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Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior

Abstract Projection neurons in the striatum give rise to two output systems, the "direct" and "indirect" pathways, which antagonistically regulate basal ganglia output. While all striatal projection neurons utilize GABA as their principal neurotransmitter, they express different opioid peptide co-transmitters and also different dopamine receptor subtypes. Neurons of the direct pathway express the peptide dynorphin and the D1 dopamine receptor, whereas indirect pathway neurons express the peptide enkephalin and the D2 receptor. In the present review, we summarize our findings on the function of dynorphin and enkephalin in these striatal output pathways. In these studies, we used D1- or D2-receptor-mediated induction of immediate-early genes as a cellular response in direct or indirect projection neurons, respectively, to investigate the role of these opioid peptides. Our results suggest that the specific function of dynorphin and enkephalin is to dampen excessive activation of these neurons by dopamine and other neurotransmitters. Levels of these opioid peptides are elevated by repeated, excessive activation of these pathways, which appears to be an adaptive or compensatory response. Behavioral consequences of increased opioid peptide function in striatal output pathways may include behavioral sensitization (dynorphin) and recovery of motor function (enkephalin).

Key words Basal ganglia · Dopamine · Dynorphin · Enkephalin · Opioid · Striatum · Substantia nigra

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Introduction

The striatum (caudate nucleus, putamen, nucleus accumbens) is among the brain regions with the highest levels of opioid peptides and receptors. The striatum is the most prominent portion of the basal ganglia, a group of forebrain nuclei that is part of the extrapyramidal motor system. This nucleus receives and processes inputs from most areas of the cerebral cortex, some regions of the thalamus, and other brain areas and projects to the two principal output nuclei of the basal ganglia, the pallidum and the substantia nigra, which in turn provide input back to the cortex (via the thalamus) and to the superior colliculus and the pedunculopontine nucleus. The importance of these circuits for normal behavior is emphasized, for example, by the devastating functional deficits seen in neurodegenerative disorders involving the basal ganglia, such as Parkinson's disease and Huntington's disease. Thus, balanced activity in striatal output pathways is required for normal motor functioning (Albin et al. 1989; Alexander et al. 1990; DeLong 1990). Furthermore, basal ganglia circuits are thought to be involved in psychotic disorders, such as schizophrenia, and mediate behavioral effects of drugs of abuse.

It has long been recognized that the striatum contains the opioid peptides dynorphin and enkephalin and their binding sites, kappa, mu, and delta opioid receptors. The present review summarizes a series of studies, in which we have investigated the functional role of these opioid systems in the striatum. In these studies, we have employed pharmacologically induced expression of certain genes, so-called immediate-early genes (IEGs), in neurochemically identified neuron populations as a functional marker to study the actions of opioid peptides in the striatum. Gene expression was measured with quantitative in situ hybridization histochemistry. Our results indicate that these opioid peptides act in the striatum to regulate the function of the striatal projection neurons in which they are expressed.

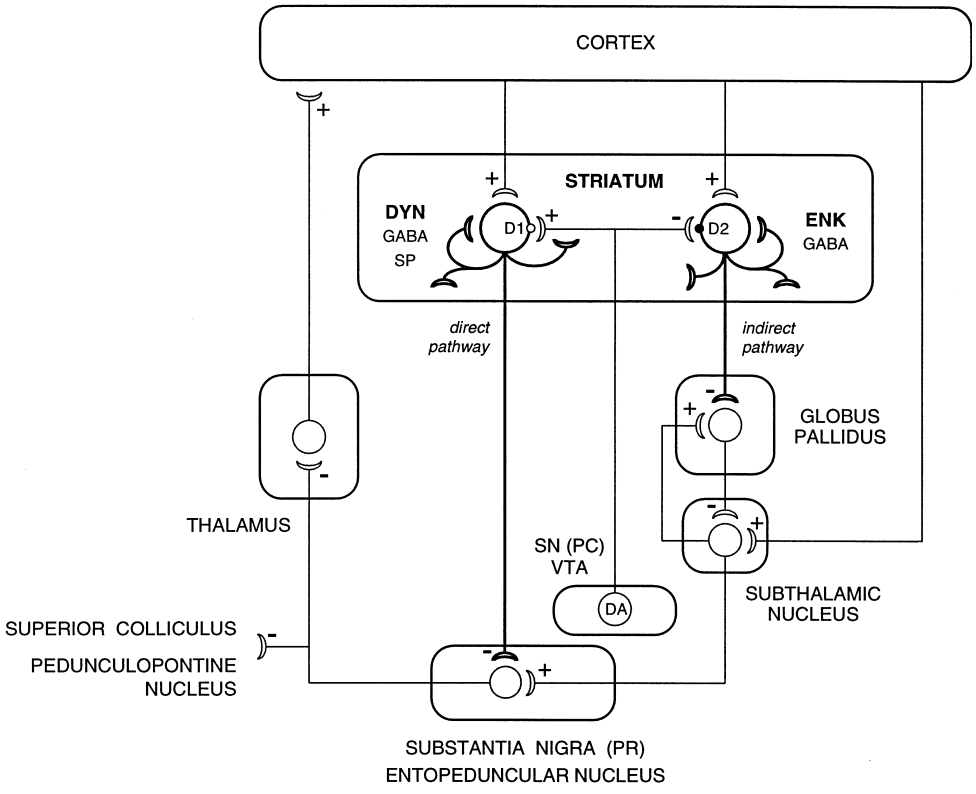

Fig. 1 Simplified diagram of basal ganglia nuclei and connections. Note the distribution of dopamine-receptor subtypes (*D1*, *D2*) and neuropeptides between the two striatal output pathways. Neurons that project to the substantia nigra and/or the entopeduncular nucleus – the "direct" pathway – contain dynorphin (*DYN*) and substance P (*SP*) in addition to the inhibitory neurotransmitter GABA. Neurons that project to the globus pallidus – the first segment of the "indirect" pathway – contain enkephalin (*ENK*) and GABA. *DA* Dopamine, *SN* substantia nigra, *PC* pars compacta, *PR* pars reticulata, *VTA* ventral tegmental area

Striatal projection neurons contain opioid peptides

The following provides a short overview of the structural details of the striatum and its connections, that are relevant for this review (Fig. 1) (for more extensive reviews of the functional organization of the basal ganglia, see, e.g., Gerfen 1992; Gerfen and Wilson 1996). About 90–95% of striatal neurons are projection neurons. These neurons can be divided into two subtypes depending on their projection targets. One subtype – striatonigral neurons – projects to the substantia nigra and/or the entopeduncular nucleus (internal segment of the globus pallidus in primates) and also gives off minor axon collaterals to the globus pallidus (external segment of the globus pallidus in primates) (Kawaguchi et al. 1990). The other subtype – striatopallidal neurons – projects only to the globus pallidus. Striatonigral neurons constitute the so-called "direct" pathway from the striatum to the basal-ganglia output nuclei, while striatopallidal neurons are the first segment of the "indirect" pathway (Albin et al. 1989). In addition to their efferent axon, these projection neurons feature extensive axon collaterals within the striatum (Park et al. 1980; Wilson and Groves 1980; Kawaguchi et al. 1990). Striatonigral and striatopallidal neurons are intermingled throughout the striatum and are approximately equal in number (Gerfen and Young 1988).

Besides the excitatory inputs from cortex and thalamus, the striatum receives further important input from dopamine neurons in the substantia nigra pars compacta and ventral tegmental area. Dopamine differentially affects striatal projection neurons based on the dopamine receptor subtypes they express. Thus, the two main subtypes of dopamine receptors, D1 and D2 receptors, are largely segregated between striatonigral and striatopallidal neurons, with D1 receptors being mainly expressed in the former and D2 receptors in the latter, and with only a minor portion of neurons containing both receptors (Gerfen et al. 1990; Le Moine et al. 1990, 1991; Curran and Watson 1995; Le Moine and Bloch 1995). While these earlier findings of differential dopamine receptor expression were obtained in studies that colocalized receptor mRNAs with other markers, recent immunohistochemical studies with receptor-selective antibodies confirmed the general segregation of D1 and D2 dopamine-receptor subtypes between the two striatal output pathways (Hersch et al. 1995; Yung et al. 1995, 1996).

Both subtypes of striatal projection neurons use the inhibitory neurotransmitter GABA (Kita and Kitai 1988). However, they also differ in a number of neuropeptides they express (Fig. 1). Striatonigral neurons generally contain dynorphin and substance P, whereas striatopallidal neurons express enkephalin (Brownstein et al. 1977; Vincent et al. 1982; Beckstead and Kersey 1985; Gerfen and Young 1988; Reiner and Anderson 1990; Curran and Watson 1995; Le Moine and Bloch 1995).

Dopamine receptors regulate neuropeptide gene expression in striatal neurons

A great body of work has demonstrated that dopamine regulates the expression of neuropeptides in striatal pro-

jection neurons (for reviews, see Gerfen 1992; Angulo and McEwen 1994; Gerfen et al. 1996). Consistent with the differential distribution of dopamine receptors, gene regulation by dopamine is opposite in the two subtypes of projection neurons. For example, dopamine depletion by the neurotoxin 6-hydroxydopamine (6-OHDA) leads to a *decrease* in substance P and dynorphin expression in striatonigral neurons (Young et al. 1986; Voorn et al. 1987; Gerfen et al. 1990, 1991; Li et al. 1990; Engber et al. 1992), an effect that can be reversed with selective D1-receptor agonists (e.g., Gerfen et al. 1990; Engber et al. 1992). In contrast, dopamine denervation results in *increased* levels of enkephalin mRNA and peptide in striatopallidal neurons (Young et al. 1986; Voorn et al. 1987; Gerfen et al. 1990, 1991; Li et al. 1990; Engber et al. 1992). This effect is reversible with D2-receptor agonist treatment (e.g., Gerfen et al. 1990; Engber et al. 1992). Also, blockade of D2 receptors with neuroleptics increases enkephalin expression (e.g., Hong et al. 1978; Tang et al. 1983). Consistent with these findings, transgenic animals that lack functional D1 receptors (D1-receptor knockouts) display decreased expression of substance P and dynorphin, with minimal or no effects on enkephalin expression (Drago et al. 1994, 1996; Xu et al. 1994). On the other hand, mice that lack D2 receptors show mostly increased enkephalin expression (Baik et al. 1995). These results demonstrate opposite gene regulation by D1- and D2-receptor stimulation in striatonigral and striatopallidal neurons, respectively. However, synergistic interactions between D1 and D2 receptors also exist and can be demonstrated at the level of gene expression (e.g., Paul et al. 1992; LaHoste et al. 1993; Gerfen et al. 1995; Keefe and Gerfen 1995; see also Gerfen et al. 1996). It is thought that these interactions are mediated by axon collaterals of projection neurons or by interneurons that connect the two types of projection neurons (Gerfen et al. 1995; Wang and McGinty 1996).

While selective dopamine receptor agonists are most effective in altering gene regulation in striatal neurons after dopamine depletion (see above), indirect dopamine receptor agonists such as cocaine and amphetamine are very potent in changing such gene expression in normal animals. These drugs, also called psychomotor stimulants, promote dopamine release and/or inhibit reuptake and thus produce increased extracellular levels of dopamine (Di Chiara and Imperato 1988a; Hurd et al. 1989; Kuczenski and Segal 1992) and, consequently, enhanced dopamine-receptor stimulation. Numerous studies have shown that treatment with cocaine or amphetamine increases mRNA and peptide levels for dynorphin and to some degree substance P in the striatonigral pathway, with minor effects on enkephalin expression (Hanson et al. 1987, 1988; Li et al. 1988; Sivam 1989; Smiley et al. 1990; Trujillo et al. 1990; Hurd and Herkenham 1992; Hurd et al. 1992; Steiner and Gerfen 1993; Spangler et al. 1993; Daunais and McGinty 1994; Wang et al. 1994).

Our series of studies, summarized below, provides evidence for the functional significance of increased dynorphin expression in striatonigral neurons following treatment with such dopamine agonists and indicates a role for enkephalin in the regulation of striatopallidal neurons.

IEG induction in striatal projection neurons as a marker for dopamine receptor responses

Pharmacologic treatments that target dopamine receptors also increase the expression of IEGs, including c-*fos* and *zif 268* (*NGFI-A*), in striatal neurons. Such IEGs encode transcription factors that are part of the cellular machinery mediating transsynaptic gene regulation (Morgan and Curran 1989; Sheng and Greenberg 1990; Robertson 1992). IEGs are rapidly and transiently activated by a variety of experimental treatments, and it is assumed that such gene expression is correlated to some degree with neuronal activity, at least in some neuronal systems (Sagar et al. 1988; Dragunow and Faull 1989; Melzer and Steiner 1997).

As is the case for neuropeptide expression, D1 and D2 receptors exert opposite effects on IEG expression in striatal projection neurons. Stimulation of D1 receptors induces IEG expression principally in striatonigral neurons. This was first shown with selective D1-receptor agonist treatment in rats with 6-OHDA lesions by colocalizing Fos-like immunoreactivity with retrograde tracers injected into the substantia nigra (Robertson et al. 1990, 1992). Moreover, in such dopamine-depleted animals, D1-agonist-induced IEG expression occurs in striatal neurons that do not contain enkephalin mRNA (Gerfen et al. 1995). In normal rats, the indirect dopamine-receptor agonists cocaine and amphetamine also induce IEGs in the striatum (Graybiel et al. 1990; Young et al. 1991; Berretta et al. 1992; Cole et al. 1992; Hope et al. 1992; Moratalla et al. 1992; Bhat and Baraban 1993; Steiner and Gerfen 1993; Daunais and McGinty 1994; Wang et al. 1994). Various colocalization techniques have been used to demonstrate that IEG induction by these drugs also predominantly occurs in striatonigral neurons (Cenci et al. 1992; Johansson et al. 1994; Kosofsky et al. 1995). Furthermore, such gene induction is mediated by D1 receptors. Thus, systemic (Graybiel et al. 1990; Young et al. 1991; Cole et al. 1992; Moratalla et al. 1992; Bhat and Baraban 1993) and intrastriatal administration (Steiner and Gerfen 1995) of selective D1-receptor antagonists block IEG expression induced by psychomotor stimulants in striatal neurons. Consistently, eliminating D1 receptors with gene targeting also eliminates the IEG response to psychomotor stimulants in the striatum (Drago et al. 1996; Moratalla et al. 1996). However, recent studies showed that D2-receptor activation contributes to a full IEG response to cocaine and amphetamine (Ruskin and Marshall 1994) or the selective D1-receptor agonist SKF-38393 (Gerfen et al. 1995) in striatonigral neurons, an example for synergistic interactions between D1 and D2 receptors.

In contrast to psychomotor stimulant-induced IEG expression in striatonigral neurons, which is mediated by *stimulation* of D1 receptors, *blockade* of D2 receptors increases IEG expression in striatopallidal neurons (Dragunow et al. 1990; Robertson et al. 1992; Robertson and Fi-

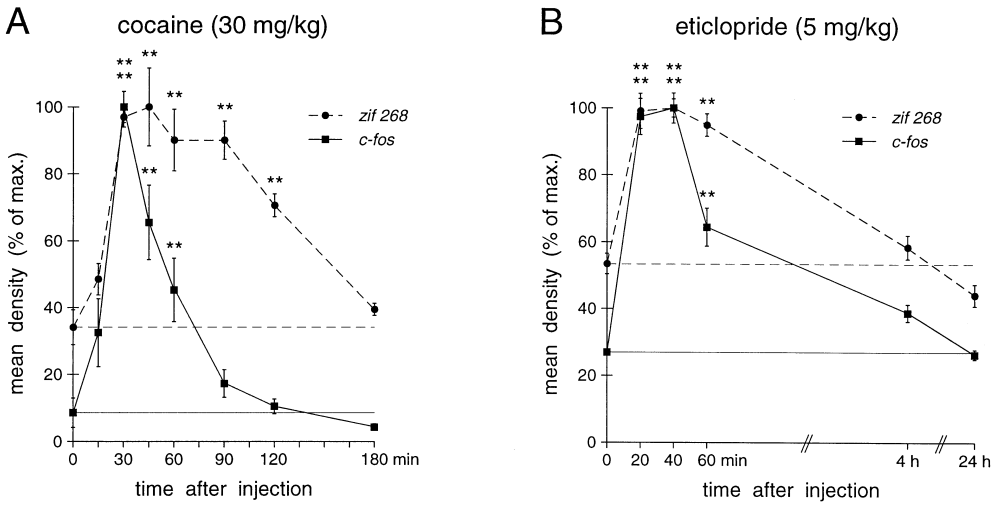

Fig. 2 Time course of c-*fos* and *zif 268* mRNA levels in the striatum after administration of the psychomotor stimulant cocaine (30 mg/kg, i.p.) (**A**) or the selective D2-receptor antagonist eticlopride (5 mg/kg, i.p.) (**B**). Gene expression was determined by in situ hybridization histochemistry, and the hybridization signals were measured with densitometry on film autoradiograms. Mean density values (mean±SEM, in % of maximal values) measured in the dorsal striatum at various time points after the drug injection are shown. ** $P<0.01$, versus 0 min; ANOVA, Dunnett's test

biger 1992). Since this IEG response is also, at least in part, dependent on glutamate (NMDA) receptor activation or cortical input (Dragunow et al. 1990; Ziólkowska and Höllt 1993; Boegman and Vincent 1996; Vargo and Marshall 1997), it is thought to reflect disinhibition of striatopallidal neurons by blockade of inhibitory D2 receptors.

IEG induction in striatal neurons after dopamine receptor agonist and antagonist treatments is very rapid and transient. Figure 2 shows the time course of c-*fos* and *zif 268* expression in the striatum following high doses of cocaine (30 mg/kg, i.p.) or the selective D2-receptor antagonist eticlopride (5 mg/kg, i.p.), doses we typically used in the experiments summarized below. Levels of c-*fos* and *zif 268* mRNAs peak about 30 min after cocaine injection and return to baseline within 90 min to 3 h (Fig. 2A). Following eticlopride administration, these mRNAs reach peak levels also at 20–40 min after the injection and return to baseline within 4 h (Fig. 2B).

In our studies, we have employed dopamine receptor-mediated IEG induction as a cellular response to investigate how striatal opioid systems regulate the two striatal output pathways. On the one hand, we examined cocaine- and D1-receptor-agonist-induced IEG expression as a marker for D1-receptor responses in striatonigral neurons. On the other, IEG induction by the D2-receptor antagonist eticlopride was used to investigate the effects of opioid-receptor stimulation on striatopallidal neurons.

Dynorphin regulates responsiveness of striatonigral neurons

IEG induction by cocaine is related to dynorphin expression in striatal neurons

Negative correlation between acute IEG response to cocaine and local dynorphin expression

The initial observation that led to this series of studies was the finding that the magnitude of IEG induction by

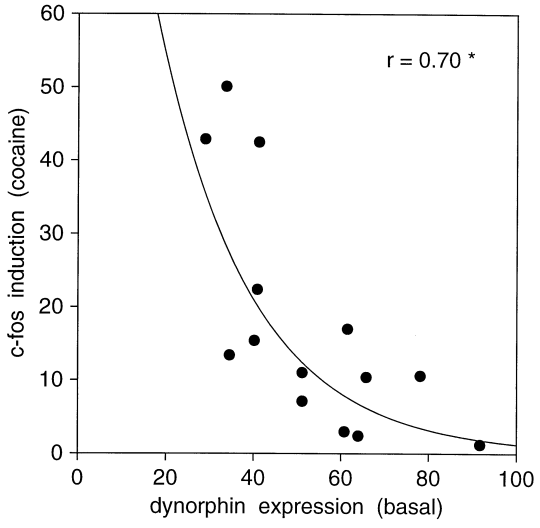

Fig. 3 Correlation between c-*fos* induction by cocaine and local dynorphin expression in the striatum. *Scatter plot* depicts dynorphin mRNA levels (mean density values, arbitrary units) measured in vehicle-treated rats and c-*fos* mRNA levels in rats killed 30 min after a cocaine injection (30 mg/kg) for 14 striatal regions, each represented by a *filled circle* (for regions, see Steiner and Gerfen 1993). *r* Pearson's correlation coefficient; * $P<0.05$, *t*-test

cocaine is related to the level of dynorphin expression in a given striatal region (Fig. 3) (Steiner and Gerfen 1993). IEG induction by cocaine displays distinct regional variations. This response is most pronounced in the dorsal central striatum and considerably weaker in ventral and medial striatal regions (Fig. 4A). A comparison with regional levels of neuropeptide expression revealed a significant negative correlation between cocaine-induced c-*fos* mRNA levels and basal levels of dynorphin mRNA, as determined in 14 regions on three rostrocaudal levels of the striatum (Fig. 3; Steiner and Gerfen 1993). Thus, IEG induction is high in striatal areas with low levels of dynorphin mRNA and low in areas with high levels of dynorphin expression. This relationship is unique in that

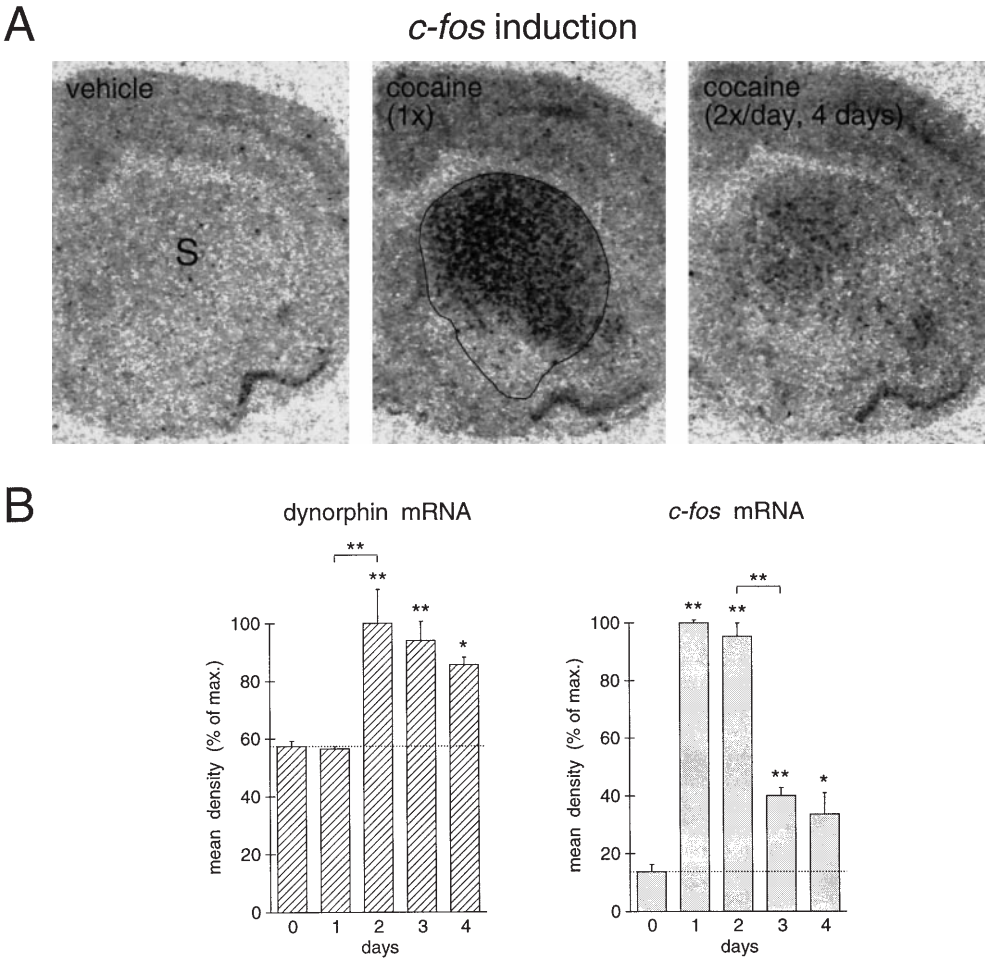

Fig. 4A, B Inverse changes in dynorphin expression and c-*fos* induction in the striatum during repeated cocaine treatment. **A** Film autoradiograms depict the expression of c-*fos* mRNA in coronal sections through the striatum (*S*) in animals that received vehicle injections (*left*), vehicle injections for 3 days followed by one cocaine injection (30 mg/kg) on day 4 (*middle*), or cocaine twice daily for 4 days (*right*). All animals were killed 30 min after the last injection. Maximal hybridization signal is *black*. The striatum is outlined in the *middle panel*. **B** Time course of changes in dynorphin (*left*) and c-*fos* mRNA levels (*right*) during repeated cocaine treatment (adapted from Steiner and Gerfen 1993). Mean density values (mean+SEM, in % of maximal values) measured in the dorsal striatum are shown. Rats received repeated vehicle injections (0-day group), vehicle injections followed by one cocaine injection (30 mg/kg) (1-day group), or cocaine injections twice daily for 2, 3, or 4 days. ** $P<0.01$, * $P<0.05$; versus 0 days or as indicated, ANOVA, Tukey-Kramer test

there is no correlation between the c-*fos* response and the expression of the neuropeptides substance P or enkephalin (Steiner and Gerfen 1993). Therefore, local dynorphin levels seem to determine the magnitude of this D1 receptor response in the striatum.

Inverse changes in the IEG response and in dynorphin expression during repeated cocaine treatment

As described above, repeated treatment with cocaine results in increased expression of dynorphin in the striatum. If dynorphin regulated the IEG response to cocaine, then such increased dynorphin expression could be expected to affect the inducibility of IEGs by cocaine. To test this notion, we treated animals twice daily for 4 days with cocaine (30 mg/kg, i.p.), which is sufficient to produce increased dynorphin expression (Steiner and Gerfen 1993), and examined the IEG response on the last treatment day. Our results showed that this drug treatment results in significantly suppressed c-*fos* induction by cocaine (Fig. 4) (Steiner and Gerfen 1992, 1993). This effect is most dramatic in the dorsal striatum, where acute IEG induction by cocaine is strongest. Suppressed IEG induction after repeated cocaine treatment has also been shown in the nucleus accumbens (Hope et al. 1992) and has been confirmed for the dorsal striatum (e.g., Daunais and McGinty 1994; Hope et al. 1994; Rosen et al. 1994).

We then investigated the temporal relationship between changes in dynorphin expression and those in the IEG response. Again, rats were treated with cocaine twice daily for up to 4 days. Results demonstrated that the changes in dynorphin and c-*fos* expression followed an inverse time course (Fig. 4B; Steiner and Gerfen 1993). Dynorphin mRNA levels were significantly increased from treatment day 2 on and remained elevated thereafter. In contrast, c-*fos* induction was maximal after the first cocaine administration. On the second day (i.e., after the third drug injection), the IEG response was still comparable to the acute response. However, from treatment day 3 on, this response was significantly attenuated (Fig. 4). Importantly, repeated cocaine treatment for up to 10 days did not completely suppress the IEG response (Steiner and Gerfen, unpublished results). Thus, this D1 receptor response is blunted, but not eliminated, by repeated cocaine treatment.

Our studies showed that suppressed gene induction in striatonigral neurons is not limited to IEGs. Acute cocaine administration also increases the expression of substance P in these neurons within 30 min (Steiner and Gerfen

1993; Drago et al. 1996). During repeated cocaine treatment, this substance P response was also suppressed in parallel to that of IEGs (Steiner and Gerfen 1993).

Together, these findings demonstrate that such psychostimulant treatments attenuate these types of cellular responses in striatonigral neurons. The temporal relationship between the increase in dynorphin synthesis and the decrease in IEG (and substance P) induction is consistent with a causal relationship between increased dynorphin function and suppressed responsiveness of these neurons.

Increase in dynorphin expression after repeated cocaine treatment is related to the magnitude of acute IEG response

The distinct regional variation in the magnitude of acute c-*fos* induction by cocaine allowed us to ask whether there is a relationship between this acute D1-receptor response in striatonigral neurons and the subsequent increase in dynorphin expression in these neurons. Regional comparisons revealed that the same striatal areas that showed the strongest acute c-*fos* response to cocaine displayed the strongest increase in dynorphin expression with repeated treatment, while areas with less initial c-*fos* induction showed a reduced increase in dynorphin mRNA (for 14 striatal regions, $r=0.91$, $P<0.01$, Steiner and Gerfen 1993). Thus, the magnitude of the initial D1-receptor response predicted the magnitude of the subsequent increase in dynorphin expression.

These results, together with the finding of blunted gene induction after repeated cocaine treatment, suggest that the increase in dynorphin expression is an adaptive or compensatory response to repeated, excessive D1-receptor stimulation in striatonigral neurons, a mechanism that seems to dampen the effects of subsequent D1-receptor stimulation (Steiner and Gerfen 1993).

IEG induction by cocaine is inhibited by systemic and intrastriatal administration of a dynorphin agonist

The results summarized above showed that the magnitude of D1-receptor-mediated IEG induction in striatal neurons is negatively correlated with the level of local dynorphin expression in the striatum. To demonstrate a possible causal relationship between dynorphin function and the IEG response, we next tested whether dynorphin receptor stimulation could indeed inhibit cocaine-induced IEG expression. Dynorphin is considered an endogenous ligand of kappa opioid receptors, which are known to have inhibitory effects in the nervous system (Chavkin et al. 1982; Corbett et al. 1982). We examined whether the kappa-receptor agonist spiradoline (U-62066) affects the IEG response to cocaine. Spiradoline had been shown to inhibit D1-, but not D2-, receptor-agonist-induced turning behavior after 6-OHDA lesions, indicating an inhibitory effect of this drug on striatonigral activity (Engber et al. 1991). First, spiradoline was administered systemically. Animals received different doses of spiradoline (0.5–10 mg/kg, i.p.) 15 min before the cocaine injection (30 mg/kg). Our results showed that systemic spiradoline reduced c-*fos* and *zif 268* induction by cocaine in striatal neurons in a dose-dependent manner (Steiner and Gerfen 1995). Systemic spiradoline also inhibited the acute behavioral activation by cocaine (Steiner and Gerfen 1995). Moreover, recent studies showed that the initiation of behavioral "sensitization" by cocaine, behavioral changes that occur with repeated dopamine-agonist treatments (see below), could be prevented by administration of kappa-receptor agonists in conjunction with the repeated cocaine treatment (Heidbreder et al. 1993, 1995).

While our results were consistent with an inhibitory action of dynorphin/kappa receptors on gene regulation in striatonigral neurons, the fact that spiradoline was given systemically disallowed conclusions regarding the site of action of this drug. Moreover, our studies also showed that effects of systemic spiradoline were not restricted to the striatum. For example, IEG mRNA levels were also suppressed throughout the cortex in these experiments (Steiner and Gerfen 1995). This raised the possibility that cortical input to the striatum was affected by systemic spiradoline, possibly by kappa-receptor stimulation outside the striatum. Several studies have shown that both dopamine-agonist-induced behavior and gene expression in striatal neurons are dependent in part on input from the cortex or glutamate-receptor stimulation (Karler et al. 1989, 1994; Cenci and Björklund 1993; Torres and Rivier 1993; Wang et al. 1994; Hanson et al. 1995; Vargo and Marshall 1995; Konradi et al. 1996; for a review, see Wang and McGinty 1996). Therefore, to ensure that kappa-receptor action in the striatum was tested, we developed methods that allowed the measurement of striatal gene regulation after intrastriatal infusion of kappa agonists.

In these studies, spiradoline (1–50 nmol) was infused into the central striatum prior to cocaine administration with the help of chronically implanted guide cannulas. Our results showed that spiradoline infusion suppressed cocaine-induced IEG expression in the striatum in a dose-dependent manner (Fig. 5; Steiner and Gerfen 1995). Increasing concentrations of the kappa agonist inhibited IEG induction in increasing regions around the infusion cannula. These results demonstrate that kappa receptors located in the striatum are involved in the inhibition of cocaine responses in striatonigral neurons.

Further studies (Steiner and Gerfen 1999) indicated that kappa receptors selectively regulate IEG induction in striatonigral neurons, as kappa-agonist infusions into the striatum did not affect IEG induction by D2-receptor antagonists (see below), which occurs in striatopallidal neurons.

Taken together, our results indicate that dynorphin acts as a negative feedback mechanism to regulate striatonigral neuron function.

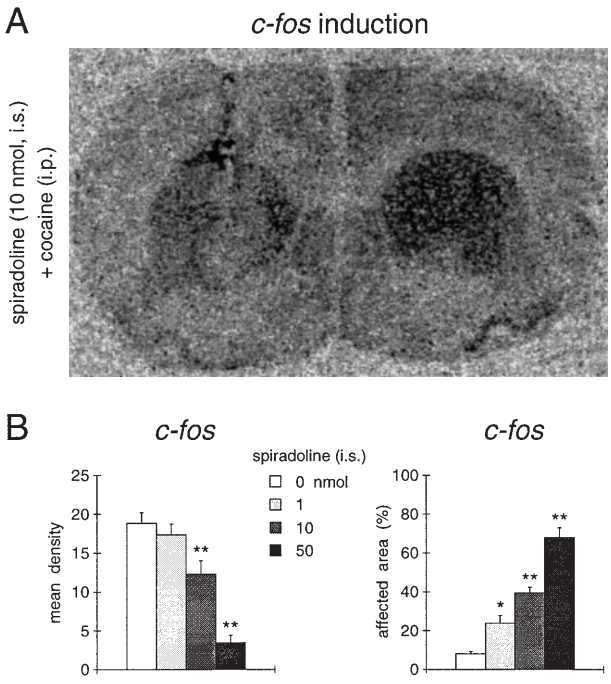

Fig. 5A, B Suppression of cocaine-induced c-*fos* expression in the striatum by the kappa-receptor agonist spiradoline (Steiner and Gerfen 1995). **A** Film autoradiogram shows an example of the inhibitory effect of spiradoline (10 nmol) infused into the striatum (*left side*) on c-*fos* induction by cocaine (30 mg/kg, i.p.). **B** Mean density values (mean+SEM, arbitrary units) measured across the entire striatum on the infused side (*left*) and size of the affected area (% of total striatal area) (*right*) are depicted for animals having received an intrastriatal infusion of spiradoline (0–50 nmol) prior to cocaine administration. ** $P<0.01$, *$P<0.05$; versus vehicle control (0 nmol), ANOVA, Dunnett's test

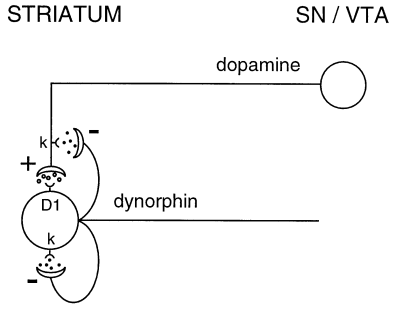

Fig. 6 Potential mechanisms mediating the inhibitory regulation of dopamine input to striatonigral neurons by dynorphin/kappa opioid receptors in the striatum. Presynaptic kappa receptors on terminals of dopamine neurons appear to inhibit dopamine release. Postsynaptic kappa receptors on striatonigral neurons seem to participate by inhibiting postsynaptic effects of D1-receptor stimulation in some neurons. The contributions of these two mechanisms appear to vary between dorsal and ventral striatal regions, with a predominant regulation of dopamine release in the dorsal striatum and additional postsynaptic kappa-receptor effects in the ventral striatum (see text) (Steiner and Gerfen 1996). *k* Kappa receptor, *SN* substantia nigra, *VTA* ventral tegmental area

Mechanisms by which dynorphin could inhibit IEG induction in striatonigral neurons

Several direct and indirect mechanisms by which dynorphin could affect striatonigral neurons are conceivable. Such mechanisms include:

1. Dynorphin could inhibit dopamine release, which would be expected to reduce IEG induction by cocaine in striatonigral neurons:

a. Dopamine neurons express kappa receptors (Meng et al. 1993; Minami et al. 1993; De Paoli et al. 1994; Mansour et al. 1994b). Released in the substantia nigra from terminals of striatonigral neurons (You et al. 1994), dynorphin could act on inhibitory kappa receptors on dopamine neurons to inhibit nigrostriatal activity and dopamine release in the striatum (Reid et al. 1988).

b. Released in the striatum upon D1-receptor stimulation (You et al. 1994) from axon collaterals (Wilson and Groves 1980; Kawaguchi et al. 1990) of striatonigral neurons or perhaps even from their dendrites (Drake et al. 1994; Simmons et al. 1995), dynorphin could locally inhibit dopamine release by interacting with kappa receptors on dopamine terminals (presynaptic kappa receptors) (Fig. 6). Evidence for such a mechanism has been provided by both in vivo microdialysis and in vitro studies (Mulder et al. 1984; Di Chiara and Imperato 1988b; Werling et al. 1988; Spanagel et al. 1992).

c. Dynorphin in the striatum may play a role in acetylcholine-regulated dopamine release (Gauchy et al. 1991).

2. Dynorphin could act on postsynaptic kappa receptors on striatonigral neurons to inhibit IEG induction (Fig. 6). Striatal projection neurons also express kappa receptors (Meng et al. 1993; Minami et al. 1993; Mansour et al. 1994b), although the subtype of these neurons remains to be determined. Released in the striatum, dynorphin could conceivably directly inhibit effects of D1-receptor stimulation in striatonigral neurons by acting on kappa receptors expressed by these neurons (postsynaptic kappa receptors). For example, inhibitory effects of dynorphin/kappa agonists on D1-receptor-stimulated adenylate-cyclase activity (Gentleman et al. 1983) or on Ca^{2+} currents (Gross and Macdonald 1987; Attali et al. 1989) have been reported. Both Ca^{2+}- and cAMP-dependent second messenger pathways, among others, are important mediators of IEG induction (Morgan and Curran 1989; Sheng and Greenberg 1990). In support of such a direct mechanism, dynorphin-immunoreactive terminals have been found to form synapses with dendrites of dynorphin-containing neurons in the ventral striatum (Van Bockstaele et al. 1995).

How is dopamine release affected by repeated treatment with psychomotor stimulants? Several studies have shown that cocaine- or amphetamine-induced dopamine overflow in the striatum is indeed reduced for several days after repeated treatment with cocaine or amphetamine (Hurd et al. 1989; Segal and Kuczenski 1992a, b; Kalivas and Duffy 1993). Increased dynorphin release in the substantia nigra could provide additional negative

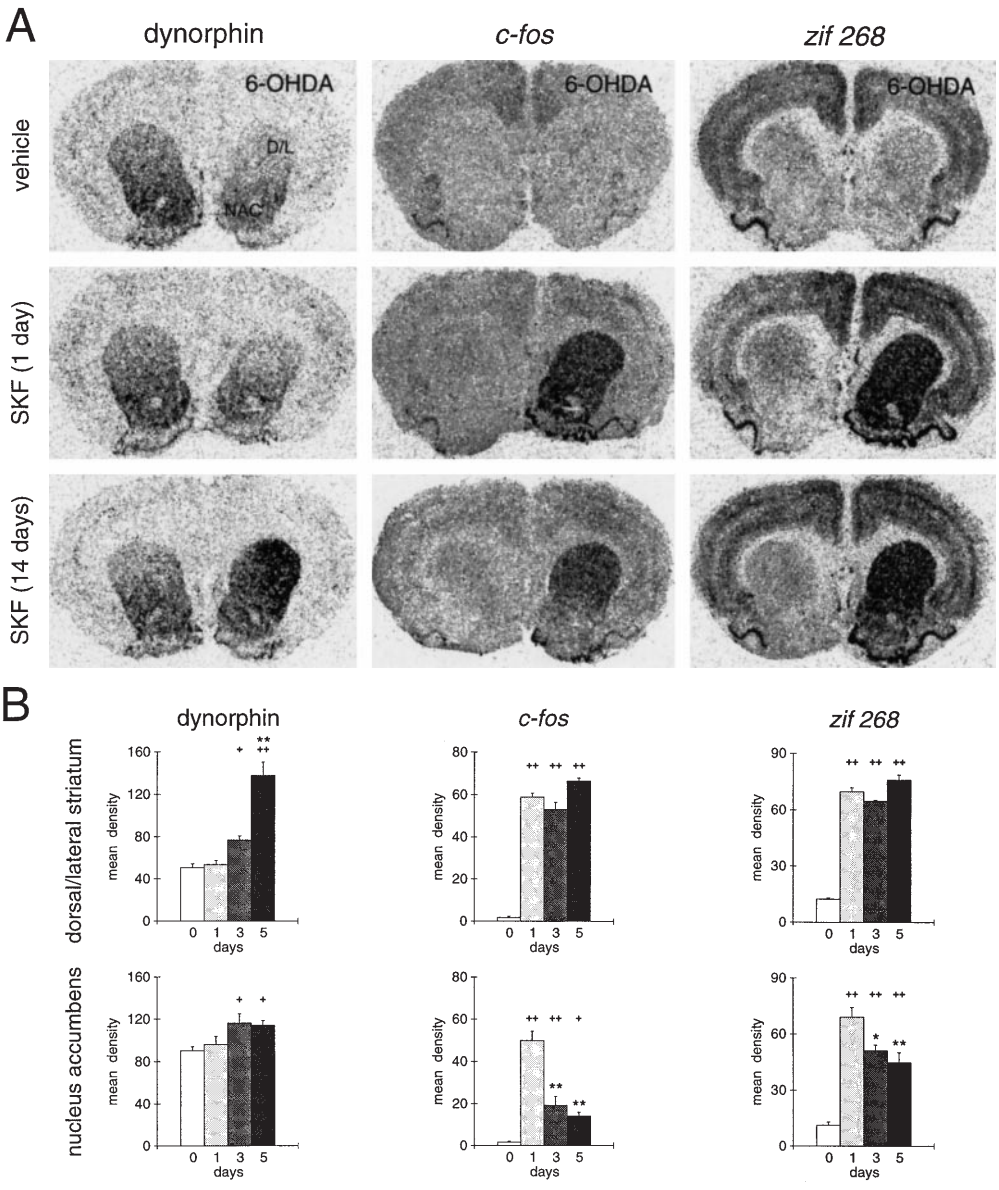

Fig. 7A, B Changes in dynorphin (*left*), c-*fos* (*middle*), and *zif 268* expression (*right*) in the striatum after repeated treatment with the selective D1-receptor agonist SKF-38393 in animals with unilateral dopamine depletion by 6-OHDA (right side) (adapted from Steiner and Gerfen 1996). **A** Film autoradiograms depict gene expression in coronal sections through the rostral striatum of animals that received vehicle injections once daily for 14 days (*top*), vehicle injections followed by one injection of the D1-receptor agonist SKF-38393 (2.0 mg/kg, i.p.) (*middle*), or D1-agonist treatment once daily for 14 days (*bottom*). Maximal hybridization signal is *black*. *D/L* Dorsal/lateral striatum, *NAC* nucleus accumbens. **B** Mean density values (mean+SEM, arbitrary units) for dynorphin, c-*fos* and *zif 268* expression measured in the dorsal/lateral striatum (*top*) or in the nucleus accumbens (*bottom*) are given for animals treated with vehicle injections (0-day group) or with SKF-38393 (2.0 mg/kg, i.p.) once daily for 1, 3, or 5 days. $^{++}$, ** $P<0.01$; $^{+}$,* $P<0.05$; $^{+}$ versus 0-day group, * versus 1-day group; ANOVA, Tukey-Kramer test

feedback to the dopamine system and contribute to such a reduction in dopamine release. However, our results, obtained with intrastriatal infusions of the dynorphin agonist spiradoline, together with the observed correlations between local dynorphin expression and the IEG response in different striatal regions strongly indicate that dynorphin acts via local mechanisms in the striatum (Fig. 6). In the following paragraphs, studies are summarized that investigated such local mechanisms in different striatal regions.

Pre- and postsynaptic kappa opioid receptors in the striatum are involved

Using dopamine depletion by 6-OHDA, we tested the effects of eliminating dopamine terminals (i.e., *presynaptic* kappa receptors) on the ability of dynorphin and agonists to inhibit IEG induction in striatal neurons (Steiner and Gerfen 1996). First, we asked whether, in the absence of presynaptic kappa receptors, upregulated dynorphin levels could still suppress IEG induction, as was seen in the normal striatum. Since cocaine can not be used in the 6-OHDA model, IEGs in striatonigral neurons were induced with the selective D1-receptor agonist SKF-38393 (Robertson et al. 1990, 1992). After 6-OHDA lesions, repeated treatment with SKF-38393 produces increased dynorphin mRNA and peptide levels in the striatum (e.g., Gerfen et al. 1990; Engber et al. 1992). Thus, rats received injections of SKF-38393 (2 mg/kg) once daily for up to 14 days, which resulted in increased dynorphin expression throughout the striatum (Fig. 7) (Steiner and Gerfen 1996). The IEG inducibility was tested on the last treatment day. Acute D1-agonist treatment produces strong, uniform IEG induction throughout the dopamine-depleted dorsal and ventral striatum (Fig. 7). After repeated SKF-

38393 treatment, the IEG response was *sustained* in dorsal and lateral areas of the striatum despite a considerable increase in dynorphin expression in these regions (Steiner and Gerfen 1996). In contrast, in the ventral striatum (nucleus accumbens), the IEG response was significantly reduced in these animals (Fig. 7). These regional variations match the known distribution of striatal neurons that express kappa receptors (i.e., *postsynaptic* kappa receptors). Such kappa-receptor expression is minimal in dorsal/lateral and substantially higher in ventral striatal regions (Meng et al. 1993; Minami et al. 1993; Mansour et al. 1994b). Thus, in contrast to normal animals, the IEG response was undiminished in regions with low levels of postsynaptic kappa receptors and was only reduced in areas with significant amounts of postsynaptic kappa receptors in the absence of presynaptic kappa receptors (dopamine terminals).

Therefore, our results suggest that, in the dorsal/lateral striatum with little or no postsynaptic kappa receptors, dynorphin normally predominantly inhibits dopamine release. This is inferred from the lack of suppression of the IEG response after eliminating dopamine terminals, despite considerably increased dynorphin levels. On the other hand, in the ventral striatum, where D1-receptor-mediated IEG induction in 6-OHDA-lesioned animals is significantly reduced after increased dynorphin expression, postsynaptic kappa receptors seem to be able to counteract the effects of D1-receptor stimulation to some extent.

In a further study, this idea was confirmed with the dynorphin agonist spiradoline. Animals without dopamine terminals (i.e., after 6-OHDA lesions) received an injection of spiradoline prior to acute SKF-38393 administration. Results showed that spiradoline had minimal effects in the dorsal/lateral striatum, but significantly suppressed D1-agonist-induced IEG expression in ventral striatal regions (Steiner and Gerfen 1996).

These results indicate that both pre- and postsynaptic kappa receptors are involved in the regulation of dopamine-mediated IEG induction in the striatonigral pathway (Fig. 6). In the dorsal/lateral striatum, inhibitory regulation of dopamine release seems to be the predominant mechanism. However, in the ventral striatum, postsynaptic kappa receptors on striatal neurons contribute to inhibition of dopamine receptor responses in striatonigral neurons.

Increased dynorphin expression in the striatonigral pathway as an adaptive mechanism and potential behavioral significance

Normal behavior is dependent on concerted and balanced activity in the two striatal output pathways. Our findings indicate that dynorphin regulates the function of striatonigral neurons by acting as a negative feedback system. Moreover, our results and those of others also demonstrate that repeated, excessive stimulation of D1 dopamine receptors produces increased expression of dynorphin in these neurons. Increased dynorphin expression appears to be an adaptive or compensatory response to such repeated dopamine receptor activation. Similar adaptive changes in gene regulation after repeated drug exposure have been demonstrated in other neuronal systems and may represent a homeostatic mechanism to maintain an equilibrium in cellular function (Hyman and Nestler 1996; Koob 1996). Our results indicate that increased dynorphin function in striatonigral neurons acts to blunt or dampen subsequent dopamine input and/or the effects of D1-receptor stimulation. However, while such an effect may be "compensatory" at the cellular level, increased negative feedback will affect the dynamics of activity in this pathway and thus alter network properties and, consequently, behavior.

Repeated exposure to psychomotor stimulants such as cocaine and amphetamine is known to produce severe behavioral alterations, including addiction and dependence. Some of the most obvious behavioral changes seen in animal models are collectively termed "behavioral sensitization" (e.g., Kalivas and Stewart 1991). Acute administration of psychomotor stimulants and other dopamine agonists in moderate doses results in behavioral activation, which includes increased rates of exploration, locomotion, rearing, and sniffing in the rat (for a review, see, e.g., Johanson and Fischman 1989). With repeated drug treatment, the motor-activating effects become more "pronounced". For example, repeated cocaine administration produces increased levels of locomotor activity and increasingly repetitive ("stereotyped") behavior, in which certain behavioral elements are repeated over and over for a prolonged period of time. This effect is called "behavioral sensitization".

Such stimulant-induced behavioral changes are most likely the result of complex neuronal alterations and interactions in more than one brain system. It is generally agreed that the acute motor-activating effects of these drugs are mediated by the dopamine neurotransmission in the forebrain, although other neurotransmitters are also likely involved (Wise and Bozarth 1987; Koob and Bloom 1988; Robinson and Berridge 1993; Koob 1996). After repeated cocaine or amphetamine treatments, changes in various aspects of the immediate dopamine transmission have been described (e.g., Kalivas and Stewart 1991; Kuhar and Pilotte 1996). However, it has become clear that such alterations alone can not account for the behavioral changes produced by psychostimulants and that neuronal changes occurring downstream to the immediate dopamine transmission, such as changes in dopamine-receptive neurons, likely contribute to altered function of the affected pathways and behavior.

It is an intriguing question how much increased dynorphin function could contribute to the behavioral changes seen after repeated dopamine-agonist treatments. Dynorphin expression is also increased in human cocaine addicts (Hurd and Herkenham 1993). In humans and experimental animals, dynorphin agonists seem to be dysphoric or aversive, and it has thus been suggested that increased inhibition of the dopamine transmission by aug-

mented dynorphin function could contribute to the dysphoric state experienced after cessation of the cocaine action, an effect that may contribute to motivational aspects of drug withdrawal and addiction (Hyman et al. 1996; Koob 1996).

In rats, repeated treatment with cocaine for a few days is sufficient to produce increased locomotor activity and the appearance of stereotypies, such as focussed sniffing and head bobbing (e.g., Segal and Kuczenski 1992b; Heidbreder et al. 1993, 1995; Kalivas and Duffy 1993). In our earlier studies, we have not systematically investigated behavioral correlates of altered gene regulation. However, some observations suggest that increased dynorphin function may be related to the appearance of behavioral sensitization. For example, in our time-course studies with cocaine (Steiner and Gerfen 1993), we noted that rats showed increased rates of stereotyped head bobbing at time points when the IEG response to cocaine was suppressed and dynorphin expression was increased. Moreover, recent studies on cocaine effects in D3 dopamine-receptor knockout mice revealed more pronounced IEG induction in the striatum by acute cocaine administration and an accelerated increase in dynorphin expression and accelerated development of behavioral sensitization with repeated cocaine treatment in these mutants (Carta et al., in preparation).

A relationship between behavioral sensitization and increased dynorphin function is also indicated by results of repeated dopamine-agonist treatment in dopamine-depleted animals. After unilateral 6-OHDA lesion, the increase in dynorphin expression produced by repeated D1-agonist treatment is accompanied by increasing rates of turning behavior (e.g., Steiner and Gerfen 1996). In a recent study, we observed a significant positive correlation between dynorphin mRNA levels in the dorsal, but not ventral, striatum and the rate of contraversive turning produced by repeated treatment with the D1-receptor agonist SKF-38393 (r=0.85, P<0.01) (Steiner and Gerfen 1996). Similar results have been reported by others (Bejar et al. 1995). Our results summarized above indicate an inhibitory function of dynorphin released in the striatum. However, these latter results suggest that dynorphin released in the substantia nigra (You et al. 1994) may have additional inhibitory effects in this target nucleus (e.g., Thompson and Walker 1990), which could contribute to functional changes in this basal-ganglia output pathway. Future experimentation will have to elucidate the exact behavioral consequences of altered dynorphin function in the striatonigral pathway.

Summary

Our findings demonstrate that dynorphin-receptor stimulation in the striatum inhibits IEG expression in striatonigral neurons induced by cocaine or D1-receptor agonists. These results indicate that dynorphin acts as a negative feedback mechanism to regulate striatonigral neuron function. Increased dynorphin expression produced by repeated D1-receptor stimulation appears to be a compensatory mechanism, which blunts the effects of dopamine input in these neurons. Such enhanced negative feedback likely alters the dynamics of this pathway, which may contribute to behavioral sensitization and other behavioral alterations occurring during repeated treatments with dopamine agonists, including psychomotor stimulants.

Enkephalin regulates responsiveness of striatopallidal neurons

Striatopallidal neurons express enkephalin. We have started to investigate whether enkephalin could play a similar autoregulatory role in the striatopallidal pathway as we propose for dynorphin in the striatonigral pathway. Our results indicate that some aspects of enkephalin function in the striatum are similar to that of dynorphin, whereas others differ.

IEG induction by D2-receptor blockade in striatal neurons is inhibited by enkephalin receptors

Blockade of D2 receptors has been shown to induce IEGs in striatopallidal neurons (Dragunow et al. 1990; Robertson et al. 1992). To investigate enkephalin effects on striatopallidal neurons, we have employed such D2 receptor antagonist-induced IEG expression as a cellular response. In these experiments, expression of c-*fos* and *zif 268* mRNAs was measured after treatment with the selective D2-receptor antagonist eticlopride (1–5 mg/kg). Enkephalin is an endogenous ligand of delta and mu opioid receptors (Paterson et al. 1983; Mansour et al. 1995), both of which are expressed in the striatum (Mansour et al. 1987, 1994a; Delfs et al. 1994). We first asked whether this IEG response could be inhibited by intrastriatal infusion of the delta-receptor-preferring agonist DADLE. DADLE (0.03–3 μg) was infused via chronically implanted guide cannulas in freely moving animals. Our results showed that DADLE infusion into the striatum prior to eticlopride injection suppressed IEG induction in striatal neurons in a dose-dependent manner (Fig. 8) (Steiner et al. 1995; Steiner and Gerfen 1999). Increasing doses of DADLE inhibited IEG expression in increasing areas around the infusion cannula.

Experiments with the nonselective opioid receptor antagonist naloxone confirmed that this DADLE effect was indeed mediated by opioid receptors. Thus, systemic injection of naloxone (1–10 mg/kg) prior to intrastriatal infusion of DADLE attenuated the suppressive effect of the delta-receptor agonist on IEG induction by eticlopride in a dose-dependent manner (Steiner and Gerfen 1999). Similarly, coinfusion of naloxone (20 μg) together with DADLE into the striatum reduced the delta-agonist effect on gene induction by the D2 antagonist.

The opioid receptors involved were further characterized by assessing the effects of intrastriatal infusion of the more selective delta receptor agonist deltorphin-II

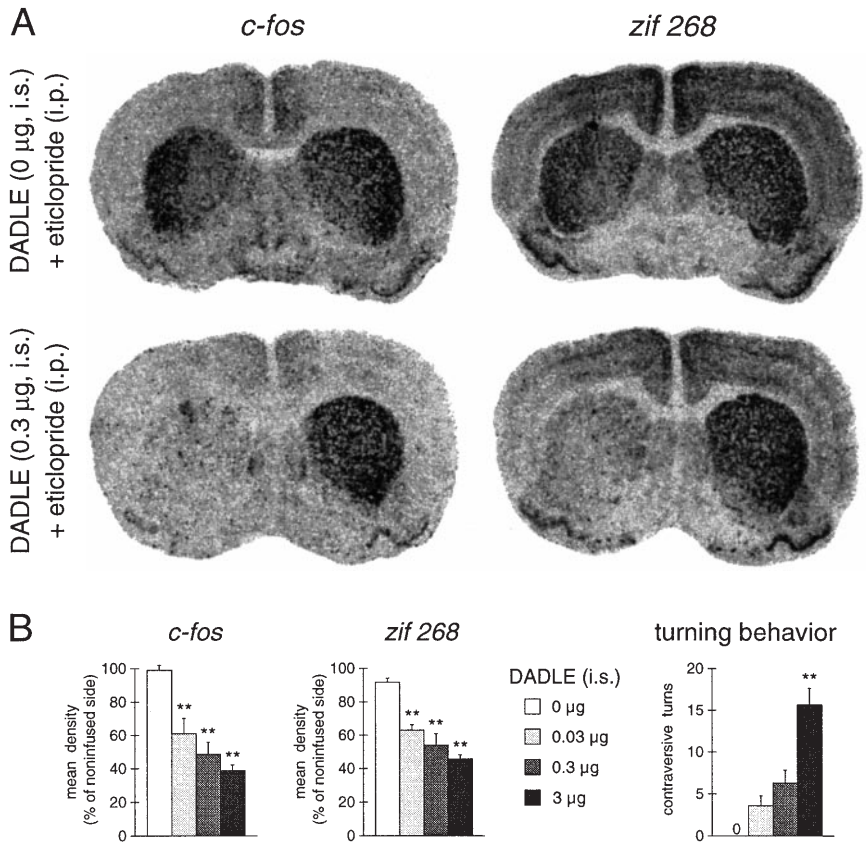

Fig. 8A, B Suppression of eticlopride-induced immediate-early gene expression in the striatum by the delta-receptor agonist DADLE (Steiner and Gerfen 1999). **A** Film autoradiograms show c-*fos* (*left*) and *zif 268* expression (*right*) in coronal brain sections of rats that received a unilateral infusion of vehicle (*top*), or DADLE (0.3 µg) (*bottom*) into the striatum (left side), followed by administration of the selective D2-receptor antagonist eticlopride (1 mg/kg, i.p.). These rats were killed 45 min later. **B** Mean density values (mean+SEM, in % of noninfused side) measured across the infused striatum, for c-*fos* (*left*) and *zif 268* expression (*middle*), are depicted for animals that received an infusion of DADLE (0–3 µg) prior to eticlopride injection. Turning behavior (*right*) was measured during minutes 41–42 after eticlopride administration. The number (mean+SEM) of contraversive full turns is shown. ** $P<0.01$, versus vehicle control (0 µg); ANOVA, Dunnett's test

(2 µg) and of the classical mu-receptor agonist DAMGO (0.5 µg) on D2-receptor-mediated IEG induction. Moreover, these effects were compared with those of a high dose of the kappa-receptor agonist U-50488 (10 µg). Results showed that both the delta-receptor agonist and the mu-receptor agonist suppressed IEG induction by eticlopride (Steiner and Gerfen 1999). In contrast, infusion of the kappa-receptor agonist did not affect this IEG response. These results demonstrate that acute stimulation of both enkephalin receptors (delta and mu), but not of the dynorphin receptor (kappa), in the striatum suppresses IEG induction in striatopallidal neurons.

Thus, enkephalin in the striatum does appear to function as a negative feedback mechanism to regulate the responsiveness of striatopallidal neurons. The exact underlying mechanisms are presently unknown. Recent colocalization studies indicate that enkephalin could act directly on opioid receptors on striatopallidal neurons (Guttenberg et al. 1996; Svingos et al. 1996, 1998; Wang et al. 1996). However, indirect mechanisms may also play a role (see Steiner and Gerfen 1999 for a discussion).

Behavioral effects produced by intrastriatal infusion of opioid receptor agonists and antagonists provide further characterization of delta- and mu-receptor actions in the striatum and indicate that enkephalin also affects other striatal neuron populations. Unilateral infusion of the delta-agonist DADLE induced contraversive turning behavior in a dose-dependent manner (Fig. 8), an effect that was also blocked by naloxone (Steiner and Gerfen 1999). Such turning behavior was not affected by the subsequent administration of the D2 antagonist. Similar results were obtained with the more selective delta agonist deltorphin-II (Steiner and Gerfen 1999), consistent with earlier findings (Longoni et al. 1991). Infusion of the mu agonist DAMGO also produced contraversive turning. However, in contrast to delta-agonist-induced behavior, mu-agonist-induced turning was blocked by subsequent D2-antagonist treatment (Steiner and Gerfen 1999). This dissociation in opioid effects indicates that, in addition to gene regulation in striatopallidal neurons, other striatal mechanisms are also affected by enkephalin agonists. Moreover, whereas both delta- and mu-receptor stimulation suppresses IEG induction in striatopallidal neurons, such other mechanisms are differentially regulated by delta and mu receptors. The neuronal basis for these differential behavioral effects is not known, but they are likely related to differential localization of these opioid receptor subtypes on striatal neurons or afferent terminals. For example, while delta-receptor expression and binding are relatively uniform throughout the striatum, mu-receptor expression and binding are highly enriched in the patch compartment (Mansour et al. 1987, 1994a, 1995; Delfs et al. 1994). Moreover, cholinergic interneurons seem to express high levels of delta-, but not mu-, receptor mRNAs (Le Moine et al. 1994; Mansour et al. 1994a), and acetylcholine release is controlled by delta-, but not mu-, receptors (Mulder et al. 1984; Schoffelmeer et al. 1988). Future studies will have to address the mechanisms

by which enkephalin receptors regulate striatopallidal neuron function and behavior.

Increased enkephalin expression as an adaptive response?

Enkephalin mRNA and peptide levels in the striatum are increased after dopamine depletion or repeated D2-receptor-antagonist treatment (see above). Thus, it seems possible that upregulated synthesis of this opioid peptide is also an adaptive or compensatory response to chronic lack of D2-receptor activation and, presumably, resulting disinhibition of striatopallidal neurons. For example, a recent study reported suppressed inducibility of c-*fos* and other IEGs in the striatum after repeated treatment with the D2-receptor antagonist haloperidol (Konradi et al. 1993), suggesting that increased enkephalin function could play a role in long-term inhibition of IEG induction by D2-receptor blockade. We demonstrated such a suppressed IEG response (c-*fos*, *zif 268*) after repeated treatment with the selective D2-receptor antagonist eticlopride, and also compared the time courses of changes in enkephalin expression and IEG induction (Steiner and Gerfen 1999). Similar to changes in dynorphin expression and IEG induction in striatonigral neurons during repeated cocaine administration (see Fig. 4), enkephalin mRNA levels increase with repeated daily eticlopride treatment, whereas the IEG response to the D2-receptor antagonist is maximal after the first drug administration and is significantly suppressed after subsequent treatments (Steiner and Gerfen 1999).

Having demonstrated that enkephalin-receptor activation in the striatum inhibits acute D2-receptor-antagonist-induced IEG expression, and that this effect can be blocked with naloxone (Steiner and Gerfen 1999), we asked whether the suppressed IEG inducibility after repeated D2 antagonist treatment could be the consequence of enhanced enkephalin function in the striatum. Thus, animals were treated with eticlopride (5 mg/kg) once daily for 4 days, which produced increased enkephalin expression and a suppressed IEG response. On the last treatment day, the effects of systemic (10–50 mg/kg) or intrastriatal (20–50 μg) administration of naloxone on IEG induction by eticlopride were examined. Results showed that neither systemic nor intrastriatal administration of naloxone significantly affected the suppressed IEG response in this situation (Steiner and Gerfen 1999). Since such naloxone treatments attenuated the enkephalin-agonist effects on IEG induction by acute eticlopride (see above) and also influenced behavior after repeated eticlopride treatment (see below), our results indicate that the suppressed IEG response in the chronic situation can not simply be attributed to increased enkephalin action in the striatum.

Evidence for a compensatory role of increased enkephalin function is provided by behavioral findings after repeated treatment with D2-receptor antagonists and in animal models of Parkinson's disease, such as the 6-OHDA lesion model. Acute dopamine depletion by neurotoxins such as 6-OHDA results in akinesia, sensorimotor neglect, and other functional deficits (for a review see, e.g., Schwarting and Huston 1996b). In animals with partial loss of dopamine neurons, substantial functional recovery occurs during the first weeks after the lesion. Such recovery is thought to be mediated mainly by various compensatory alterations in residual dopamine neurons that can produce and maintain near-normal extracellular dopamine levels in the striatum (Schwarting and Huston 1996a, b). In animals with near-total dopamine depletion (<5–15% residual dopamine tissue content), such presynaptic compensatory mechanisms are insufficient and extracellular dopamine levels are reduced. Nevertheless, such animals also show some degree of behavioral recovery. In such animals, various neuronal alterations in postsynaptic, dopamine-receptive neurons have been described that may act as compensatory mechanisms, including dopamine-receptor supersensitivity. The dramatic increase in IEG induction after D1-receptor stimulation in striatonigral neurons of the dopamine-depleted striatum (see Fig. 7) reflects such postsynaptic neuronal adaptations.

The increase in enkephalin synthesis in striatopallidal neurons is another example of such postsynaptic neuronal changes. Increased enkephalin expression is only seen in animals with near-total dopamine depletion (<10% residual tissue content; Li et al. 1990; Nisenbaum et al. 1996) and is the result of insufficient stimulation of D2 receptors, as this increase can be reversed by D2-receptor agonist supplement (Gerfen et al. 1990; Engber et al. 1992). Enkephalin expression mostly increases during the first

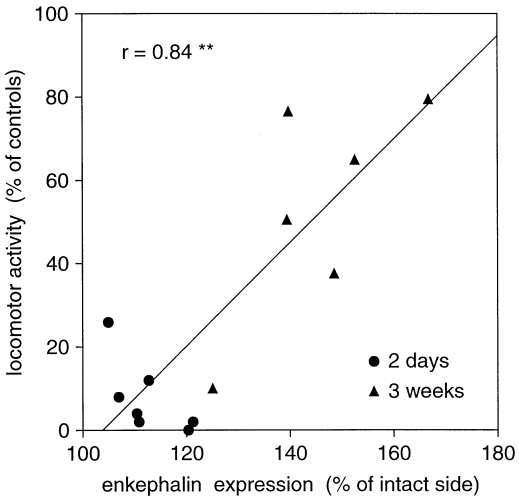

Fig. 9 Correlation between enkephalin expression in the dopamine-depleted striatum and locomotor activity in an exploratory test at different time points after a 6-OHDA lesion (Steiner and Kitai, unpublished results). *Scatter plot* shows enkephalin mRNA levels on the side of the lesion (expressed as % of values in the intact striatum) at a mid-striatal level and locomotor activity (line crossings, in % of values in sham-lesion controls) in individual animals tested 2 days or 3 weeks after unilateral 6-OHDA injection into the rostral substantia nigra (for methods, see Steiner and Gerfen 1996). Spontaneous behavior was examined for 10 min in a novel open field (60×60 cm, divided into 3×3 squares by lines on the floor). ** $P<0.001$

week after the lesion (Nisenbaum et al. 1994). During the same time period, rats with near-total dopamine depletion show recovery from lesion-induced akinesia (Fornaguera et al. 1994a, b). Functional recovery is not complete, however, as such animals (with unilateral lesions) do not recover from ipsiversive turning and scanning asymmetries (Fornaguera et al. 1994a, b).

In a recent study, we found a positive correlation between behavioral recovery and changes in enkephalin expression in rats with a unilateral 6-OHDA lesion (Steiner and Kitai, unpublished results). Rats examined 2 days after 6-OHDA administration displayed severe hypokinesia in an open field test (locomotor activity, mean, 7.7% of sham-lesion controls; Fig. 9) and had minimally increased enkephalin expression in the striatum on the side of the lesion (112.5% of intact side). In contrast, animals tested 3 weeks after the lesion showed significantly more locomotor activity (53.2% of controls; $P<0.01$) and displayed significantly higher enkephalin mRNA levels (145.3%; $P<0.01$). Although the 3-week group showed significant recovery from akinesia, these rats had not recovered from turning asymmetries, confirming earlier observations (Fornaguera et al. 1994a, b). These results are consistent with a compensatory role for increased enkephalin levels, which may counteract consequences of lost D2-receptor stimulation and thus allow some recovery of function.

Such a role for enkephalin was also indicated by findings in our studies with acute and repeated treatment with the D2-receptor antagonist eticlopride. Acute administration of D2-receptor antagonists produces catalepsy and akinesia. We measured locomotor activity and rearing behavior in an open field test to determine behavioral correlates of altered gene regulation by the D2-antagonist treatments. These tests showed that, with repeated eticlopride treatment that produced increased enkephalin expression, animals recovered from akinesia to some extent. Thus, the degree of akinesia was maximal after the first eticlopride injection. With repeated administration, rats displayed progressively more locomotor activity and rearing (Steiner and Gerfen 1999). This effect is well-known and is sometimes called "tolerance" to repeated D2-receptor-antagonist treatment (e.g., Ezrin-Waters and Seeman 1977; Campbell and Baldessarini 1981; Masuda et al. 1982; Hess et al. 1988). To test whether recovery from akinesia after repeated eticlopride was related to increased enkephalin function, we investigated the behavioral effects of blocking enkephalin receptors with the opioid-receptor antagonist naloxone. Results showed that naloxone indeed reinstated akinesia after repeated eticlopride treatment, while having minimal effects on locomotion in normal animals (Steiner and Gerfen 1999). These results indicate that increased enkephalin levels after repeated D2-antagonist treatment do act as an adaptive mechanism to "normalize" some aspects of basal-ganglia function. Whereas enkephalin apparently is not involved in long-term suppression of gene expression in striatopallidal neurons, this neuropeptide may act by influencing other striatal functions (see above) or by affecting neuronal processes in target areas of the striatopallidal projection, such as regulating GABA release from striatopallidal terminals (Dewar et al. 1987; Maneuf et al. 1994).

Summary

Our results demonstrate that enkephalin-receptor stimulation in the striatum inhibits IEG expression in striatopallidal neurons induced by acute D2-receptor blockade, indicating that enkephalin acts as a negative feedback mechanism to regulate striatopallidal neuron function in the normal brain. While increased enkephalin levels do not appear to play a role in the suppressed IEG response after repeated D2-antagonist treatment, this opioid peptide seems to be involved in other neuronal mechanisms that mediate recovery of function after such chronic dopamine antagonist treatments or after dopamine depletion.

Conclusions

In this series of studies, we have investigated the function of the opioid peptides dynorphin and enkephalin in neurons of the striatonigral and striatopallidal output pathways, respectively. We have used IEG induction by dopamine-receptor agonists and antagonists as a cellular response to examine the role of these opioid peptides in the regulation of these striatal projection neurons. Our results indicate that both opioid peptides function, at least in part, as autoregulatory mechanisms to modulate the pathways they are contained in. The synthesis of both neuropeptides is upregulated by chronic drug treatments and/or lesions that tend to activate these pathways. Whereas psychomotor stimulants produce increased dynorphin expression, repeated neuroleptic treatment or dopamine depletion result in increased enkephalin expression. Our findings indicate that these changes in gene regulation are adaptive responses in these neurons – probably based on homeostatic principles – which seem to counteract the perturbations produced by the drug exposure/lesion. The consequences of these cellular adaptive mechanisms for the function of the affected basal-ganglia pathways and behavior, however, appear to differ for the two opioid peptides. While increased enkephalin expression seems to play a role in recovery of function after loss of D2-receptor stimulation, increased dynorphin expression may contribute to behavioral dysfunction produced by psychomotor stimulants, including addiction, dependence, and behavioral sensitization. An understanding of the role of these opioid peptides in the behavioral consequences of such drug use/treatments may lead to improved therapeutic approaches for the treatment of such behavioral alterations.

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References

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375

Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119–146

Angulo JA, McEwen BS (1994) Molecular aspects of neuropeptide regulation and function in the corpus striatum and nucleus accumbens. Brain Res Brain Res Rev 19:1–28

Attali B, Saya D, Nah S-Y, Vogel Z (1989) κ opiate agonists inhibit Ca^{2+} influx in rat spinal cord-dorsal root ganglion cocultures. J Biol Chem 264:347–353

Baik J-H, Picetti R, Saiardi A, Thiriet G, Dierich A, Depaulis A, Le Meur M, Borrelli E (1995) Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 377:424–428

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

Bejar JF, Harrison MB, Trugman JM (1995) Correlation of striatal dynorphin mRNA expression and behavioral sensitization following repeated D1 agonist injection. Soc Neurosci Abstr 21: 2089

Berretta S, Robertson HA, Graybiel AM (1992) Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. J Neurophysiol 68:767–777

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

Boegman RJ, Vincent SR (1996) Involvement of adenosine and glutamate receptors in the induction of c-*fos* in the striatum by haloperidol. Synapse 22:70–77

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

Campbell A, Baldessarini RJ (1981) Tolerance to behavioral effects of haloperidol. Life Sci 29:1341–1346

Cenci MA, Björklund A (1993) Transection of corticostriatal afferents reduces amphetamine- and apomorphine-induced striatal Fos expression and turning behaviour in unilaterally 6-hydroxydopamine-lesioned rats. Eur J Neurosci 5:1062–1070

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

Cole AJ, Bhat RV, Patt C, Worley PF, Baraban JM (1992) Di, dopamine receptor activation of multiple transcription factor genes in rat striatum. J Neurochem 58:1420–1426

Corbett AD, Paterson SJ, McKnight AT, Magnan J, Kosterlitz HW (1982) $Dynorphin_{1-8}$ and $dynorphin_{1-9}$ are ligands for the kappa-subtype of opiate receptor. Nature 299:79–81

Curran EJ, Watson SJ (1995) Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: a double in situ hybridization study. J Comp Neurol 361:57–76

Daunais JB, McGinty JF (1994) Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. Synapse 18:35–45

Delfs JM, Kong H, Mestek A, Chen Y, Yu L, Reisine T, Chesselet MF (1994) Expression of mu opioid receptor mRNA in rat brain: an in situ hybridization study at the single cell level. J Comp Neurol 345:46–68

DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285

De Paoli AM, Hurley KM, Yasada K, Reisine T, Bell G (1994) Distribution of κ opioid receptor mRNA in adult mouse brain: an in situ hybridization histochemistry study. Mol Cell Neurosci 5: 327–335

Dewar D, Jenner P, Marsden CD (1987) Effects of opioid agonist drugs on the in vitro release of 3H-GABA, 3H-dopamine and 3H-5HT from slices of rat globus pallidus. Biochem Pharmacol 36:1738–1741

Di Chiara G, Imperato A (1988a) 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

Di Chiara G, Imperato A (1988b) Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J Pharmacol Exp Ther 244:1067–1080

Drago J, Gerfen CR, Lachowicz JE, Steiner H, Hollon TR, Love PE, Ooi GT, Grinberg A, Lee EJ, Huang SP, Bartlett PF, Jose PA, Sibley DR, Westphal H (1994) Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc Natl Acad Sci USA 91:12564–12568

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

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) D_2 dopamine receptor antagonists induce Fos and related proteins in rat striatal neurons. Neuroscience 37:287–294

Drake CT, Terman GW, Simmons ML, Milner TA, Kunkel DD, Schwartzkroin PA, Chavkin C (1994) Dynorphin opioids present in dentate granule cells may function as retrograde inhibitory neurotransmitters. J Neurosci 14:3736–3750

Engber TM, Boldry RC, Chase TN (1991) The kappa-opioid receptor agonist spiradoline differentially alters the rotational response to dopamine D_1 and D_2 agonists. Eur J Pharmacol 200: 171–173

Engber TM, Boldry RC, Kuo S, Chase TN (1992) Dopaminergic modulation of striatal neuropeptides: differential effects of D_1 and D_2 receptor stimulation on somatostatin, neuropeptide Y, neurotensin, dynorphin and enkephalin. Brain Res 581:261–268

Ezrin-Waters C, Seeman P (1977) Tolerance of haloperidol catalepsy. Eur J Pharmacol 41:321–327

Fornaguera J, Carey RJ, Dai H, Huston JP, Schwarting RKW (1994a) Differentiation of motor inactivation from movement asymmetry effects in an animal model of hemi-parkinsonism. Neuroreport 6:173–176

Fornaguera J, Carey RJ, Huston JP, Schwarting RKW (1994b) Behavioral asymmetries and recovery in rats with different degrees of unilateral striatal dopamine depletion. Brain Res 664:178–188

Gauchy C, Desban M, Krebs MO, Glowinski J, Kemel 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. Trends Neurosci 15:133–139

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 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) D_1 and D_2 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

Gerfen CR, Keefe KA, Gauda EB (1995) D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate-early gene response in D1-containing neurons. J Neurosci 15:8167–8176

Gerfen CR, Keefe KA, Steiner H (1996) D1 and D2 dopamine receptor-mediated gene regulation in the striatum. In: Merchant KM (ed) Pharmacological regulation of gene expression in the CNS. CRC, Boca Raton, pp 3–24

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

Guttenberg ND, Klop H, Minami M, Satoh M, Voorn P (1996) Colocalization of mu opioid receptor is greater with dynorphin than enkephalin in rat striatum. Neuroreport 7:2119–2124

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

Hanson GR, Singh N, Merchant K, Johnson M, Gibb JW (1995) The role of NMDA receptor systems in neuropeptide responses to stimulants of abuse. Drug Alcohol Depend 37:107–110

Heidbreder CA, Goldberg SR, Shippenberg TS (1993) The kappa-opioid receptor agonist U-69593 attenuates cocaine-induced behavioral sensitization in the rat. Brain Res 616:335–338

Heidbreder CA, Babovic-Vuksanovic D, Shoaib M, Shippenberg TS (1995) Development of behavioral sensitization to cocaine: influence of kappa opioid receptor agonists. J Pharmacol Exp Ther 275:150–163

Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237

Hess EJ, Norman AB, Creese I (1988) Chronic treatment with dopamine receptor antagonists: behavioral and pharmacologic effects on D1 and D2 dopamine receptors. J Neurosci 8:2361–2370

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

Hope B, Kosofsky B, Hyman SE, Nestler EJ (1992) Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci USA 89: 5764–5768

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 13: 1235–1244

Hurd YL, Herkenham M (1992) Influence of a single injection of cocaine, amphetamine or GBR 12909 on mRNA expression of striatal neuropeptides. Brain Res Mol Brain Res 16:97–104

Hurd YL, Herkenham M (1993) Molecular alterations in the neostriatum of human cocaine addicts. Synapse 13:357–369

Hurd YL, Weiss F, Koob GF, And NE, Ungerstedt U (1989) Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: an in vivo microdialysis study. Brain Res 498:199–203

Hurd YL, Brown EE, Finlay JM, Fibiger HC, Gerfen CR (1992) Cocaine self-administration differentially alters mRNA expression of striatal peptides. Brain Res Mol Brain Res 13:165–170

Hyman SE, Nestler EJ (1996) Initiation and adaptation: a paradigm for understanding psychotropic drug action. Am J Psychiatry 153:151–162

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, Boca Raton, pp 115–131

Johanson C-E, Fischman MW (1989) The pharmacology of cocaine related to its abuse. Pharmacol Rev 41:3–52

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

Kalivas PW, Duffy P (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J Neurosci 13:266–275

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 16:223–244

Karler R, Calder LD, Chaudhry IA, Turkanis SA (1989) Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci 45:599–606

Karler R, Calder LD, Thai LH, Bedingfield JB (1994) A dopaminergic-glutamatergic basis for the action of amphetamine and cocaine. Brain Res 658:8–14

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

Keefe KA, Gerfen CR (1995) D1–D2 dopamine receptor synergy in striatum: effects of intrastriatal infusions of dopamine agonists and antagonists on immediate early gene expression. Neuroscience 66:903–913

Kita H, Kitai ST (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. Brain Res 447:346–352

Konradi C, Kobierski LA, Nguyen TV, Heckers S, Hyman SE (1993) The cAMP-response-element-binding protein interacts, but Fos protein does not interact, with the proenkephalin enhancer in rat striatum. Proc Natl Acad Sci USA 90:7005–7009

Konradi C, Leveque J-C, Hyman SE (1996) Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. J Neurosci 16:4231–4239

Koob GF (1996) Drug addiction: the yin and yang of hedonic homeostasis. Neuron 16:893–896

Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715–723

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 (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

Kuhar MJ, Pilotte NS (1996) Neurochemical changes in cocaine withdrawal. Trends Pharmacol Sci 17:260–264

LaHoste GJ, Yu J, Marshall JF (1993) Striatal Fos expression is indicative of dopamine D1/D2 synergism and receptor supersensitivity. Proc Natl Acad Sci USA 90:7451–7455

Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426

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

Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209

Le Moine C, Kieffer B, Gaveriaux-Ruff C, Befort K, Bloch B (1994) Delta-opioid receptor gene expression in the mouse forebrain: localization in cholinergic neurons of the striatum. Neuroscience 62:635–640

Li SJ, Sivan 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. Brain Res Mol Brain Res 8:219–225

Longoni R, Spina L, Mulas A, Carboni E, Garau L, Melchiorri P, Di Chiara G (1991) (D-Ala2) deltorphin II: D1-dependent stereotypies and stimulation of dopamine release in the nucleus accumbens. J Neurosci 11:1565–1576

Maneuf YP, Mitchell IJ, Crossman AR, Brotchie JM (1994) On the role of enkephalin cotransmission in the GABAergic striatal efferents to the globus pallidus. Exp Neurol 125:65–71

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

Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Akil H, Watson SJ (1994a) Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. J Comp Neurol 350:412–438

Mansour A, Fox CA, Meng F, Akil H, Watson SJ (1994b) κ, receptor mRNA distribution in the rat CNS: comparison to κ receptor binding and prodynorphin mRNA. Mol Cell Neurosci 5: 124–144

Mansour A, Fox CA, Akil H, Watson SJ (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 18:22–29

Masuda Y, Murai S, Itoh T (1982) Tolerance and reverse tolerance to haloperidol catalepsy induced by the difference of administration interval in mice. Jpn J Pharmacol 32:1186–1188

Melzer P, Steiner H (1997) Stimulus-dependent expression of immediate-early genes in rat somatosensory cortex. J Comp Neurol 380:145–153

Meng F, Xie G-X, Thompson RC, Mansour A, Goldstein A, Watson SJ, Akil H (1993) Cloning and pharmacological characterization of a rat κ opioid receptor. Proc Natl Acad Sci USA 90:9954–9958

Minami M, Hosoi Y, Toya T, Katao Y, Maekawa K, Katsumata S, Yabuuchi K, Onogi T, Satoh M (1993) In situ hybridization study of kappa-opioid receptor mRNA in the rat brain. Neurosci Lett 162:161–164

Moratalla R, Robertson HA, Graybiel AM (1992) Dynamic regulation of *NGFI-A* (*zif 268*, *egr1*) gene expression in the striatum. J Neurosci 12:2609–2622

Moratalla R, Xu M, Tonegawa S, Graybiel AM (1996) Cellular responses to psychomotor stimulant and neuroleptic drugs are abnormal in mice lacking the D1 dopamine receptor. Proc Natl Acad Sci USA 93:14928–14933

Morgan JI, Curran T (1989) Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. Trends Neurosci 12:459–462

Mulder AH, Wardeh G, Hogenboom F, Frankhuyzen AL (1984) Kappa- and delta-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. Nature 308:278–280

Nisenbaum LK, Kitai ST, Crowley WR, Gerfen CR (1994) Temporal dissociation between changes in striatal enkephalin and substance P messenger RNAs following striatal dopamine depletion. Neuroscience 60:927–937

Nisenbaum LK, Crowley WR, Kitai ST (1996) Partial striatal dopamine depletion differentially affects striatal substance P and enkephalin messenger RNA expression. Brain Res Mol Brain Res 37:209–216

Park MR, Lighthall JW, Kitai ST (1980) Recurrent inhibition in the rat neostriatum. Brain Res 194:359–369

Paterson SJ, Robson LE, Kosterlitz HW (1983) Classification of opioid receptors. Br Med Bull 39:31–36

Paul ML, Graybiel AM, David J-C, Robertson HA (1992) D1-like and D2-like dopamine receptors synergistically activate rotation and c-*fos* expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J Neurosci 12:3729–3742

Reid M, Herrera-Marschitz M, Hökfelt T, Terenius L, Ungerstedt U (1988) Differential modulation of striatal dopamine release by intranigral injection of γ-aminobutyric acid (GABA), dynorphin A and substance P. Eur J Pharmacol 147:411–420

Reiner A, Anderson KD (1990) The patterns of neurotransmitter and neuropeptide cooccurrence among striatal projection neurons: conclusions based on recent findings. Brain Res Brain Res Rev 15:251–265

Robertson GS, Fibiger HC (1992) Neuroleptics increase c-*fos* expression in the forebrain: contrasting effects of haloperidol and clozapine. Neuroscience 46:315–328

Robertson GS, Vincent SR, Fibiger HC (1990) Striatonigral projection neurons contain D_1 dopamine receptor-activated c-*fos*. Brain Res 523:288–290

Robertson GS, Vincent SR, Fibiger HC (1992) D_1 and D_2 dopamine receptors differentially regulate c-*fos* expression in striatonigral and striatopallidal neurons. Neuroscience 49:285–296

Robertson HA (1992) Immediate-early genes, neuronal plasticity, and memory. Biochem Cell Biol 70:729–737

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247–291

Rosen JB, Chuang E, Iadarola MJ (1994) Differential induction of Fos protein and a Fos-related antigen following acute and repeated cocaine administration. Brain Res Mol Brain Res 25: 168–172

Ruskin DN, Marshall JF (1994) Amphetamine- and cocaine-induced *fos* in the rat striatum depends on D2 dopamine receptor activation. Synapse 18:233–240

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) Mu-, delta- and kappa-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

Schwarting RKW, Huston JP (1996a) Unilateral 6-hydroxydopamine lesions of meso-striatal dopamine neurons and their physiological sequelae. Prog Neurobiol 49:215–266

Schwarting RKW, Huston JP (1996b) The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 50:275–331

Segal DS, Kuczenski R (1992a) In vivo microdialysis reveals a diminished amphetamine-induced DA response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment. Brain Res 571:330–337

Segal DS, Kuczenski R (1992b) Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. Brain Res 577:351–355

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

Simmons ML, Terman GW, Gibbs SM, Chavkin C (1995) L-type calcium channels mediate dynorphin neuropeptide release from dendrites but not axons of hippocampal granule cells. Neuron 14:1265–1272

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

Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci USA 89:2046–2050

Spangler R, Unterwald EM, Kreek MJ (1993) 'Binge' cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. Brain Res Mol Brain Res 19:323–327

Steiner H, Gerfen CR (1992) Time course of alterations in substance P, dynorphin, enkephalin and c-*fos* gene expression in striatal neurons during chronic cocaine treatment. Soc Neurosci Abstr 18:1044

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 (1995) Dynorphin opioid inhibition of cocaine-induced, D1 dopamine receptor-mediated immediate-early gene expression in the striatum. J Comp Neurol 353:200–212

Steiner H, Gerfen CR (1996) Dynorphin regulates D1 dopamine receptor-mediated responses in the striatum: relative contributions of pre- and postsynaptic mechanisms in dorsal and ventral striatum demonstrated by altered immediate-early gene induction. J Comp Neurol 376:530–541

Steiner H, Gerfen CR (1999) Enkephalin regulates acute D2 dopamine receptor antagonist-induced immediate-early gene expression in striatal neurons. Neuroscience (in press)

Steiner H, Mariotti R, Gerfen CR (1995) Enkephalin inhibits immediate-early gene expression induced by blockade of D2 dopamine receptors in striatum. Soc Neurosci Abstr 21:1904

Svingos AL, Moriwaki A, Wang JB, Uhl GR, Pickel VM (1996) Ultrastructural immunocytochemical localization of mu-opioid receptors in rat nucleus accumbens: extrasynaptic plasmalemmal distribution and association with Leu5-enkephalin. J Neurosci 16:4162–4173

Svingos AL, Clarke CL, Pickel VM (1998) Cellular sites for activation of delta-opioid receptors in the rat nucleus accumbens shell: relationship with Met5-enkephalin. J Neurosci 18:1923–1933

Tang F, Costa E, Schwartz JP (1983) Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. Proc Natl Acad Sci USA 80:3841–3844

Thompson LA, Walker JM (1990) Inhibitory effects of the kappa opiate U50,488 in the substantia nigra pars reticulata. Brain Res 517:81–87

Torres G, Rivier C (1993) Cocaine-induced expression of striatal c-*fos* in the rat is inhibited by NMDA receptor antagonists. Brain Res Bull 30:173–176

Trujillo KA, Day R, Akil H (1990) Regulation of striatonigral prodynorphin peptides by dopaminergic agents. Brain Res 518:244–256

Van Bockstaele EJ, Gracy KN, Pickel VM (1995) Dynorphin-immunoreactive neurons in the rat nucleus accumbens: ultrastructure and synaptic input from terminals containing substance P and/or dynorphin. J Comp Neurol 351:117–133

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

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

Wang JQ, McGinty JF (1996) 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, Boca Raton, pp 81–113

Wang JQ, Daunais JB, McGinty JF (1994) NMDA receptors mediate amphetamine-induced upregulation of zif/268 and preprodynorphin mRNA expression in rat striatum. Synapse 18:343–353

Wang H, Moriwaki A, Wang JB, Uhl GR, Pickel VM (1996) Ultrastructural immunocytochemical localization of mu opioid receptors and Leu-5-enkephalin in the patch compartment of the rat caudate-putamen nucleus. J Comp Neurol 375:659–674

Werling LL, Frattali A, Portoghese 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

Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194:599–615

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492

Xu M, Moratalla R, Gold LH, Hiroi N, Koob GF, Graybiel AM, Tonegawa S (1994) Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 79:729–742

You Z-B, Herrera-Marschitz M, Nylander I, Goiny M, O'Connor WT, Ungerstedt U, Terenius L (1994) The striatonigral dynorphin pathway of the rat studied with in vivo microdialysis. II. Effects of dopamine D_1 and D_2 receptor agonists. Neuroscience 63:427–434

Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal c-Fos-immunoreactive proteins via dopaminergic D_1 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

Yung KK, Bolam JP, Smith AD, Hersch SM, Ciliax BJ, Levey AI (1995) Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. Neuroscience 65:709–730

Yung KK, Smith AD, Levey AI, Bolam JP (1996) Synaptic connections between spiny neurons of the direct and indirect pathways in the neostriatum of the rat: evidence from dopamine receptor and neuropeptide immunostaining. Eur J Neurosci 8:861–869

Ziólkowska B, Höllt V (1993) The NMDA receptor antagonist MK-801 markedly reduces the induction of c-*fos* gene by haloperidol in the mouse striatum. Neurosci Lett 156:39–42