1). At 1 h after drug administration, statistically significant increases in mRNA levels were found for 5 and 10 mg/kg, but not for 0.5–2 mg/kg. After 10 mg/kg, *c-fos* expression was induced in 14 of the 18 sectors of the caudate-putamen (5 mg/kg: 10/18; Table 1a), but in none of the five sectors of the nucleus accumbens (Figure 3). After 2 mg/kg, there was a minor *c-fos* response in some of the animals (not statistically significant as a group; Table 1a). Induction of *c-fos* was most pronounced in the dorsal sectors, especially at middle to caudal striatal levels (Figure 2), and was generally weaker in the medial and lateral sectors, and weakest or absent in the ventral sectors (Table 1a; see also Figures 4 and 7).

Changes in substance P expression displayed similar characteristics (Figures 2 and 3, Table 1b). At 1 h after injection of 10 mg/kg, substance P mRNA levels were significantly increased in 13 of 18 caudate-putamen sectors (5 mg/kg: 2/18; Table 1b); no changes were seen in the accumbal sectors (Figure 3). The increase in substance P expression was also most robust in the dorsal sectors of the middle and caudal striatum (Figure 2) and weakest or absent in the ventral sectors (see also Figures 4 and 7).

In contrast to *c-fos* and substance P, dynorphin mRNA levels were not significantly changed by methylphenidate (Figure 2), with the exception of borderline increases ($P < 0.05$) after 10 mg/kg in the dorsolateral sector on the rostral level and the ventrolateral on the caudal level (data not shown). Similarly, no statistically significant changes in

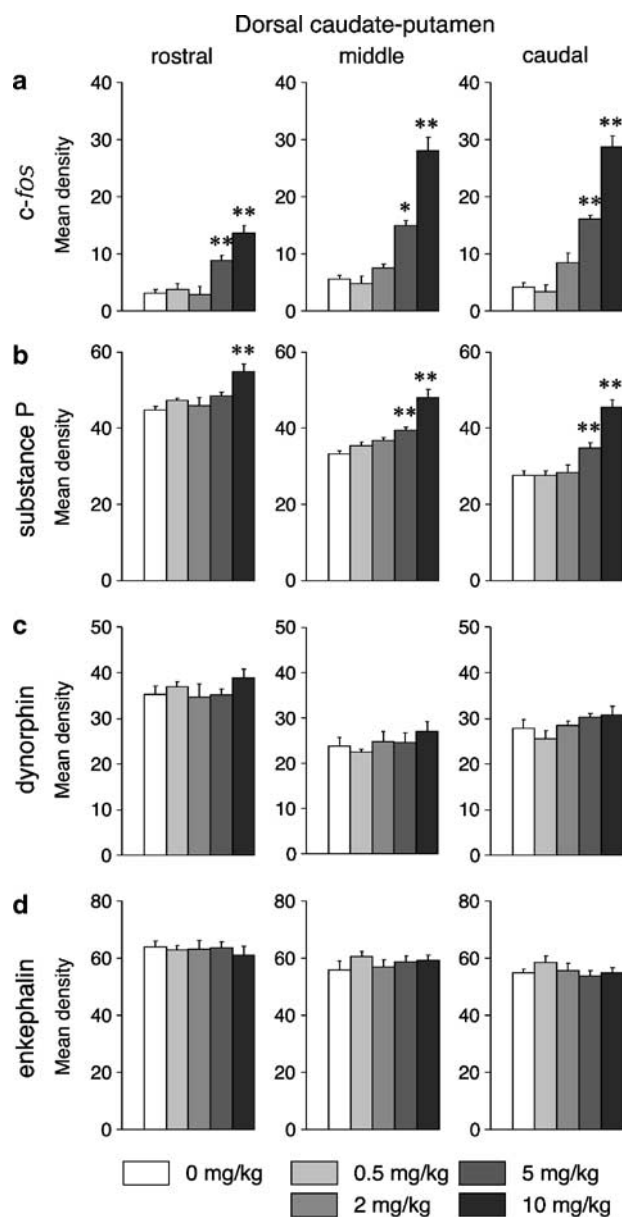


Figure 2 Dose-dependent effects of methylphenidate on *c-fos* (a), substance P (b), dynorphin (c), and enkephalin (d) expression in the dorsal caudate-putamen. Mean density values (mean ± SEM, arbitrary units) measured in the dorsal sector on rostral, middle, and caudal striatal levels are shown for rats treated with 0–10 mg/kg (i.p.) of methylphenidate and killed 1 h later. ** $P < 0.01$, * $P < 0.05$ vs 0 mg/kg.

enkephalin expression were found, either in the dorsal sectors (Figure 2) or in any other sector (data not shown).

Time Course of Changes in Gene Expression after Acute Methylphenidate Administration

The time courses for changes in *c-fos* and neuropeptide expression after injection of 5 mg/kg methylphenidate are depicted in Figures 5–7. Figure 7 presents regional maps for the different time points and genes. In all, 14 of 18 caudate-putamen sectors (Figure 7, Table 2a) and one of five nucleus accumbens sectors (Figures 6 and 7) displayed a significant *c-fos* response. *C-fos* mRNA levels were typically increased

by 20 min after drug administration (12 of 15 responding sectors; Figure 5, Table 2a), peaked at 40 min in all 15 sectors (Figure 4), were in most cases still increased at 1 h (12 of 15 sectors), and returned to baseline by 3 h in all sectors. Overall, the *c-fos* response was again most robust in the dorsal sectors of the middle and caudal striatum (Figures 4, 5 and 7, Table 2a), weaker rostrally, and weakest or absent in ventral sectors. The only significant *c-fos* response in the nucleus accumbens was found in the lateral part of the shell at 40 min after drug injection (Figures 6 and 7).

Of the 18, 13 caudate-putamen sectors showed a significant change in substance P expression after administration of 5 mg/kg methylphenidate (Figures 4 and 7, Table 2b). Again, no changes were found in the nucleus accumbens (Figures 6 and 7). Substance P expression changed with a delayed time course compared to *c-fos* expression (Figures 5 and 7, Table 2b). Substance P mRNA levels were significantly increased by 20 min only in five of the most responsive sectors, among them the three dorsal sectors (Figure 5). Levels peaked at 40–60 min in all 13 sectors (Figure 4), were in most cases still increased at 3 h (eight of 13), and returned to baseline by 24 h in all sectors. Interestingly, the increase in substance P mRNA levels peaked earlier in caudal sectors (40 min) than in rostral sectors (around 1 h) (Figures 5 and 7).

Dynorphin expression was minimally affected by methylphenidate (Figure 7). Levels of mRNA displayed a statistically significant increase only in two of the 23 sectors, in the medial ($P < 0.05$; data not shown) and dorsal (Figure 5) sectors on the middle level at 1 h after drug administration. Similarly, minimal effects were found for enkephalin expression. On the middle level, a borderline increase was seen in the dorsal sector at 20 min (Figure 5), whereas the ventral sector showed a decrease ($P < 0.05$; data not shown) after 24 h. No other effects were found for dynorphin or enkephalin expression (not shown).

Correlations between Changes in *c-fos* and Substance P Expression

The relationship between the changes in *c-fos* and substance P expression across the 23 striatal sectors was further characterized by correlation analyses. For these correlations, the difference between a treatment group (dose or time point) and the control (0 mg/kg or 0 min, respectively) was calculated for a given sector and expressed as the percentage of the maximal difference found in the 23 sectors for all doses, or time points (Figure 8). In the dose-response study, the changes in *c-fos* and substance P expression were compared for each methylphenidate dose (Figure 8a). After injection of 0.5 mg/kg, *c-fos* and substance P values were not correlated ($r = 0.216$; $P > 0.05$). In contrast, a significant positive correlation between the changes in *c-fos* and substance P mRNA levels was found for 2 mg/kg ($r = 0.562$; $P < 0.01$), indicating that this dose produced changes in the expression of both genes in at least some of the striatal sectors. Highly significant correlations between *c-fos* and substance P expression were found for 5 mg/kg ($r = 0.781$; $P < 0.001$) and 10 mg/kg ($r = 0.905$; $P < 0.001$) (Figure 8a).

For the time-course study, changes in *c-fos* and substance P expression were compared for each time point. Highly significant positive correlations were found for 20 min

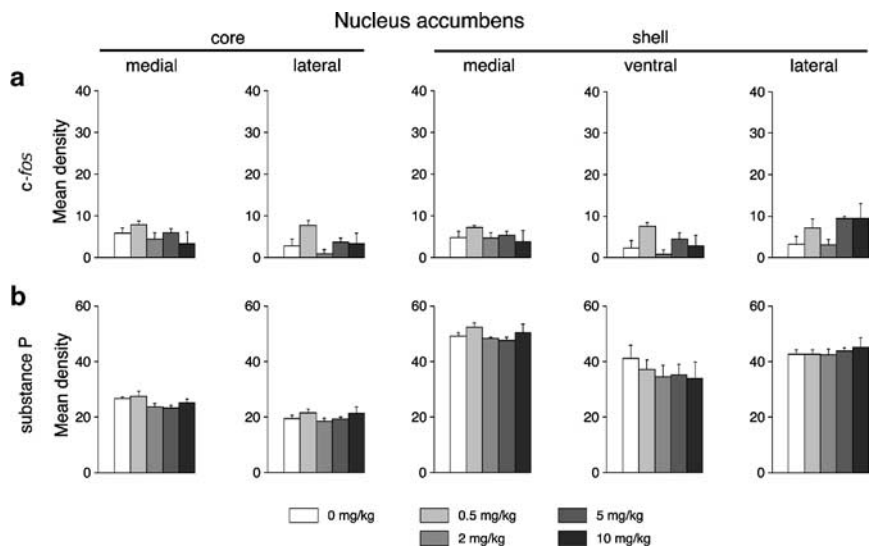


Figure 3 Effects of methylphenidate on *c-fos* (a) and substance P (b) expression in the nucleus accumbens. Mean density values (mean \pm SEM) measured in the medial and lateral core, and in the medial, ventral, and lateral shell are given for rats treated with 0–10 mg/kg of methylphenidate and killed 1 h later.

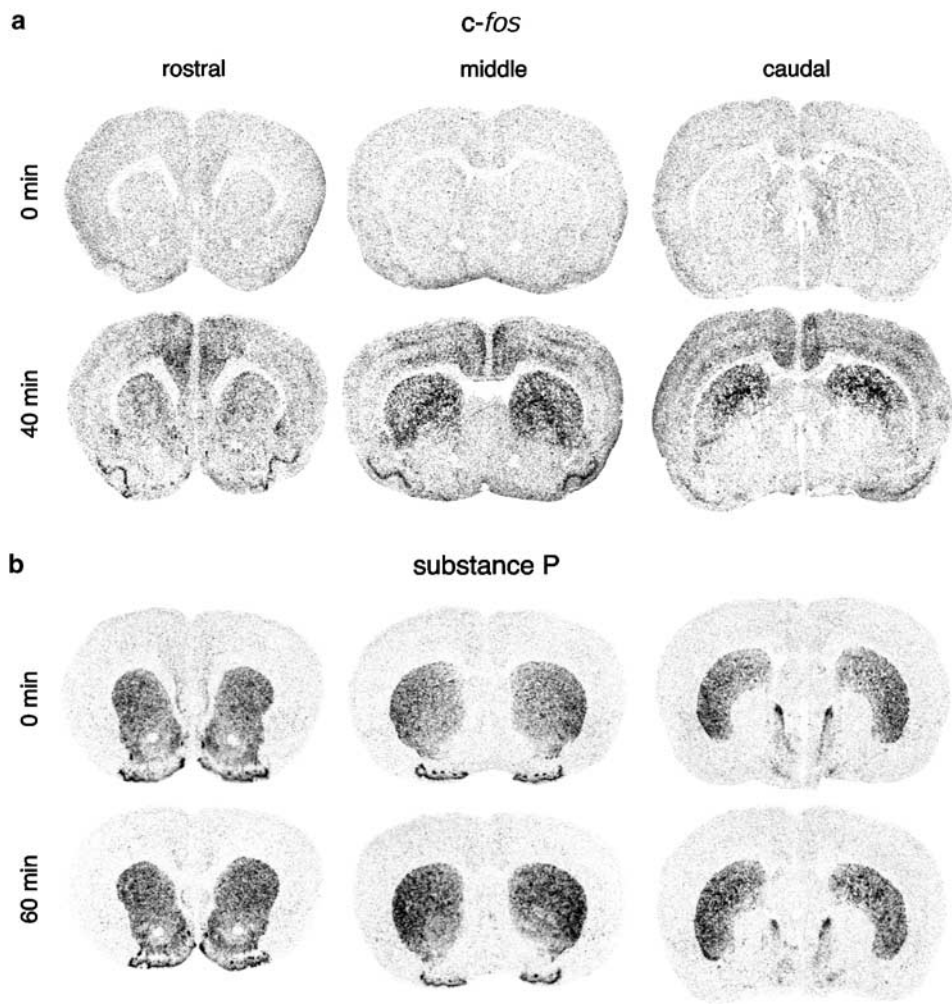


Figure 4 Methylphenidate-induced expression of *c-fos* (a) and substance P (b) in the rostral, middle, and caudal striatum. Illustrations of film autoradiograms depict gene expression at 0 and 40 min (*c-fos*) or 60 min (substance P) after methylphenidate injection (5 mg/kg). Maximal hybridization signal is black.

Table 1 Dose-Dependent Effects of Methylphenidate on the Expression of (a) *c-Fos* and (b) Substance P in the Caudate-Putamen

	Doses (mg/kg)				
	0	0.5	2	5	10
<i>(a)</i>					
<i>Rostral</i>					
Medial	3.7 ± 1.2	5.8 ± 0.7	4.1 ± 1.3	9.4 ± 1.6*	9.9 ± 1.4**
Dorsomedial	3.7 ± 0.8	4.9 ± 0.5	4.0 ± 1.1	10.0 ± 1.2**	14.0 ± 1.3**
Dorsal	3.1 ± 0.8	3.8 ± 1.0	2.9 ± 1.4	8.8 ± 0.9**	13.7 ± 1.2**
Dorsolateral	3.5 ± 1.2	3.6 ± 1.1	2.9 ± 1.1	7.6 ± 1.0*	12.4 ± 0.8**
Ventral	2.3 ± 1.5	6.2 ± 1.1	2.4 ± 0.9	5.9 ± 0.9	6.2 ± 1.5
<i>Middle</i>					
Medial	6.9 ± 0.9	6.8 ± 1.6	10.0 ± 1.1	14.8 ± 0.6**	19.7 ± 1.0**
Dorsal	5.6 ± 0.7	4.8 ± 1.3	7.5 ± 0.7	15.0 ± 0.9*	28.1 ± 2.3**
Dorsolateral	4.3 ± 0.7	2.9 ± 1.1	4.9 ± 1.1	8.3 ± 1.0	18.3 ± 2.1**
Ventrolateral	3.2 ± 1.0	3.0 ± 1.5	4.4 ± 1.3	8.9 ± 1.7*	22.6 ± 2.1**
Ventral	5.8 ± 1.4	5.3 ± 1.4	5.0 ± 1.1	6.8 ± 1.7	9.1 ± 1.9
Central	4.2 ± 0.8	3.5 ± 1.4	6.0 ± 1.2	13.7 ± 2.5**	20.2 ± 1.5**
<i>Caudal</i>					
Medial	5.8 ± 0.9	6.0 ± 0.9	9.6 ± 2.5	10.2 ± 1.2	14.9 ± 2.9**
Dorsal	4.2 ± 0.8	3.4 ± 1.2	8.4 ± 1.8	16.1 ± 0.7**	28.7 ± 2.0**
Dorsolateral	0.4 ± 0.9	0.8 ± 1.0	2.9 ± 1.4	5.2 ± 0.9*	14.9 ± 1.3**
Ventrolateral	0.0 ± 0.5	1.3 ± 1.4	1.4 ± 1.4	0.6 ± 0.7	11.8 ± 2.8**
Ventral	0.9 ± 1.1	2.9 ± 1.8	1.4 ± 2.1	0.2 ± 0.3	0.4 ± 1.2
Ventral central	0.9 ± 0.7	1.3 ± 1.5	2.0 ± 1.2	2.5 ± 0.7	5.5 ± 1.5
Dorsal central	0.0 ± 0.9	2.4 ± 1.0	3.0 ± 1.3	3.2 ± 0.4	12.3 ± 2.0**
<i>(b)</i>					
<i>Rostral</i>					
Medial	33.3 ± 1.4	34.4 ± 1.6	33.9 ± 1.1	35.2 ± 0.7	39.7 ± 0.9**
Dorsomedial	41.8 ± 0.9	43.0 ± 0.8	43.1 ± 1.3	43.4 ± 0.9	49.3 ± 1.4**
Dorsal	44.8 ± 1.0	47.2 ± 0.7	46.0 ± 2.0	48.4 ± 1.0	54.9 ± 2.0**
Dorsolateral	46.6 ± 0.7	48.1 ± 1.6	46.6 ± 1.5	51.4 ± 1.3	55.1 ± 1.9**
Ventral	38.8 ± 1.4	40.6 ± 1.3	38.3 ± 1.5	39.4 ± 0.8	40.4 ± 1.8
<i>Middle</i>					
Medial	24.9 ± 1.2	26.4 ± 1.2	30.0 ± 1.0	29.8 ± 1.3	35.3 ± 2.3**
Dorsal	33.3 ± 0.8	35.3 ± 1.0	36.7 ± 0.8	39.5 ± 0.8**	48.1 ± 2.0**
Dorsolateral	47.1 ± 1.5	47.5 ± 0.9	48.7 ± 0.6	48.7 ± 0.8	56.7 ± 2.8**
Ventrolateral	48.7 ± 1.2	47.4 ± 1.4	48.9 ± 0.4	48.6 ± 1.1	59.4 ± 3.0**
Ventral	26.7 ± 1.2	28.2 ± 1.5	26.9 ± 0.8	27.1 ± 1.2	28.6 ± 0.9
Central	34.7 ± 1.9	34.8 ± 1.1	36.7 ± 1.3	38.1 ± 1.2	44.5 ± 1.1**
<i>Caudal</i>					
Medial	17.5 ± 1.7	17.5 ± 0.9	18.4 ± 1.4	19.8 ± 0.3	29.3 ± 2.4**
Dorsal	27.7 ± 1.1	27.7 ± 1.0	28.3 ± 2.0	34.9 ± 1.2**	45.6 ± 1.9**
Dorsolateral	36.4 ± 0.9	37.9 ± 1.6	37.7 ± 2.1	39.3 ± 0.7	49.6 ± 2.9**
Ventrolateral	46.6 ± 1.1	46.7 ± 2.3	44.9 ± 2.2	45.8 ± 1.7	55.3 ± 5.5
Ventral	33.4 ± 1.5	33.6 ± 1.7	32.4 ± 1.4	32.8 ± 0.8	32.7 ± 3.1
Ventral central	26.7 ± 1.8	29.8 ± 0.8	26.6 ± 1.9	27.7 ± 1.1	30.7 ± 1.9
Dorsal central	19.5 ± 1.6	18.9 ± 0.9	19.3 ± 1.8	20.1 ± 1.5	26.7 ± 1.1**

Mean density values (mean ± SEM, arbitrary units) measured in the 18 sectors of the caudate-putamen, on rostral, middle, and caudal striatal levels, are shown for rats treated with 0 (vehicle), 0.5, 2, 5, or 10 mg/kg (i.p.) of methylphenidate and killed 1 h later.

* $P < 0.05$ vs 0 mg/kg.

** $P < 0.01$ vs 0 mg/kg.

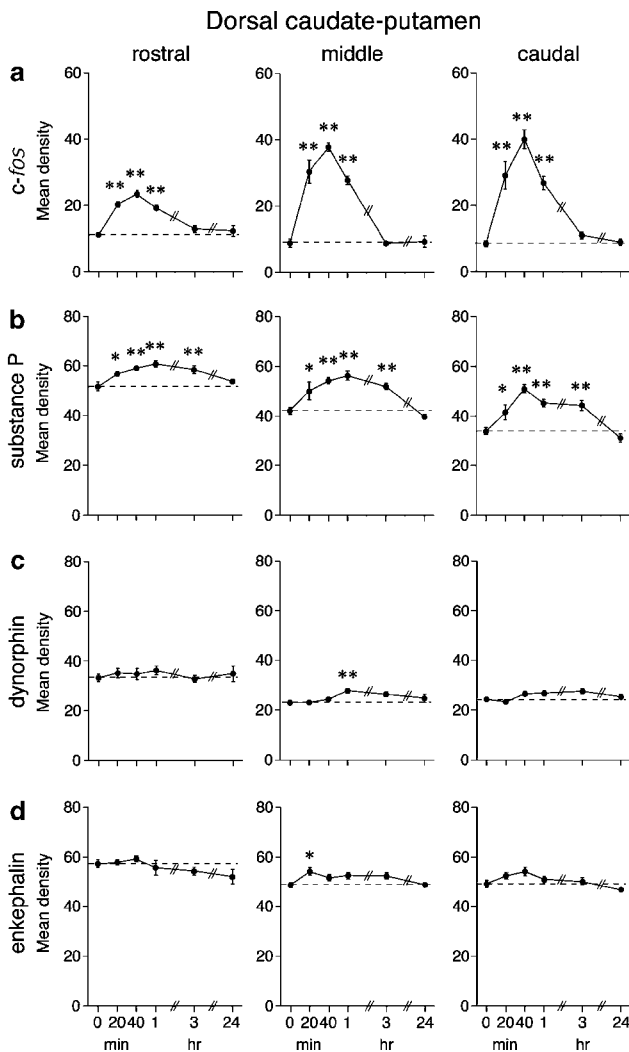


Figure 5 Time course of methylphenidate-induced expression of *c-fos* (a), substance P (b), dynorphin (c), and enkephalin (d) in the dorsal caudate-putamen. Mean density values (mean \pm SEM) measured in the dorsal sector on rostral, middle, and caudal striatal levels are shown for rats that received 5 mg/kg of methylphenidate and were killed either immediately (0 min) or at 20, 40 min, 1, 3, or 24 h after drug administration. ** $P < 0.01$, * $P < 0.05$ vs 0 min.

($r = 0.879$; $P < 0.001$), 40 min ($r = 0.960$; $P < 0.001$), and 60 min ($r = 0.850$; $P < 0.001$) (Figure 8b). In contrast, 24 h after drug administration, a significant negative correlation between changes in *c-fos* and substance P mRNA levels was observed ($r = -0.572$; $P < 0.01$) (Figure 8b). Further analysis indicated that this effect was largely due to substance P mRNA levels in maximally responding sectors (40 min) that were below baseline at 24 h (Figure 8b). This was confirmed by significant negative correlations for substance P mRNA levels at 24 h vs substance P mRNA levels at 40 min ($r = -0.599$, $P < 0.01$), as well as vs *c-fos* mRNA levels at 40 min ($r = -0.592$, $P < 0.01$). These results indicate suppressed substance P expression in the most responsive (dorsal) sectors 24 h after acute methylphenidate administration. In contrast, no relationships between acute drug responses and enkephalin mRNA levels at 24 h after injection were seen (enkephalin (24 h) vs *c-fos* (40 min), $r = -0.158$, $P > 0.05$; vs substance P (40 min), $r = -0.127$, $P > 0.05$).

DISCUSSION

Psychostimulants that produce dopamine overflow in the striatum, including cocaine and amphetamine, alter gene regulation in striatal output pathways. Our previous results in adolescent rats (Brandon and Steiner, 2003) and present findings in adults demonstrate that the psychostimulant methylphenidate is no exception, but differs in certain molecular effects from other psychostimulants. First, we show that acute administration of methylphenidate increased the expression of the transcription factor (immediate-early gene) *c-fos* and the neuropeptide substance P in a dose-dependent and regionally distinct manner. These effects emerged with 2 mg/kg and were very robust after 5–10 mg/kg. For both genes, mRNA levels were elevated within 20 min of drug administration and remained increased for more than 1 (*c-fos*) or 3 h (substance P). These molecular changes displayed a distinct topography, with maximal increases in dorsal, sensorimotor sectors on middle to caudal striatal levels, and lesser or no effects in rostral and ventral striatal sectors. Second, in contrast to these prominent effects on *c-fos* and substance P, and unlike the effects of cocaine and amphetamine, acute methylphenidate produced only minimal changes in the expression of the opioid peptides dynorphin and enkephalin. Overall, our results indicate that methylphenidate treatment causes neuroadaptations in neurons of the direct striatal output pathway.

Methylphenidate Affects Transcription Factors

Our present results and those of others demonstrate that methylphenidate alters the expression of transcription factors in the striatum in a manner similar to other psychostimulants (Harlan and Garcia, 1998; Torres and Horowitz, 1999). Several studies reported acute effects of methylphenidate on *c-fos* expression. Increased Fos protein levels were shown in different species for a range of doses and routes of administration (cat, 2.5 mg/kg, p.o. (Lin *et al*, 1996); mouse, 5 and 20 mg/kg, s.c. (Penner *et al*, 2002); 30 mg/kg, i.p. (Trinh *et al*, 2003); rat, 2–20 mg/kg, s.c. (Chase *et al*, 2003)). Previously, we demonstrated increased *c-fos* and *zif 268* mRNA levels after 2–10 mg/kg (i.p.) in adolescent rats (Brandon and Steiner, 2003). In our present study, we assessed the effects of 0.5–10 mg/kg (i.p.) in adults and also found increased *c-fos* expression after 2 mg/kg and higher doses. While 5 and 10 mg/kg produced pronounced effects, increased gene expression after 2 mg/kg was revealed only by a more powerful two-marker correlation analysis (*c-fos* vs substance P). A similar effect was found for *zif 268* and Homer 1a with this dose (Yano and Steiner, 2005). Thus, this threshold dose of 2 mg/kg appeared to produce more robust changes in adolescents (Brandon and Steiner, 2003; Chase *et al*, 2003) than in adults. A more robust acute molecular response in adolescents (see also Andersen *et al*, 2001) would be consistent with greater neuroadaptive changes after repeated drug treatment in this age group (Ehrlich *et al*, 2002). For example, our previous study showed significantly increased dynorphin expression after repeated methylphenidate treatment in adolescents (Brandon and Steiner, 2003). However, such possible age differences will need to be affirmed by direct comparisons.

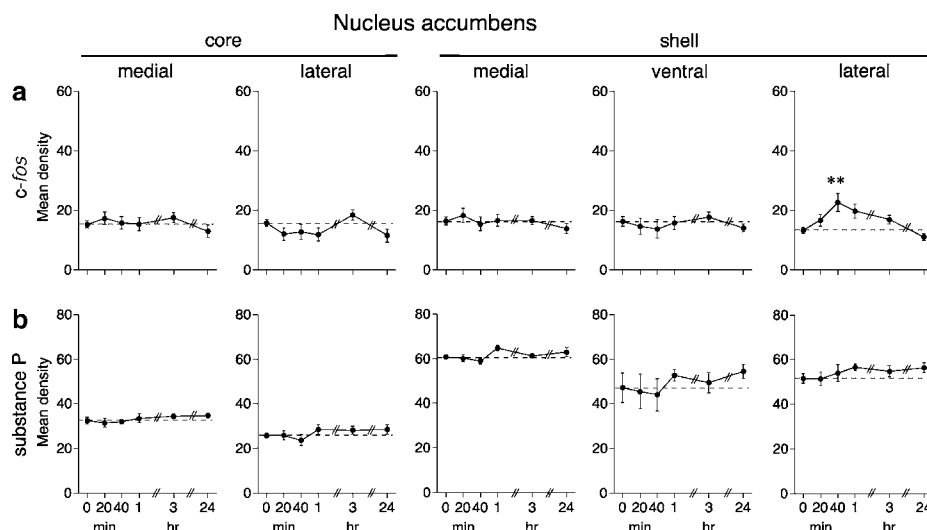


Figure 6 Time course of methylphenidate-induced expression of *c-fos* (a) and substance P (b) in the nucleus accumbens. Mean density values (mean \pm SEM) measured in the medial and lateral core, and in the medial, ventral, and lateral shell are given for rats that received 5 mg/kg of methylphenidate and were killed either immediately (0 min) or at 20, 40 min, 1, 3, or 24 h after drug administration. $**P < 0.01$ vs 0 min.

In addition to the above dose–response relationship, our study is the first to describe the time course of changes in *c-fos* expression after acute methylphenidate administration. Levels of *c-fos* mRNA were significantly increased by 20 min, peaked around 40 min, and returned to baseline between 1 and 3 h after drug injection. This time course is very similar to that of *c-fos* expression after acute cocaine administration (eg Steiner and Gerfen, 1998).

Previous studies also assessed the effects of repeated methylphenidate treatment on immediate-early gene expression. It was found that the inducibility of *c-fos* and *zif 268* by cocaine (Brandon and Steiner, 2003) or by methylphenidate (Chase *et al*, 2003) was suppressed after 7- or 14-day repeated methylphenidate treatments. These effects are also principally similar to those of repeated cocaine (eg Willuhn *et al*, 2003) and amphetamine treatments (for reviews, see Harlan and Garcia, 1998; Torres and Horowitz, 1999). Such blunted gene induction is thought to reflect drug-induced neuroadaptations (Steiner and Gerfen, 1998). A number of signaling molecules/mechanisms involved in gene regulation are affected by methylphenidate treatment. These include, for example, the dopamine transporter (Izenwasser *et al*, 1999; Moll *et al*, 2001) and the vesicular monoamine transporter (Sandoval *et al*, 2002); levels of GluR2 glutamate receptors (Andersen *et al*, 2002) and D1 dopamine receptors (Papa *et al*, 2002); protein kinase A and dopamine-stimulated adenylyl cyclase activities (Crawford *et al*, 1998); as well as the transcription factor CREB (Andersen *et al*, 2002). Several of these play a role in the regulation of dopamine-mediated *c-fos* and *zif 268* expression in the striatum, and such changes could thus underlie altered gene induction after repeated methylphenidate treatment.

Prominent Effects on Substance P, but not Opioid Peptide, Expression by Acute Methylphenidate

Psychostimulants such as cocaine and amphetamine increase substance P expression in the striatum (Harlan and Garcia, 1998; Steiner and Gerfen, 1998). For example,

substance P mRNA levels are elevated within 30 min of acute cocaine injection (Steiner and Gerfen, 1993; Drago *et al*, 1996; Brandon and Steiner, 2003) and remain elevated for more than 2–3 h (eg Hurd and Herkenham, 1992; Adams *et al*, 2001). Our present study is the first to demonstrate that acute methylphenidate administration also increases the expression of substance P in the striatum. Changes in mRNA levels followed a similar time course, as they were increased within 20 min, peaked at 40–60 min, and returned to baseline only after 3 h. In maximally responding (dorsal) sectors, substance P expression was slightly suppressed 24 h after methylphenidate injection. Interestingly, the time course showed a shift between rostral and caudal sectors. All rostral sectors showed peak increases around 60 min, some (medial) with near-maximal levels even at 3 h. In contrast, increases in caudal, sensorimotor sectors peaked at 40 min, coincident with peak locomotor activation by methylphenidate (Gerasimov *et al*, 2000). This effect suggests a relationship between substance P expression and behavior-associated neuronal activity in the caudal striatum. Together, our results demonstrate a very robust effect of acute methylphenidate on striatal substance P expression.

These substance P effects contrasted with the minimal effects on the opioid peptides dynorphin and enkephalin. Increases in dynorphin mRNA levels are generally more pronounced after repeated psychostimulant treatments due to accumulation (eg Hurd *et al*, 1992; Steiner and Gerfen, 1993; Daunais and McGinty, 1994; Spangler *et al*, 1996; Willuhn *et al*, 2003), and in fact increased dynorphin expression was seen after repeated methylphenidate treatment (in adolescent rats; Brandon and Steiner, 2003). However, unlike acute methylphenidate administration, both acute cocaine and amphetamine result in significantly enhanced dynorphin expression. Elevated mRNA levels can be seen as early as 30 min after drug injection (Brandon and Steiner, 2003; Willuhn *et al*, 2003), are prominent at 2–3 h (Hurd and Herkenham, 1992; Smith and McGinty, 1994; Wang *et al*, 1995; Adams *et al*, 2003), and have been found

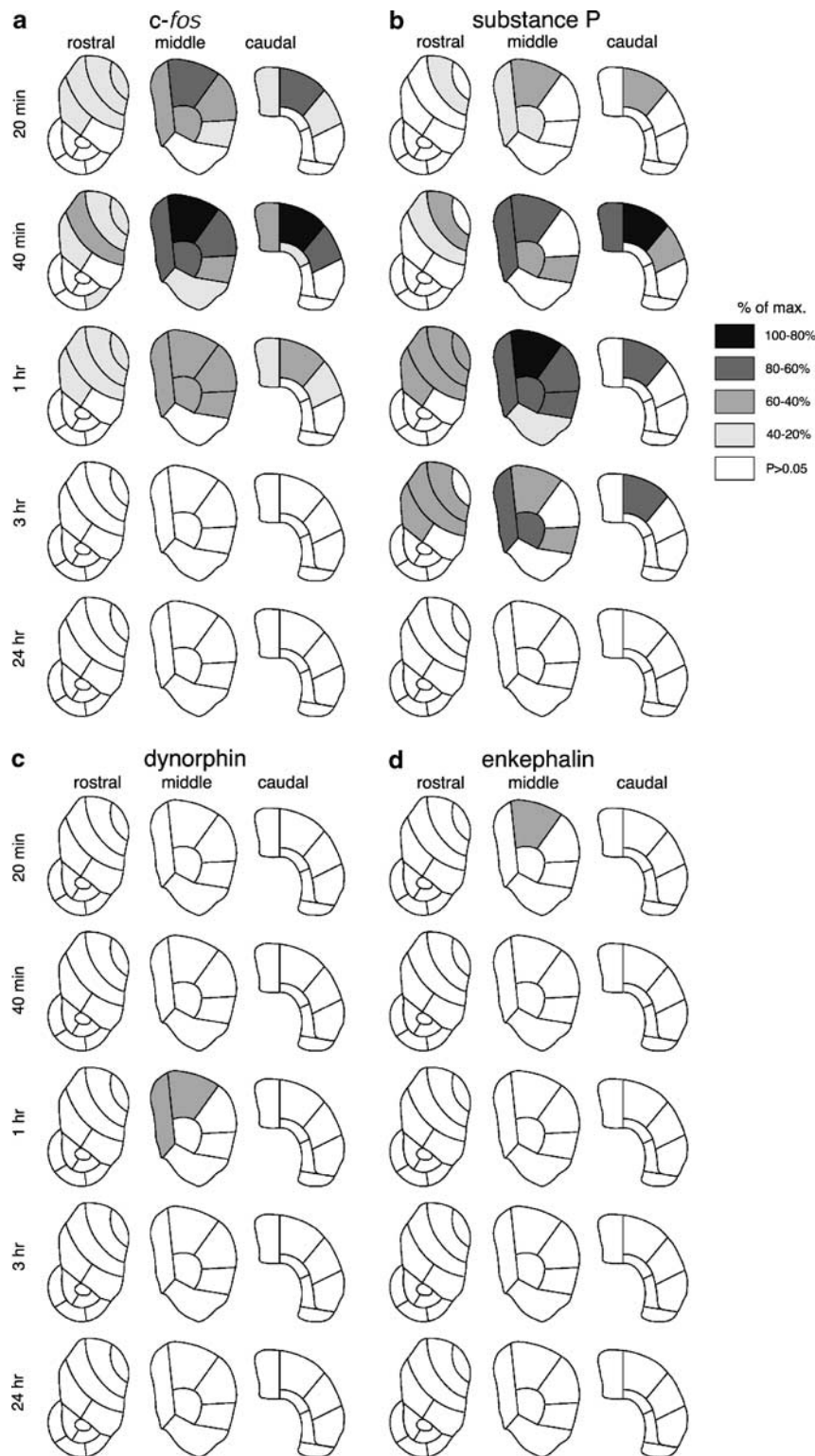


Figure 7 Topography of methylphenidate-induced changes in the expression of *c-fos* (a), substance P (b), dynorphin (c), and enkephalin (d) in the 23 striatal sectors. The increases in gene expression ($P < 0.05$ vs basal expression, 0 min) on rostral, middle, and caudal striatal levels are depicted for rats that received an injection of methylphenidate (5 mg/kg) and were killed 20, 40 min, 1, 3, or 24 h later. These values are expressed as the percentage of the maximal increase (% of max.) in the 23 sectors for each gene, and are coded as indicated for *c-fos* and substance P, or are shaded for dynorphin and enkephalin.

to last for 18 (Smith and McGinty, 1994) to 30 h (Wang *et al*, 1995). In our present study, only borderline increases were observed at 1 h after methylphenidate injection. These

results indicate differential molecular changes after treatment with methylphenidate as compared to cocaine and amphetamine.

Table 2 Time Course of Methylphenidate-Induced Changes in (a) c-Fos and (b) Substance P Expression in the Caudate-Putamen

	Time points					
	0 min	20 min	40 min	1 h	3 h	24 h
<i>(a)</i>						
<i>Rostral</i>						
Medial	13.0±0.7	20.2±1.5**	24.2±1.3**	17.9±1.6*	13.6±1.5	12.9±0.8
Dorsomedial	11.6±0.7	20.2±0.6**	24.9±1.3**	19.8±1.2**	12.5±1.0	12.1±1.1
Dorsal	11.2±0.7	20.3±0.9**	23.5±1.0**	19.2±0.8**	12.9±1.0	12.3±1.6
Dorsolateral	10.2±0.9	19.8±0.9**	22.7±1.6**	17.8±0.3**	12.0±1.1	12.1±1.5
Ventral	13.5±0.8	15.6±1.4	18.3±1.5	14.6±1.5	15.7±1.4	13.8±1.7
<i>Middle</i>						
Medial	11.0±0.9	25.8±2.1**	33.3±1.8**	25.3±1.3**	10.0±1.0	11.3±1.7
Dorsal	8.9±1.2	30.4±3.6**	37.8±1.3**	27.8±1.4**	8.8±0.3	9.3±1.7
Dorsolateral	10.5±1.6	24.3±2.8**	29.6±2.2**	23.7±1.1**	8.0±1.1	10.5±2.2
Ventrolateral	13.3±1.5	23.8±2.4**	30.9±2.2**	26.5±2.1**	11.6±1.0	14.4±1.9
Ventral	14.3±1.3	18.2±1.1	20.9±1.7*	18.6±1.9	13.5±1.1	15.4±2.1
Central	11.2±1.2	25.1±3.6**	33.4±1.9**	26.8±2.1**	9.1±0.8	10.7±2.2
<i>Caudal</i>						
Medial	10.3±1.0	22.8±1.9**	26.4±1.1**	20.4±1.4**	13.4±1.1	11.9±1.1
Dorsal	8.5±0.9	29.1±4.1**	40.0±2.8**	26.8±2.0**	11.0±1.0	9.0±1.0
Dorsolateral	8.9±1.0	18.6±2.0**	28.1±0.8**	19.5±1.8**	12.8±1.6	10.7±1.2
Ventrolateral	9.4±1.9	12.2±0.8	16.3±1.2	13.6±1.8	15.4±2.2	11.6±2.6
Ventral	10.2±2.3	8.0±2.1	6.4±0.2	9.6±2.4	17.2±3.0	13.6±3.6
Ventral central	8.1±1.2	9.6±1.4	10.4±1.1	9.9±1.8	12.0±1.6	9.0±1.9
Dorsal central	6.8±1.1	12.0±2.3	17.8±1.3**	8.5±1.6	10.2±1.6	6.9±1.1
<i>(b)</i>						
<i>Rostral</i>						
Medial	41.6±1.4	42.8±2.0	45.6±1.0	49.1±1.2**	48.4±2.1*	43.3±1.7
Dorsomedial	48.8±1.3	52.8±0.9	55.4±1.0**	57.3±1.3**	55.7±1.5**	48.1±1.8
Dorsal	51.7±1.7	56.8±1.0*	59.2±0.5**	60.8±1.3**	58.5±1.6**	53.8±0.7
Dorsolateral	53.8±1.4	58.1±1.0	59.3±1.1	61.4±1.8**	58.2±2.1	55.6±2.2
Ventral	48.3±1.4	49.7±2.2	47.5±0.7	49.6±1.2	50.5±1.0	49.9±1.8
<i>Middle</i>						
Medial	30.0±1.0	35.0±1.9*	42.0±1.7**	42.7±1.4**	40.8±0.9**	30.1±0.5
Dorsal	42.1±1.3	50.0±3.6*	54.2±1.3**	56.2±1.9**	51.9±1.2**	39.7±0.9
Dorsolateral	58.3±1.7	63.3±2.8	65.3±2.5	69.6±2.1**	63.9±1.6	57.1±1.2
Ventrolateral	57.6±1.1	63.8±2.3	64.7±2.1*	69.8±2.3**	65.0±1.4*	58.6±1.8
Ventral	32.8±0.4	32.7±1.3	34.8±1.1	37.2±1.3*	34.8±0.9	31.5±0.8
Central	40.2±0.9	45.9±2.2*	49.6±1.2**	53.1±0.7**	51.2±1.4**	37.8±1.2
<i>Caudal</i>						
Medial	21.2±1.0	27.7±2.6	31.4±2.0**	27.3±1.3	27.4±2.4	20.2±1.1
Dorsal	33.9±1.4	41.5±3.0*	50.9±1.7**	45.2±1.6**	44.3±2.0**	31.2±1.7
Dorsolateral	49.6±1.8	51.2±1.7	57.3±0.6*	51.6±2.1	53.1±2.3	45.2±1.2
Ventrolateral	58.3±1.7	59.1±2.8	61.6±0.6	60.5±1.5	62.7±1.8	58.4±0.7
Ventral	39.5±2.6	40.7±1.6	37.0±3.4	41.7±0.8	40.4±1.4	38.1±2.3
Ventral central	32.7±1.4	35.2±0.9	35.9±1.4	36.8±1.2	33.9±1.5	32.7±2.0
Dorsal central	24.0±1.4	27.3±2.4	28.6±1.2	26.8±1.5	27.2±1.6	23.1±1.2

Mean density values (mean±SEM) measured in the 18 sectors of the caudate-putamen, on rostral, middle, and caudal striatal levels, are given for rats that received an injection of 5 mg/kg of methylphenidate and were killed either immediately (0 min) or 20, 40 min, 1, 3, or 24 h later.

* $P < 0.05$ vs 0 min.

** $P < 0.01$ vs 0 min.

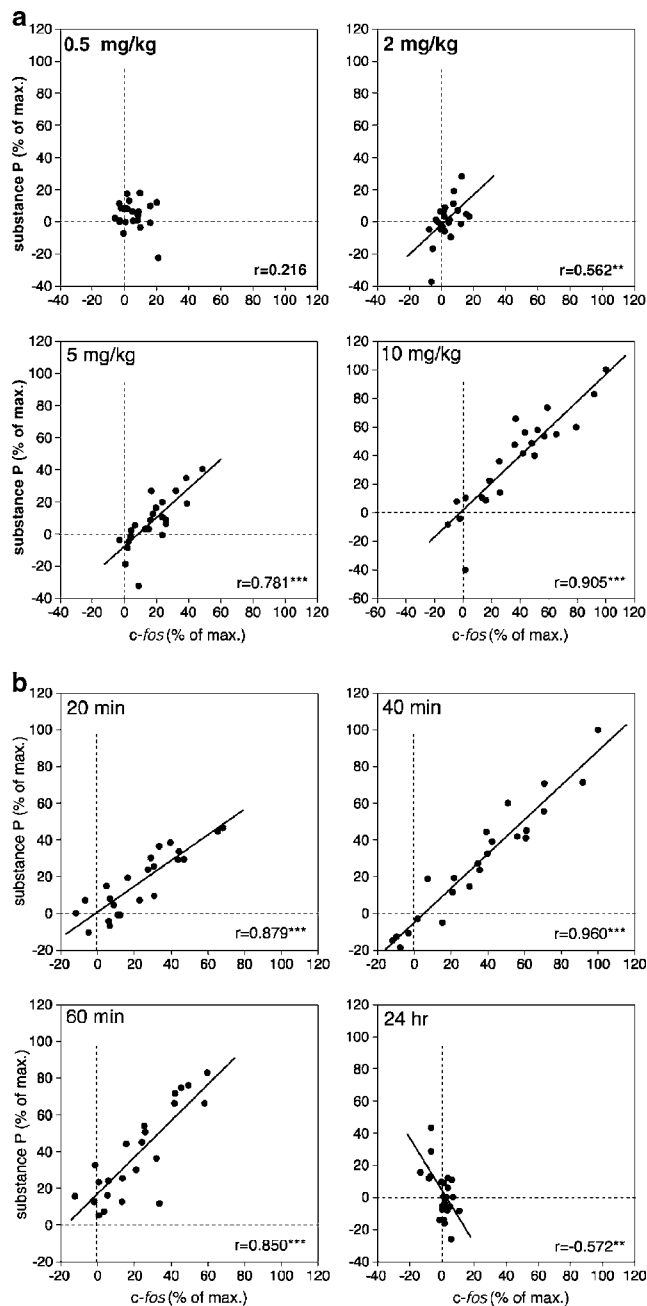


Figure 8 Correlations between the methylphenidate-induced increases in c-fos and substance P expression in the dose-response study (a) and in the time-course study (b). The scatterplots depict the relationship between increases in c-fos and substance P mRNA levels (dose or time point minus 0 mg/kg or 0 min, respectively) in the individual sectors, for each dose (0.5–10 mg/kg) and the time points 20, 40, 60 min and 24 h. The values are expressed as the percentage of the maximal increase (% of max.) in the 23 sectors for each gene. $***P < 0.001$, $**P < 0.01$.

Similar differential effects were found for enkephalin expression. Perhaps best known are the increases in striatal enkephalin expression (indirect pathway) produced by dopamine depletion or chronic antipsychotic treatment/D2 receptor blockade (cf. Steiner and Gerfen, 1998). For example, enkephalin mRNA levels begin to rise within 1 h and remain elevated for more than 24 h after a D2 receptor antagonist injection (Steiner and Gerfen, 1999). Similarly,

increases in striatal enkephalin expression have also been consistently found after acute and repeated cocaine and amphetamine treatment (Steiner and Gerfen, 1993; Wang and McGinty, 1996a; Spangler *et al*, 1997; Mathieu-Kia and Besson, 1998; Brandon and Steiner, 2003; see Wang and McGinty, 1996b, for review), although these changes were typically smaller than those for substance P and dynorphin expression. In contrast, neither repeated (Brandon and Steiner, 2003) nor acute methylphenidate treatment (present study) produced consistent changes in enkephalin expression.

The basis for this differential regulation of immediate-early genes and substance P vs opioid peptides is not clear, but may be related to the transmitter systems affected by methylphenidate. One important difference between methylphenidate and cocaine/amphetamine is the low affinity of methylphenidate for the serotonin transporter (Schwartz *et al*, 1985; Gatley *et al*, 1996). Indeed, in contrast to cocaine and amphetamine, methylphenidate does not produce increased extracellular levels of serotonin (Kuczenski and Segal, 1997; Kankaanpää *et al*, 2002). Gene expression in striatal neurons is regulated by complex interactions between several transmitter systems, most prominently dopamine, glutamate, and acetylcholine (Hyman *et al*, 1996; Wang and McGinty, 1996b). Serotonin also affects striatal gene expression (eg Bhat and Baraban, 1993; Torres and Rivier, 1994; Gardier *et al*, 2000), including that of dynorphin and enkephalin (Morris *et al*, 1988; Walker *et al*, 1996; Mijster *et al*, 1998; Basura and Walker, 1999; Keefe *et al*, 2002). It is thus conceivable that the relatively minor effects of methylphenidate on opioid peptide expression, as observed in our present and previous studies (Brandon and Steiner, 2003), reflect a more important role for serotonin in the regulation of these genes, as compared to substance P and the immediate-early genes.

Opioid peptides in striatal output pathways are thought to serve as negative feedback mechanisms to maintain systems homeostasis (Steiner and Gerfen, 1993, 1998; Hyman and Nestler, 1996). Psychostimulant-induced changes in opioid peptide expression are considered neuroadaptations that may underlie certain withdrawal symptoms and other behavioral effects in psychostimulant addiction (Hyman and Nestler, 1996; Nestler, 2001). Our results suggest that methylphenidate is less likely than other psychostimulants to trigger such molecular adaptations.

Functional Domains Affected by Methylphenidate

One purpose of our present study was to map the topography of acute methylphenidate-induced gene regulation in the striatum and to compare it to the topography of cocaine-induced gene regulation (Willuhn *et al*, 2003). The goal was to determine whether there is an association of methylphenidate-induced molecular changes with particular corticostriatal circuits (functional domains). As in our previous study (Willuhn *et al*, 2003), we divided the striatum (caudate-putamen and nucleus accumbens) into sectors that were mostly defined by their predominant cortical inputs (Figure 1; see Materials and methods and Willuhn *et al*, 2003).

Our most important findings can be summarized as follows:

- (1) Similar to the effects of cocaine (Willuhn *et al*, 2003), the most pronounced increases in gene expression occurred in sectors receiving inputs from the sensorimotor cortex on middle to caudal striatal levels (Figure 7).
- (2) In the sensorimotor striatum, the dorsal sectors that receive the densest input from the medial agranular cortex (M2 in Figure 1) (Reep *et al*, 1987, 2003; Berendse *et al*, 1992; Kincaid and Wilson, 1996; Reep and Corwin, 1999), in addition to inputs from the somatosensory, visual, and primary motor cortex (eg Reep *et al*, 1987, 2003; McGeorge and Faull, 1989; Brown and Sharp, 1995; Alloway *et al*, 1999), displayed maximal changes in gene regulation. Surrounding sectors that are, to some lesser extent, also targets of medial agranular projections (Reep *et al*, 1987, 2003; Berendse *et al*, 1992; Kincaid and Wilson, 1996; Reep and Corwin, 1999), also consistently showed changes in gene expression. These findings match cocaine effects (Willuhn *et al*, 2003). The medial agranular cortex has mixed prefrontal/premotor features (eg connections with the mediodorsal as well as the ventral lateral and ventromedial thalamic nuclei; neurons that project to brain stem and spinal cord; Donoghue and Wise, 1982; Reep *et al*, 1987, 2003; Passingham *et al*, 1988; Berendse *et al*, 1992; Cowan and Wilson, 1994; Reep and Corwin, 1999; see also Preuss, 1995; Uylings *et al*, 2003), and thus can be considered a prefrontal/motor interface. It remains to be seen whether the observed methylphenidate-induced molecular changes are driven by medial agranular cortical input (Vargo and Marshall, 1995, 1997) or other striatal inputs/mechanisms with a similar regional distribution. However, our findings indicate that sensorimotor circuits under the influence of medial agranular input are particularly prone to psychostimulant-induced neuroplasticity.
- (3) Consistently also affected, albeit to some lesser degree, were medial and rostral striatal sectors that receive inputs from several prefrontal regions including the anterior cingulate, prelimbic and lateral orbital cortex (Berendse *et al*, 1992), similar to cocaine effects (Willuhn *et al*, 2003).
- (4) Generally, small or no changes in gene regulation were seen in ventral striatal sectors that receive inputs mostly from the dorsal agranular insular cortex (Berendse *et al*, 1992), on all three rostrocaudal levels.
- (5) Well appreciated are cocaine-induced molecular changes in the nucleus accumbens (eg Nestler, 2001), although such gene regulation effects are generally modest compared to those in the sensorimotor striatum (see Willuhn *et al*, 2003). Similar differential effects between caudate-putamen and nucleus accumbens were demonstrated here and before (Brandon and Steiner, 2003), and were also found after doses as high as 30 mg/kg (i.p.) (Trinh *et al*, 2003). We assessed effects on gene expression in five nucleus accumbens sectors on a level that contains core and shell subdivisions. In contrast to cocaine (Willuhn *et al*, 2003), acute (present study) or

repeated methylphenidate treatment (Brandon and Steiner, 2003) did not induce significant changes in gene regulation in these sectors, with one notable exception. The lateral part of the shell displayed a robust *c-fos* response at 40 min after methylphenidate injection (a similar effect was seen for *zif 268* and *Homer 1a* expression; Yano and Steiner, 2005). These findings are consistent with our previous findings after repeated methylphenidate treatment. First, the lateral shell was the only accumbal sector with significantly increased dynorphin expression (Brandon and Steiner, 2003). Second, in this subregion only, the *c-fos* response to a cocaine challenge was enhanced (rather than suppressed, as in the dorsal striatum, see above) after the 7-day repeated methylphenidate treatment (Brandon and Steiner, 2003). The lateral part of the shell, at this rostrocaudal level, is apparently the only subregion of the nucleus accumbens that receives medial agranular (Reep *et al*, 1987) and ventral agranular insular (Berendse *et al*, 1992) cortical inputs, in addition to inputs from the piriform and entorhinal cortex, hippocampus, amygdala, and other regions (eg McGeorge and Faull, 1989; Brog *et al*, 1993; Wright and Groenewegen, 1996). Future work will have to elucidate the functional significance of methylphenidate-induced molecular changes in this accumbal region.

Functional Considerations

Our present and previous (Brandon and Steiner, 2003) studies demonstrate several methylphenidate effects on striatal gene regulation; some of these (*c-fos*, substance P) are similar to those of other psychostimulants, others (dynorphin, enkephalin) notably differ. Pronounced alterations in gene expression were found for *c-fos* and substance P, whereas effects on dynorphin and enkephalin expression were modest to minimal. Substance P is a marker for neurons of the direct striatal output pathway (striatonigral neurons), whereas enkephalin is expressed in indirect pathway (striatopallidal) neurons. Psychostimulants such as cocaine and amphetamine induce immediate-early genes predominantly in direct pathway neurons (Cenci *et al*, 1992; Johansson *et al*, 1994; Kosofsky *et al*, 1995), especially under treatment conditions as used in the present study (home cage treatment; eg Badiani *et al*, 1999; Uslaner *et al*, 2001). Our findings thus indicate that methylphenidate induces neuronal plasticity in the direct pathway. The lack of enkephalin effects suggests that, at least under the present treatment conditions, methylphenidate is more selective than cocaine and amphetamine in this regard (see above), although our study does not rule out other methylphenidate-induced molecular changes in indirect pathway neurons. Activity in the direct pathway provides facilitatory input back to the cortex (cf. Steiner and Kitai, 2000). The functional consequences of methylphenidate-induced molecular changes in the direct pathway remain to be demonstrated, but are most likely related to the corticostriatal circuits/functional domains affected by this drug. Our findings indicate that methylphenidate preferentially affects sensorimotor domains, somewhat less frontostriatal

(associative) domains, and least limbic (ventral striatal) domains.

CONCLUSIONS

Altered gene regulation in basal ganglia-cortical circuits induced by methylphenidate may underlie some of the behavioral changes produced by this psychostimulant (see Introduction). Behavioral changes in animal models have been shown with doses as low as 2 mg/kg (i.p.). Similarly, methylphenidate-induced gene regulation in rats emerged with 2 mg/kg and higher doses (present study; Brandon and Steiner, 2003; Chase *et al*, 2003). This dose is considered to be within or above the range of therapeutically relevant doses (Gerasimov *et al*, 2000; Swanson and Volkow, 2003). Although therapeutic doses of methylphenidate can produce dopamine overflow in the striatum in humans (Volkow *et al*, 2001), it remains to be seen whether or not prolonged clinical use induces molecular changes in the brain. However, recreational users are likely exposed to higher peak levels due to different amounts/routes of intake (Kollins *et al*, 2001; Swanson and Volkow, 2003) and may thus be more at risk for such gene regulation effects and their behavioral consequences.

ACKNOWLEDGEMENTS

This work was supported by Grant DA11261 (HS) and an Individual National Research Service Award F30 DA017998 (MY) from the National Institute on Drug Abuse. We thank Joel Beverley for excellent technical assistance, and Dr Gloria Meredith for critical comments on the manuscript.

REFERENCES

- Acheson AW, Thompson AC, Kristal MB, Baizer JS (2001). Methylphenidate induces *c-fos* expression in juvenile rats. *Soc Neurosci Abstr* 27: 223.4.
- Adams DH, Hanson GR, Keefe KA (2001). Differential effects of cocaine and methamphetamine on neurotensin/neuromedin N and preprotachykinin messenger RNA expression in unique regions of the striatum. *Neuroscience* 102: 843–851.
- Adams DH, Hanson GR, Keefe KA (2003). Distinct effects of methamphetamine and cocaine on preprodynorphin messenger RNA in rat striatal patch and matrix. *J Neurochem* 84: 87–93.
- Alloway KD, Crist J, Mutic JJ, Roy SA (1999). Corticostriatal projections from rat barrel cortex have an anisotropic organization that correlates with vibrissal whisking behavior. *J Neurosci* 19: 10908–10922.
- Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon WJ (2002). Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nat Neurosci* 5: 13–14.
- Andersen SL, LeBlanc CJ, Lyss PJ (2001). Maturation increases in *c-fos* expression in the ascending dopamine systems. *Synapse* 41: 345–350.
- Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1999). Environmental modulation of amphetamine-induced *c-fos* expression in D1 versus D2 striatal neurons. *Behav Brain Res* 103: 203–209.
- Basura GJ, Walker PD (1999). Differential sensitivity of tachykinin vs enkephalin gene expression in the posterior striatum in response to acute p-chloroamphetamine treatment during postnatal development. *Dev Brain Res* 112: 155–157.
- Beckstead RM (1979). An autoradiographic examination of corticocortical and subcortical projections of the medio-dorsal-projection (prefrontal) cortex in the rat. *J Comp Neurol* 184: 43–62.
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992). Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J Comp Neurol* 316: 314–347.
- Berke JD, Hyman SE (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron* 25: 515–532.
- Bhat RV, Baraban JM (1993). Activation of transcription factor genes in striatum by cocaine: role of both serotonin and dopamine systems. *J Pharmacol Exp Ther* 267: 496–505.
- Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ (2003). Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry* 54: 1317–1329.
- Brandon CL, Marinelli M, Baker LK, White FJ (2001). Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology* 25: 651–661.
- Brandon CL, Steiner H (2003). Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum. *Eur J Neurosci* 18: 1584–1592.
- Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993). The patterns of afferent innervation of the core and shell in the 'accumbens' part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol* 338: 255–278.
- Brown LL, Sharp FR (1995). Metabolic mapping of rat striatum: somatotopic organization of sensorimotor activity. *Brain Res* 686: 207–222.
- Brown LL, Smith DM, Goldbloom LM (1998). Organizing principles of cortical integration in the rat neostriatum: corticostriate map of the body surface is an ordered lattice of curved laminae and radial points. *J Comp Neurol* 392: 468–488.
- Butcher SP, Liptrot J, Arbuthnott GW (1991). Characterisation of methylphenidate and nomifensine induced dopamine release in rat striatum using *in vivo* brain microdialysis. *Neurosci Lett* 122: 245–248.
- Carelli RM, West MO (1991). Representation of the body by single neurons in the dorsolateral striatum of the awake, unrestrained rat. *J Comp Neurol* 309: 231–249.
- Carlezon WJ, Mague SD, Andersen SL (2003). Enduring behavioral effects of early exposure to methylphenidate in rats. *Biol Psychiatry* 54: 1330–1337.
- 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.
- Chase TD, Brown RE, Carrey N, Wilkinson M (2003). Daily methylphenidate administration attenuates *c-fos* expression in the striatum of prepubertal rats. *Neuroreport* 14: 769–772.
- Cowan RL, Wilson CJ (1994). Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. *J Neurophysiol* 71: 17–32.
- Crawford CA, McDougall SA, Meier TL, Collins RL, Watson JB (1998). Repeated methylphenidate treatment induces behavioral sensitization and decreases protein kinase A and dopamine-stimulated adenylyl cyclase activity in the dorsal striatum. *Psychopharmacology* 136: 34–43.
- Daunais JB, McGinty JF (1994). Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. *Synapse* 18: 35–45.
- Donoghue JP, Herkenham M (1986). Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. *Brain Res* 365: 397–403.

- Donoghue JP, Wise SP (1982). The motor cortex of the rat: cytoarchitecture and microstimulation mapping. *J Comp Neurol* 212: 76–88.
- Drago J, Gerfen CR, Westphal H, Steiner H (1996). D1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. *Neuroscience* 74: 813–823.
- Ehrlich ME, Sommer J, Canas E, Unterwald EM (2002). Periadolescent mice show enhanced DeltaFosB upregulation in response to cocaine and amphetamine. *J Neurosci* 22: 9155–9199.
- Gardier AM, Moratalla R, Cuellar B, Sacerdote M, Guibert B, Lebrech H *et al* (2000). Interaction between the serotonergic and dopaminergic systems in *D*-fenfluramine-induced activation of *c-fos* and *jun B* genes in rat striatal neurons. *J Neurochem* 74: 1363–1373.
- Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sci* 58: 231–239.
- Gaytan O, al-Rahim S, Swann A, Dafny N (1997). Sensitization to locomotor effects of methylphenidate in the rat. *Life Sci* 61: PL101–107.
- Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D *et al* (2000). Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. *J Pharmacol Exp Ther* 295: 51–57.
- Gerfen CR, Wilson CJ (1996). The basal ganglia. In: Swanson LW, Björklund A, Hökfelt T (eds). *Handbook of Chemical Neuroanatomy*. Elsevier: Amsterdam. pp 371–468.
- Gerfen CR, Young III WS (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.
- Graybiel AM (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci* 13: 244–254.
- Graybiel AM, Canales JJ, Capper-Loup C (2000). Levodopa-induced dyskinesias and dopamine-dependent stereotypies: a new hypothesis. *Trends Neurosci* 23: S71–S77.
- Harlan RE, Garcia MM (1998). Drugs of abuse and immediate-early genes in the forebrain. *Mol Neurobiol* 16: 221–267.
- Hoffer ZS, Alloway KD (2001). Organization of corticostriatal projections from the vibrissal representations in the primary motor and somatosensory cortical areas of rodents. *J Comp Neurol* 439: 87–103.
- 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.
- Hurd YL, Herkenham M (1992). Influence of a single injection of cocaine, amphetamine or GBR 12909 on mRNA expression of striatal neuropeptides. *Mol Brain Res* 16: 97–104.
- Hurd YL, Ungerstedt U (1989). *In vivo* neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen. *Eur J Pharmacol* 166: 251–260.
- Hyman SE, Cole RL, Schwarzschild M, Cole D, Hope B, Konradi C (1996). Molecular mechanisms of striatal gene regulation: a critical role for glutamate in dopamine-mediated gene induction. In: Merchant KM (ed). *Pharmacological Regulation of Gene Expression in the CNS*. CRC Press: Boca Raton, FL. pp 115–131.
- Hyman SE, Malenka RC (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat Rev Neurosci* 2: 695–703.
- Hyman SE, Nestler EJ (1996). Initiation and adaptation: a paradigm for understanding psychotropic drug action. *Am J Psychiatry* 153: 151–162.
- Izenwasser S, Coy AE, Ladenheim B, Loeloff RJ, Cadet JL, French D (1999). Chronic methylphenidate alters locomotor activity and dopamine transporters differently from cocaine. *Eur J Pharmacol* 373: 187–193.
- Johansson B, Lindström K, Fredholm BB (1994). Differences in the regional and cellular localization of *c-fos* messenger RNA induced by amphetamine, cocaine and caffeine in the rat. *Neuroscience* 59: 837–849.
- Kankaanpää A, Meririnne E, Seppälä T (2002). 5-HT₃ receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. *Psychopharmacology* 159: 341–350.
- Keefe KA, Xochime C, Hanson GR, Adams DH (2002). Cocaine-induced predynorphin mRNA expression in the striatal matrix is dependent on serotonin. *Soc Neurosci Abstr* 28: 500.6.
- Kincaid AE, Wilson CJ (1996). Corticostriatal innervation of the patch and matrix in the rat neostriatum. *J Comp Neurol* 374: 578–592.
- Kollins SH, MacDonald EK, Rush CR (2001). Assessing the abuse potential of methylphenidate in nonhuman and human subjects: a review. *Pharmacol Biochem Behav* 68: 611–627.
- Kosofsky BE, Genova LM, Hyman SE (1995). Substance P phenotype defines specificity of *c-fos* induction by cocaine in developing rat striatum. *J Comp Neurol* 351: 41–50.
- Kuczenski R, Segal DS (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *J Neurochem* 68: 2032–2037.
- Lin JS, Hou Y, Jouvét M (1996). Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by *c-fos* immunocytochemistry in the cat. *Proc Natl Acad Sci USA* 93: 14128–14133.
- Martin-Iverson MT, Ortmann R, Fibiger HC (1985). Place preference conditioning with methylphenidate and nomifensine. *Brain Res* 332: 59–67.
- Mathieu-Kia AM, Besson MJ (1998). Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. *Mol Brain Res* 54: 141–151.
- McDougall SA, Collins RL, Karper PE, Watson JB, Crawford CA (1999). Effects of repeated methylphenidate treatment in the young rat: sensitization of both locomotor activity and stereotyped sniffing. *Exp Clin Psychopharmacol* 7: 208–218.
- McGeorge AJ, Faull RLM (1987). The organization and collateralization of corticostriate neurones in the motor and sensory cortex of the rat brain. *Brain Res* 423: 318–324.
- McGeorge AJ, Faull RLM (1989). The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience* 29: 503–537.
- Meririnne E, Kankaanpää A, Seppälä T (2001). Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and effects of dopamine D1- and D2-receptor antagonists. *J Pharmacol Exp Ther* 298: 539–550.
- Mijnster MJ, Galis-de Graaf Y, Voorn P (1998). Serotonergic regulation of neuropeptide and glutamic acid decarboxylase mRNA levels in the rat striatum and globus pallidus: studies with fluoxetine and DOI. *Mol Brain Res* 54: 64–73.
- Moll GH, Hause S, Ruther E, Rothenberger A, Huether G (2001). Early methylphenidate administration to young rats causes a persistent reduction in the density of striatal dopamine transporters. *J Child Adolesc Psychopharmacol* 11: 15–24.
- Morris BJ, Reimer S, Holtt V, Herz A (1988). Regulation of striatal prodynorphin mRNA levels by the raphe–striatal pathway. *Brain Res* 464: 15–22.
- Nestler EJ (2001). Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* 2: 119–128.
- Papa M, Diwald L, Carey MP, Esposito FJ, Gironi Carnevale UA, Sadile AG (2002). A rostro-caudal dissociation in the dorsal and ventral striatum of the juvenile SHR suggests an anterior hypo- and a posterior hyperfunctioning mesocorticolimbic system. *Behav Brain Res* 130: 171–179.
- Passingham RE, Myers C, Rawlins N, Lightfoot V, Fearn S (1988). Premotor cortex in the rat. *Behav Neurosci* 102: 101–109.

- Paxinos G, Watson C (1998). *The Rat Brain in Stereotaxic Coordinates*. Academic Press: New York.
- Penner MR, McFadyen MP, Pinaud R, Carrey N, Robertson HA, Brown RE (2002). Age-related distribution of *c-fos* expression in the striatum of CD-1 mice after acute methylphenidate administration. *Dev Brain Res* 135: 71–77.
- Preuss TM (1995). Do rats have prefrontal cortex? The Rose–Woolsey–Akert program reconsidered. *J Cogn Neurosci* 7: 1–24.
- Reep RL, Cheatwood JL, Corwin JV (2003). The associative striatum: organization of cortical projections to the dorsocentral striatum in rats. *J Comp Neurol* 467: 271–292.
- Reep RL, Corwin JV (1999). Topographic organization of the striatal and thalamic connections of rat medial agranular cortex. *Brain Res* 841: 43–52.
- Reep RL, Corwin JV, Hashimoto A, Watson RT (1987). Efferent connections of the rostral portion of medial agranular cortex in rats. *Brain Res Bull* 19: 203–221.
- Sandoval V, Riddle EL, Hanson GR, Fleckenstein AE (2002). Methylphenidate redistributes vesicular monoamine transporter-2: role of dopamine receptors. *J Neurosci* 22: 8705–8710.
- Schenk S, Izenwasser S (2002). Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine. *Pharmacol Biochem Behav* 72: 651–657.
- Schweri MM, Skolnick P, Rafferty MF, Rice KC, Janowsky AJ, Paul SM (1985). [³H]Threo-(±)-methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters. *J Neurochem* 45: 1062–1070.
- Shuster L, Hudson J, Anton M, Righi D (1982). Sensitization of mice to methylphenidate. *Psychopharmacology* 77: 31–36.
- Smith AJW, McGinty JF (1994). Acute amphetamine or methamphetamine alters opioid peptide mRNA expression in rat striatum. *Mol Brain Res* 21: 359–362.
- Spangler R, Ho A, Zhou Y, Maggos CE, Yuferov V, Kreek MJ (1996). Regulation of kappa opioid receptor mRNA in the rat brain by 'binge' pattern cocaine administration and correlation with prodynorphin mRNA. *Mol Brain Res* 38: 71–76.
- Spangler R, Zhou Y, Maggos CE, Schlussman SD, Ho A, Kreek MJ (1997). Prodynorphin, proenkephalin and kappa opioid receptor mRNA responses to acute 'binge' cocaine. *Mol Brain Res* 44: 139–142.
- Steiner H, Gerfen CR (1993). Cocaine-induced *c-fos* messenger RNA is inversely related to dynorphin expression in striatum. *J Neurosci* 13: 5066–5081.
- Steiner H, Gerfen CR (1998). Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. *Exp Brain Res* 123: 60–76.
- Steiner H, Gerfen CR (1999). Enkephalin regulates acute D2 dopamine receptor antagonist-induced immediate-early gene expression in striatal neurons. *Neuroscience* 88: 795–810.
- Steiner H, Kitai ST (2000). Regulation of rat cortex function by D1 dopamine receptors in the striatum. *J Neurosci* 20: 5449–5460.
- Swanson JM, Volkow ND (2003). Serum and brain concentrations of methylphenidate: implications for use and abuse. *Neurosci Biobehav Rev* 27: 615–621.
- Torres G, Horowitz JM (1999). Drugs of abuse and brain gene expression. *Psychosom Med* 61: 630–650.
- Torres G, Rivier C (1994). Induction of *c-fos* in rat brain by acute cocaine and fenfluramine exposure: a comparison study. *Brain Res* 647: 1–9.
- Trinh JV, Nehrenberg DL, Jacobsen JP, Caron MG, Wetsel WC (2003). Differential psychostimulant-induced activation of neural circuits in dopamine transporter knockout and wild type mice. *Neuroscience* 118: 297–310.
- Uslaner J, Badiani A, Norton CS, Day HE, Watson SJ, Akil H *et al* (2001). Amphetamine and cocaine induce different patterns of *c-fos* mRNA expression in the striatum and subthalamic nucleus depending on environmental context. *Eur J Neurosci* 13: 1977–1983.
- Uylings HB, Groenewegen HJ, Kolb B (2003). Do rats have a prefrontal cortex? *Behav Brain Res* 146: 3–17.
- Vargo JM, Marshall JF (1995). Time-dependent changes in dopamine agonist-induced striatal Fos immunoreactivity are related to sensory neglect and its recovery after unilateral prefrontal cortex injury. *Synapse* 20: 305–315.
- Vargo JM, Marshall JF (1997). Reduced eticlopride-induced Fos expression in caudate-putamen and globus pallidus after unilateral frontal cortex injury: relation to neglect. *Neuroscience* 76: 1083–1095.
- Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L *et al* (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci* 21(RC121): 1–5.
- Walker PD, Capodilupo JG, Wolf WA, Carlock LR (1996). Preprotachykinin and preproenkephalin mRNA expression within striatal subregions in response to altered serotonin transmission. *Brain Res* 732: 25–35.
- Wang JQ, McGinty JF (1996a). D1 and D2 receptor regulation of preproenkephalin and preprodynorphin mRNA in rat striatum following acute injection of amphetamine or methamphetamine. *Synapse* 22: 114–122.
- Wang JQ, McGinty JF (1996b). Glutamatergic and cholinergic regulation of immediate-early gene and neuropeptide gene expression in the striatum. In: Merchant KM (ed). *Pharmacological Regulation of Gene Expression in the CNS*. CRC Press: Boca Raton, FL. pp 81–113.
- Wang JQ, Smith AJW, McGinty JF (1995). A single injection of amphetamine or methamphetamine induces dynamic alterations in *c-fos*, *zif/268* and preprodynorphin messenger RNA expression in rat forebrain. *Neuroscience* 68: 83–95.
- Willuhn I, Sun W, Steiner H (2003). Topography of cocaine-induced gene regulation in the rat striatum: Relationship to cortical inputs and role of behavioural context. *Eur J Neurosci* 17: 1053–1066.
- Wright AK, Norrie L, Ingham CA, Hutton EA, Arbuthnott GW (1999). Double anterograde tracing of outputs from adjacent 'barrel columns' of rat somatosensory cortex. Neostriatal projection patterns and terminal ultrastructure. *Neuroscience* 88: 119–133.
- Wright AK, Ramanathan S, Arbuthnott GW (2001). Identification of the source of the bilateral projection system from cortex to somatosensory neostriatum and an exploration of its physiological actions. *Neuroscience* 103: 87–96.
- Wright CI, Groenewegen HJ (1996). Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. *Neuroscience* 73: 359–373.
- Yano M, Steiner H (2003). Temporal and regional topographies of methylphenidate-induced gene regulation in the striatum and cortex of the adult rat. *Soc Neurosci Abstr* 29: 855.17.
- Yano M, Steiner H (2005). Methylphenidate (Ritalin) induces Homer 1a and zif 268 expression in specific corticostriatal circuits. *Neuroscience*, in press.