Molecular Neurobiology

ISSN0893-7648/98/16(3): 221–267/$19.75

Drugs of Abuse and Immediate-Early Genes in the Forebrain

Richard E. Harlan* and Meredith M. Garcia

Departments of Anatomy (REH) and Otolaryngology (MMG), Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112

Abstract

A diverse array of chemical agents have been self administered by humans to alter the psychological state. Such drugs of abuse include both stimulants and depressants of the central nervous system. However, some commonalties must underlie the neurobiological actions of these drugs, since the desire to take the drugs often crosses from one drug to another. Studies have emphasized a role of the ventral striatum, especially the nucleus accumbens, in the actions of all drugs of abuse, although more recent studies have implicated larger regions of the forebrain. Induction of immediate-early genes has been studied extensively as a marker for activation of neurons in the central nervous system. In this review, we survey the literature reporting activation of immediate-early gene expression in the forebrain, in response to administration of drugs of abuse. All drugs of abuse activate immediate-early gene expression in the striatum, although each drug induces a particular neuroanatomical signature of activation. Most drugs of abuse activate immediate-early gene expression in several additional forebrain regions, including portions of the extended amygdala, cerebral cortex, lateral septum, and midline/intralaminar thalamic nuclei, although regional variations are found depending on the particular drug administered. Common neuropharmacological mechanisms responsible for activation of immediate-early gene expression in the forebrain involve dopaminergic and glutamatergic systems. Speculations on the biological significance and clinical relevance of immediate-early gene expression in response to drugs of abuse are presented.

Index Entries: Immediate-early genes; c-fos; cocaine; amphetamine; nicotine; caffeine; morphine; tetrahydrocannabinol; hallucinogens; ethanol; forebrain; striatum; extended amygdala.

Introduction

Humans have been remarkably inventive in identifying a large number of agents that alter mental status and are addictive. These agents include both stimulants of the nervous system, such as cocaine, amphetamine, caffeine, nicotine, and phencyclidine, as well as depressants of the nervous system such as opiates, barbiturates, and ethanol. When taken to an extent that daily living patterns are adversely affected, these agents can be classified as drugs

*Author to whom all correspondence and reprint requests should be addressed.

of abuse. The cost to society stemming from drugs of abuse is incalculable. Despite the wide variety of drugs self-administered by humans, some common mechanisms of action on the brain have been identified. For instance, virtually all drugs of abuse activate release of dopamine in specific forebrain structures (e.g., Di Chiara and Imperato, 1988; Koob, 1992, 1996; Self and Nestler, 1995). However, the extent to which such similarities exist across different brain regions is difficult to determine using microdialysis of multiple brain areas.

Over the last decade, a multitude of studies have explored the expression of immediate-early genes (IEGs) in the central nervous system as markers of neuronal activation. As has been well described in other reviews (e.g. Hughes and Dragunow, 1995), the immediate-early genes are a set of transcription factors that are expressed rapidly after stimulation of a variety of cell-surface receptors. The prototypical immediate-early gene is c-*fos*, which is expressed at low levels in the unstimulated brain, but which is rapidly and transiently expressed in specific neuronal systems following administration of certain stimuli. Other immediate-early genes often have substantial baseline expression patterns (e.g. Harlan and Garcia, 1995; Herdegen et al., 1995), but will show a rapid and transient change in expression in response to various stimuli. Detection of these immediate-early genes at the single-cell level with *in situ* hybridization to localize mRNA or with immunocytochemistry to label the protein products of these genes allows examination of all brain regions following administration of a specific stimulus. Following the initial observations of effects of morphine (Chang et al., 1988), and cocaine or amphetamine (Graybiel et al., 1990) on expression of c-*fos*, numerous studies have demonstrated that drugs of abuse increase expression of certain immediate-early genes in the forebrain, although each drug appears to induce a particular neuroanatomical pattern of expression, suggesting activation of distinct sets of neurons.

The purpose of this review is to provide a survey of the anatomical patterns of immediate-early gene expression induced by each drug of abuse, with particular reference to the forebrain, and especially to the largest organizational features of the forebrain, i.e., the striatum, cortex, and extended amygdala. In addition, this review will provide an update on the described mechanisms of action of the different drugs leading to expression of immediate-early genes. In this fashion, we hope to provide information on potential commonalties and dissimilarities among forebrain regions that are activated by different drugs of abuse.

Large-Scale Organization of the Forebrain

Developmental Insights into the Organization of the Forebrain

Numerous schemes have been proposed to understand the basic organization of the forebrain. Recently, we (Heimer et al., 1997) and others (e.g., Altman and Bayer, 1995; Swanson, 1995) have emphasized the advantage of developmental studies for understanding basic organizational features of the brain. During development of the prosencephalon, the telencephalon expands bilaterally and forms walls bounding the rather triangular-shaped lateral ventricles. The medial walls produce the septal complex, the hippocampal formation, and the intervening fiber systems of the fornix. The dorsal and dorsolateral walls develop into the cerebral cortex, which eventually expands ventrally to cover deeper basal forebrain structures. The ventrolateral wall forms a series of basal forebrain structures, the largest of which are the striatum and the extended amygdala, which consists of several structures, including the medial and central nuclei of the amygdala, the bed nucleus of the stria terminalis, the sublenticular extended amygdala (substantia innominata), and the stria terminalis with its embedded clusters of neurons (Heimer et al., 1997a,b; Heimer et al., 1995; Alheid, 1995). In the rat, between embryonic day 13 (E13) and

E17 or E18, the ventrolateral wall is thrown into two longitudinal ridges, termed the lateral and medial ganglionic eminences. A considerable body of evidence indicates that the lateral ganglionic eminence will develop into the striatum (e.g., Palzaban et al., 1993; Deacon et al., 1994). Derivatives of the medial ganglionic eminence are less well understood, with some authors claiming the globus pallidus (Altman and Bayer, 1995), and others suggesting the extended amygdala (Song and Harlan, 1994b). The lateral eminence is larger than the medial, extending farther rostral. Moreover, this ridge expands at a much faster rate than does the medial eminence, eventually crowding the medial structure into a smaller, and somewhat discontinuous series of structures, which we believe to include much of the extended amygdala, and possibly the globus pallidus.

Basic Organization of Striatal-Pallidal Systems

The basic organization of the striatal complex has been described by numerous investigators (reviewed in Heimer et al., 1995; Gerfen, 1992a,b; Graybiel, 1990). The striatum can be divided into two major regions: a dorsal striatum, corresponding to the caudate and putamen in species with a fairly unified internal capsule, or to the caudate-putamen (CPu) in species with a dispersed internal capsule, such as the rat; and the ventral striatum, consisting of the olfactory tubercle (Tu) and the nucleus accumbens (Acb), which is divided into a core region (AcbC) surrounding the anterior limb of the anterior commissure, and a shell (AcbSh) bordering the core on its medial, ventral and ventrolateral sides (Fig. 1). The vast majority (70–95% depending on the species) of the striatal neurons are classified as medium spiny projection neurons. These neurons are GABAergic, and also express neuropeptides. Approximately 50–60% of the medium spiny neurons express the preproenkephalin (PPE) gene (Harlan et al., 1987), which encodes a precursor protein that is cleaved into several peptides including methionine enkephalin and leucine enkephalin. A somewhat smaller percentage of medium spiny neurons express the preprotachykinin (PPT) gene (Warden and Young, 1988; Harlan et al., 1989), which encodes the precursor to the peptides substance P and neurokinin A. Many of the PPT neurons in the dorsal striatum also express the preprodynorphin (PPD) gene (Anderson and Reiner, 1990), which encodes the precursor to the opioid peptide dynorphin. However, in the ventral striatum, PPT expression is generally greater than PPD expression. The majority of PPE neurons express dopamine D_2 (Le Moine et al., 1990) and adenosine A_2 receptors (Schiffmann et al., 1991), whereas the majority of PPT/PPD neurons express dopamine D_1 receptors (Gerfen et al., 1990). Although the PPE and PPT/PPD neurons are largely separate populations, approx 10–15% of medium spiny neurons express both genes (Gerfen and Young, 1988; Song and Harlan, 1994), and some of these neurons also express the neurokinin B gene (Burgunder and Young, 1989). Additional neuropeptide genes are expressed in smaller numbers of medium spiny neurons, including neurokinin B (Warden and Young, 1988; Lucas et al., 1992), neurotensin (Alexander et al., 1989) and other neuropeptides. In addition to the medium spiny neurons, two major populations of large aspiny interneurons are found in the striatum: cholinergic neurons, which are found in somewhat greater numbers laterally, and several groups of GABAergic interneurons expressing different calcium binding proteins, or coexpressing neuronal nitric oxide synthase, and peptides such as somatostatin, vasoactive intestinal polypeptide, and neuropeptide Y (reviewed in Heimer et al., 1995; Gerfen, 1992a,b; Graybiel, 1990: Kawaguchi et al., 1995).

Superimposed on the neuropeptide organization of the striatum is a compartmental feature dividing the structure into a large matrix compartment, which comprises approx 85% of the striatal volume and which surrounds clusters of neurons comprising the patch or striosome compartment (reviewed in Graybiel, 1990; Gerfen, 1992a,b). The patch compartment includes a rim of neurons subjacent

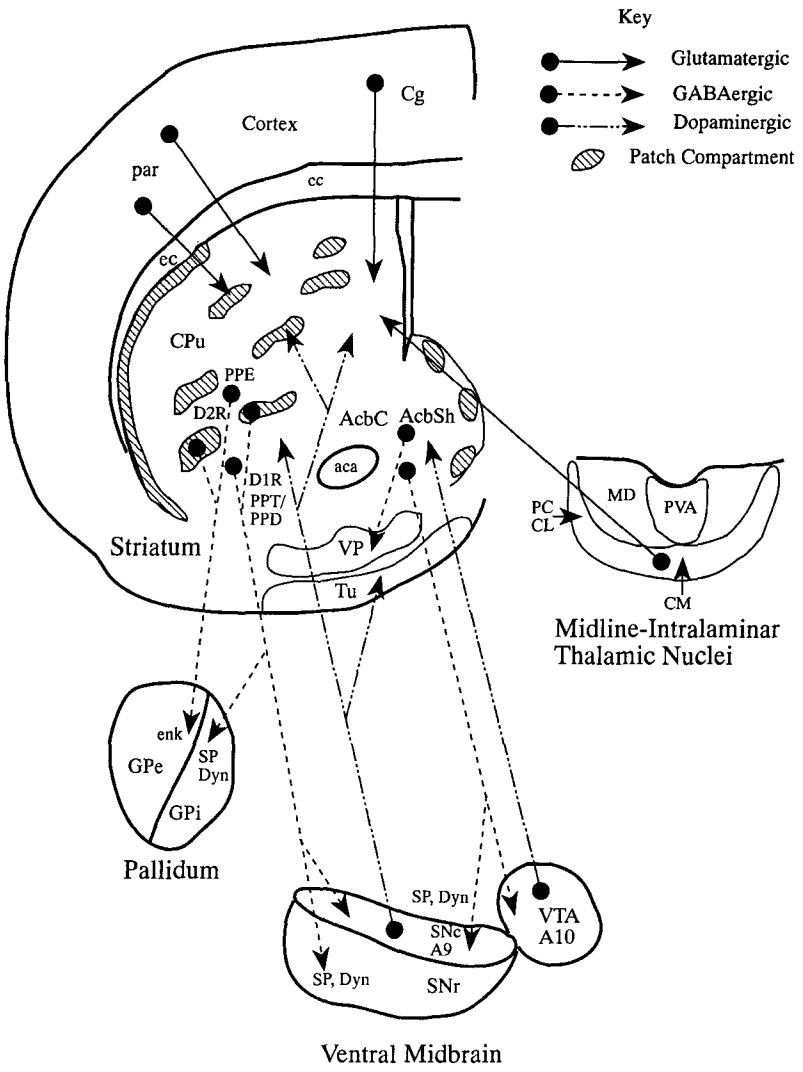

Fig. 1. Schematic diagram of the major organizational features of the striatum. The striatum consists of a dorsal portion, or caudate-putamen (CPu) and a ventral portion, which includes the nucleus accumbens (Acb) and the olfactory tubercle (Tu). The Acb is further divided into a core (AcbC) and a shell (AcbSh). The striatum is divided histochemically and hodologically into two compartments, the patch (identified by mu opiate receptors, shown in crosshatch) and the matrix. The patch compartment also includes a rim subjacent to the corpus callosum (cc) and external capsule (ec). The great majority of striatal neurons are medium spiny GABAergic projection neurons, which also express neuropeptide genes. Approximately half of the medium spiny neurons express the preproenkephalin (PPE) gene, as well as dopamine D_2 receptors (D2R) and adenosine A_2 receptors. The great majority of the PPE neurons in the CPu project to the external segment of the globus pallidus (GPe), where they release met-enkephalin (enk) and other peptides derived from the same precursor. Somewhat less than half of the medium spiny neurons express the preprotachykinin (PPT) and preprodynorphin (PPD) genes, as well as dopamine D_1 receptors (D1R). The great majority of these CPu neurons project to the substantia nigra pars reticulata (SNr), and release substance P (SP) and dynorphin (Dyn), and many send collaterals to the internal segment of the globus pallidus (GPi, or entopeduncular nucleus in the rat). The PPT/PPD neurons of the patch compartment project directly to the dopaminergic neurons of the substantia nigra pars compacta (SNc). Neurons of the Acb project to the ventral pallidum (VP), as well as to the ventral tegmental area (VTA) and SNc. Three major inputs of the striatum include dopaminergic inputs from the SNc and VTA, and glutamatergic inputs from midline/intralaminar thalamic nuclei and the cerebral cortex. These glutamatergic inputs are topographically arranged in longitudinal arrays. For instance the cingulate cortex (Cg) projects to the dorsomedial CPu, whereas the parietal cortex (par) projects to the central and lateral striatal regions. In addition, deep layers of cortex project to the patch compartment, whereas more superficial subgranular layers project to the matrix. Additional abbreviations: aca, anterior limb of the anterior commissure; CL, centrolateral thalamic nucleus; CM, central medial thalamic nucleus, MD, mediodorsal thalamic nucleus; PC, paracentral nucleus; PVA, anterior paraventricular thalamic nucleus.

to the corpus callosum and external capsule, called the subcallosal streak (Fig. 1). The neurons of the two compartments differ in inputs, outputs, and genes expressed (Fig. 2). Various markers of the patch-matrix organization have been used. The first, and perhaps still the best, marker is the mu opiate receptor, which is expressed by neurons of the patch compartment (Gerfen, 1992a,b). Matrix markers have also been used, including calbindin D_{28k}, which labels neuronal cell bodies, and somatostatin, which is found in fibers of the matrix (Gerfen, 1992a,b). However, these markers do not reveal all of the matrix, since calbindin is absent in the dorsolateral CPu, and somatostatin fibers are most enriched in the matrix of the ventral and ventrolateral CPu. Because the absence of staining of matrix markers provides a definition of the patch compartment, these matrix markers fail to identify patches in some regions of the dorsal striatum. Moreover, the markers are less useful in the AcbSh (Gerfen, 1992a,b). Whereas both PPE and PPT/PPD neurons are distributed fairly homogeneously between the patch and the matrix compartments, expression of PPD mRNA is higher in patch neurons than in matrix neurons (Fig. 2) (Gerfen and Young, 1988).

Medium spiny neurons project to pallidal structures (Fig. 1), including the ventral pallidum, the globus pallidus, the entopeduncular nucleus (corresponding to the internal segment of the globus pallidus in primates), and the substantia nigra pars reticulata (SNr). Both PPE and PPT neurons in the Acb project to the ventral pallidum, globus pallidus and entopeduncular nucleus (Napier et al., 1995). Additional projections from the ventral striatum target neurons in the ventral tegmental area and the SNc (Heimer et al., 1995). In the dorsal striatum, most of the PPE neurons project to the globus pallidus (corresponding to the external segment of the GP in primates), whereas most of the PPT/PPD neurons project to the GABAergic neurons of the SNr (Gerfen, 1992a,b), and often send a collateral to the internal segment of the globus pallidus (entopeduncular nucleus). The PPT/PPD neurons of the patch compartment project directly to the dopaminergic neurons of the SNc (Gerfen, 1984, 1985).

Superimposed on the organizations composed of neuropeptide systems, patch-matrix, and efferent projections, is a pattern of longitudinal arrays of glutamatergic inputs, topographically arranged from the neocortex (McGeorge and Faull, 1989; Gerfen, 1992; Berendse et al., 1992) and the midline/intralaminar nuclei (Berendse and Groenewegen, 1990). Thus, different patches, and different portions of the matrix, receive input from different regions of the neocortex and midline/intralaminar nuclei. Postsynaptic to these inputs, striatal neurons express multiple types of glutamate receptors, including NMDA receptors, AMPA/KA receptors, and metabotropic glutamate receptors (reviewed in Hollman and Heinemann, 1994), although different receptor types are localized to different neuronal phenotypes.

Basic Functions of Striatal-Pallidal Systems

A considerable effort has been placed on the identification of different cortico-striatal-pallidal-thalamo-cortical loops that engage different regions of cortex, striatum, pallidum, and thalamus (e.g., Chesselet and Delfs, 1996; Middleton and Strick, 1996). As a large-scale simplification, one can divide the loops into two groups, one employing the dorsal striatum and one utilizing the ventral striatum. Undoubtedly, within each of these groups specific loops engaging different parts of the cortex and striatum will eventually be identified. However, as a simplification, it may be possible to assign general functions to the loops that involve the dorsal vs the ventral striatum. An involvement of the dorsal striatum, with its inputs from sensory and motor cortex, in general behaviors and motor functions has been fairly well established (Heimer et al., 1995; Chesselet and Delfs, 1996; Middleton and Strick, 1996). Numerous studies have implicated the ventral striatum, with its cortical inputs derived from limbic

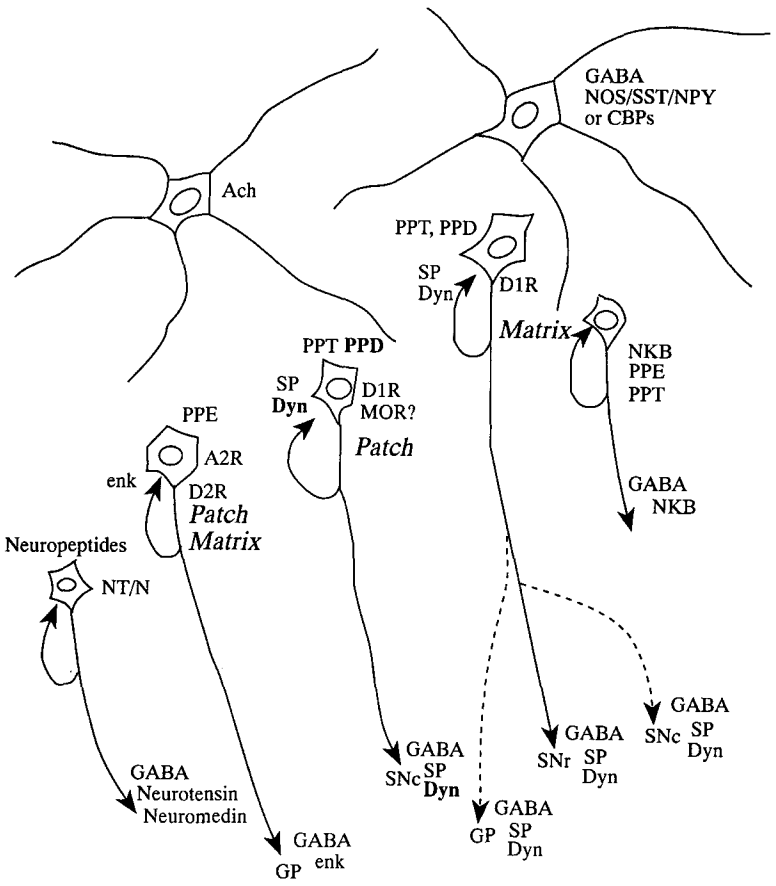

Fig. 2. Schematic diagram of the major neuronal phenotypes in the dorsal striatum. The great majority of neurons are medium spiny, GABAergic, projection neurons, which also have recurrent collaterals that project back to the parent cell body and its neighborhood. Found in both the patch and the matrix, neurons expressing the preproenkephalin (PPE) gene coexpress dopamine D_2 (D2R) and adenosine A_2 (A2R) receptors, and project primarily to the globus pallidus (GP), where they release both GABA and enkephalinergic peptides (enk). The second major medium spiny phenotype are neurons that coexpress the preprotachykinin (PPT) and preprodynorphin (PPD) genes, as well as the dopamine D_1 receptor (D1R). Whereas these neurons are found in both patch and matrix, those found in the patch compartment express higher levels of the PPD gene, and thus may release more dynorphin (dyn) from both recurrent collaterals and terminals in the substantia nigra pars compacta (SNc). These neurons may also express mu opiate receptors. PPT/PPD neurons in the matrix project to the substantia nigra pars reticulata (SNr), and send small collateral projections to the GP and the SNc. PPT expression levels and substance P (SP) release do not differ between PPT/PPD neurons of the patch and matrix. Smaller numbers of medium spiny neurons express additional neuropeptide genes, including neurokinin B (NKB, also known as preprotachykinin B), which is often coexpressed with both PPE and PPT, and NT/N, which encodes the precursor to neurotensin and neuromedin. In addition to the medium spiny projection neurons, two groups of large interneurons are also found: cholinergic (Ach) neurons and GABAergic neurons some of which coexpress nitric oxide synthase (NOS), somatostatin (SST), and neuropeptide Y (NPY), and some of which express particular calcium binding proteins (CBPs).

cortex and with subcortical loops involving specific intralaminar nuclei, in motivated behaviors, i.e., behaviors that result in a reward or the expectation of a reward (e.g., Heimer, et al., 1995, 1997).

The Extended Amygdala

A second major subdivision of the basal forebrain has been recognized in recent years as a system of interconnected regions

termed the extended amygdala (Heimer et al., 1997a,b, 1995; Alheid et al., 1995). These regions include the bed nucleus of the stria terminalis, the central and medial nuclei of the amygdala, and two intervening columns of neurons, one below the CPu and globus pallidus termed the sublenticular extended amygdala (substantia innominata), and a second embedded in the fiber systems of the stria terminalis. At the rostral pole, the extended amygdala, represented by the bed nucleus of the stria terminalis, is immediately caudal to the AcbSh, and tends to intermingle with this ventral striatal structure (Heimer et al., 1997). Indeed, the AcbSh appears to be a transition area between the striatum and the extended amygdala, and thus may subserve functions of each of these major forebrain areas. The brain regions forming the extended amygdala receive substantial inputs from visceral processing centers, such as the nucleus of the solitary tract and parabrachial nuclei (Alheid et al., 1995), and in turn project back to the hypothalamus and to brain stem autonomic regions controlling visceral functions. The extended amygdala also receives substantial cortical inputs, especially from the medial prefrontal, orbitofrontal, and insular cortex (Alheid et al., 1995; Heimer et al., 1997). These cortical regions also project to the Acb, especially the shell, and thus may serve to integrate the extended amygdala with the AcbSh as an extensive forebrain region that may help to attach emotional significance to environmental cues and to underpin motivated behaviors. In addition, the extended amygdala contains numerous neurons with receptors for estrogens and/or androgens (Heimer et al., 1997), potentially providing an added emotional context to motivated behaviors. Several recent studies have emphasized the amygdala, indeed much of the extended amygdala, in actions of drugs of abuse (e.g., Caine et al., 1995; McGregor and Roberts, 1993; Robeldo and Koob, 1993; Koob, 1996). As will be evident in this review, drugs of abuse activate IEG expression in both the striatum and subregions of the extended amygdala.

Immediate-Early Genes

A considerable body of evidence has been provided over the last decade that a system of genes is rapidly and transiently expressed in response to a variety of cellular stimuli, and that the protein products of these immediate-early genes act as transcription factors that potentially regulate expression of a wide variety of other cellular genes (reviewed in Hughes and Dragunow, 1995). The prototypical IEG is c-*fos*, which is expressed at very low levels in the unstimulated brain, but which is rapidly induced by a large variety of stimuli. Indeed, induction of c-*fos* has been used extensively to map brain regions activated in response to many different stimuli. Other members of the Fos family consist of a set of fos-related antigens (Fras), including Fra1, Fra2, FosB, and ΔFosB, which is a truncated form of FosB. Fras generally have slower kinetics of induction and decay than does c-Fos. The degree of baseline expression of these proteins varies across brain regions, but in general the levels in unstimulated animals are higher than that of c-Fos. These proteins heterodimerize with members of the Jun family of IEGs (reviewed in Hughes and Dragunow, 1995), including c-Jun, JunB, JunD, and at least one jun-related antigen (Harlan and Garcia, 1995). Members of the Jun family typically display considerable baseline expression, depending on the brain region (Harlan and Garcia, 1995; Herdegen et al., 1995). Each member of the Fos family has a different affinity for each member of the Jun family, providing considerable combinatorial possibilities. Moreover, the various heterodimers have different transcriptional activities at the consensus AP-1 DNA-binding sites (reviewed in Hughes and Dragunow, 1995). Thus, alterations in the ratios of different IEGs within a cell may profoundly affect the of regulation of expression of other genes, and may underlie long-term plastic changes associated with drug abuse. In addition to this large family of IEGs that codes for AP-1-binding transcription factors, other IEGs encoding transcription factors have been identified. Par-

ticular attention has been focused on a gene described variously as *zif/268*, *krox24*, *egr-1* or NGFI-A (Sukhatme et al., 1988). This gene has a considerable baseline expression level in the striatum and cortex, apparently as a response to synaptic activity (Worley et al., 1991; Moratalla et al., 1992; Mailleux et al., 1992), but responds rapidly and transiently to a variety of stimuli. The family of known IEGs continues to expand. However, with few exceptions, investigations of effects of drugs of abuse on IEG expression have been limited to the AP-1-binding proteins and *zif/268*.

Studies of IEG expression in the brain are useful from at least two points of view. First, the protein products of IEGs act as transcription factors that presumably alter the expression of target genes. Although studies in transfected cell lines have provided evidence for involvement of specific IEGs in regulation of expression of certain target genes (reviewed in Hughes and Dragunow, 1995), great difficulty has been encountered in attempting to demonstrate a role of IEGs in regulation of target genes in vivo. Nevertheless, it is likely that AP-1-binding proteins and other IEGs will eventually be demonstrated to have specific roles in regulating expression of certain target genes in vivo, but that this regulation will be modified by the variety of other cellular proteins expressed in each neuronal phenotype. To date, evidence has accumulated implicating IEGs in synaptic remodeling, learning, and memory, and long-term behavioral modifications (reviewed in Hughes and Dragunow, 1995).

Despite these limitations in our current understanding of the biological significance of IEG induction in the nervous system, the induction of IEGs can be used as a tool to investigate neural circuits that are activated biochemically in response to specific stimuli. Activation of IEGs can be demonstrated at the single-cell level, in all parts of the brain, from a single animal, at the same time. Thus, localization of IEGs, especially c-Fos, which has a low baseline level of expression, can become a powerful tool for determining the neuroanatomical patterns of neurons biochemically activated in response to drugs of abuse or other stimuli. It should be stressed, however, that induction of IEGs in a neuron signifies a biochemical activation of the neuron in response to stimulation of one or more signal-transduction pathways. IEG expression does not imply electrophysiological activation of the neuron; indeed, electrophysiological responses may be opposite of the biochemical activation revealed by IEG induction.

Drugs of Abuse and Expression of Immediate-Early Genes in the Forebrain

Cocaine

Neuroanatomical Patterns of IEG Induction by Cocaine

Acute administration of cocaine has been reported to increase expression of several immediate-early genes in the striatum, including c-Fos (e.g., Graybiel et al., 1990; Young et al., 1991), JunB (Hope et al., 1992; Moratalla et al., 1993) and *zif268* (Hope et al. 1992; Bhat et al., 1992; Bhat and Baraban, 1992). The *c-fos* response to cocaine was rapid, with maximal mRNA levels found at 60 min after injection (Ennulat et al., 1994). This elevation was followed by a depression to levels below those of uninjected control rats for 6–12 h (Ennulat et al., 1994). The c-Jun response to acute cocaine treatment has been reported to be weak (Kosofsky et al., 1995), if present at all (Moratalla et al., 1993; Chen et al., 1995). The most robust c-Fos, JunB, and *zif268* responses within the CPu were reported to be in the dorsocentral portion (Fig. 3), although nearly all regions of the CPu responded. There was no obvious relationship between the pattern of c-Fos induction in the CPu and the patch-matrix organization; i.e., cocaine induced expression of c-Fos in both patch and matrix (Graybiel et al., 1990). Several groups have reported induction of c-Fos in the Acb, especially in the core, although the

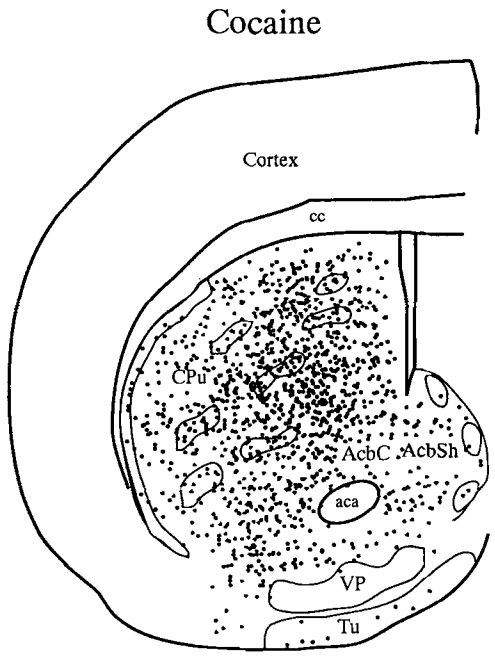

Fig. 3. Schematic diagram of the pattern of c-Fos induction in the striatum after acute injection of cocaine. Note that the most robust response was in the dorsal-central CPu, where c-Fos was induced in both the patch and matrix compartments. A smaller response was seen in the Acb, where the response was somewhat greater in the core than in the shell. The response in the Tu was weak. Although responses were seen in the cortex and septum, these were not plotted, for the sake of clarity. Abbreviations: aca, anterior limb of the anterior commissure; cc, corpus callosum; VP, ventral pallidum.

response was generally less robust than that in the CPu (Graybiel et al., 1990; Young et al., 1991; Hope et al., 1992; Brown et al., 1992; Torres and Rivier, 1992, 1994; Johansson et al., 1994). However, Bhat and Baraban (1993) reported no increase in *zif268* in the Acb following acute administration of cocaine. Expression of c-Fos in the Tu following acute treatment has been reported to be rather weak following 20 mg/kg cocaine (Graybiel et al., 1990), but strong with a higher dose (Iadarola et al., 1993).

Several other brain regions have been reported to display an immediate-early gene response to acute administration of cocaine, including the septum (Graybiel et al., 1990; Brown et al., 1992), cingulate cortex (Graybiel et al., 1990; Brown et al., 1992), claustrum (Brown et al., 1992), piriform cortex (Brown et al., 1992; Torres and Rivier 1992; Johansson et al., 1994), layer IV of parietal cortex (Johansson et al., 1994; Daunais and McGinty 1994), islands of Calleja (Young et al., 1991; Iadarola et al., 1993), paraventricular nucleus of the thalamus (Brown et al., 1992; Torres and Rivier, 1992), amygdala (probably the central nucleus; Brown et al., 1992), lateral habenula (Brown et al., 1992), suprachiasmatic nucleus (Torres and Rivier, 1992), lateral hypothalamic area (Torres and Rivier, 1992) and cerebellum (Couceyro et al., 1994). The array of IEGs induced by acute administration of cocaine may vary across brain regions, since Couceyro et al., (1994) reported that the AP-1 complex in striatum was composed of c-Fos, FosB, JunB, and JunD, but the cocaine-induced AP-1 complex in cerebellum contained only c-Fos and JunD. When rats were given enough cocaine to induce seizures, induction of immediate-early genes was much greater, and spread into several additional brain regions, including the granule-cell layer of the dentate gyrus, pyramidal layer of all hippocampal fields, olfactory bulb, posteromedial cortical amygdala, anterolateral amygdolohippocampal area, ventromedial hypothalamic nucleus, entorhinal cortex, and most areas of the neocortex (Clark et al., 1992; Helton et al., 1993).

In rats displaying a conditioned place preference induced by cocaine, placement of the rats in the conditioning chamber was sufficient to increase expression of c-Fos in several brain regions, including the cingulate cortex, piriform cortex, claustrum, olfactory tubercle, lateral septum, paraventricular thalamic nucleus, and amygdala, but not in the nucleus accumbens, dorsomedial striatum, ventral tegmental area, and substantia nigra (Brown et al., 1992; Crawford et al., 1995). Moreover, a positive correlation was found between the time spent in the cocaine-associated chamber and the number of c-Fos cells in the cingulate cortex, piriform cortex, olfactory tubercle, and lateral septum (Crawford et al., 1995). All of the brain

regions in which a cocaine-associated environment induced c-Fos are brain regions in which acute injection of cocaine induced IEGs. These results reveal a subset of nonstriatal brain regions in which the cocaine-induced IEG response may relate to the social or environmental aspects of cocaine use. It is likely that the striatal regions responding to cocaine play a different role in the overall neural response to this drug of abuse.

In a developmental study, Kosofsky et al., (1995) found that cocaine induced c-*fos* and *zif268*, but not c-*jun*, in the striatum, but not in the cortex or cerebellum at P5. By P15, cocaine induced all three immediate-early genes in the striatum and cortex, but not in the cerebellum. By P28, an adult pattern of induction was observed, with all three immediate-early genes responding in the striatum and cortex, but only c-*fos* was induced in the cerebellum. Within the CPu, the pattern of c-*fos* induction varied during development, with a patchy appearance at P5, linear arrays of labeled cells at P15, and the adult, rather homogeneous pattern centered on the dorsocentral CPu at P28. This shift in developmental pattern may reflect the migratory arrangements of early- vs later-born neurons in the CPu, since early-born neurons, which will develop primarily into the patch compartment (van der Kooy and Fishell, 1987; Snyder-Keller, 1995), undergo a secondary migration in groups or linear arrays during development of the CPu (Song and Harlan 1994).

Several groups have characterized the predominant striatal phenotype that displays an IEG response to acute cocaine treatment. Cenci et al., (1992) injected retrograde tracers into either the substantia nigra or the globus pallidus, then injected cocaine (25 mg/kg) 10 d later, followed by sacrifice 2 h later for immunocytochemical localization of c-Fos. The number of double-labeled cells as a percentage of the c-Fos immunoreactive neurons or of the retrogradely labeled cells was greater for transport from substantia nigra than from globus pallidus; 36.2% of striatal neurons projecting to the substantia nigra contained detectable cocaine-induced c-Fos, as did 18.4% of the neurons projecting to the globus pallidus. Ruskin and Marshall (1994) reported that cocaine (40 mg/kg) induced c-Fos expression in 10–25% of striatal neurons that were retrogradely labeled following injection of fluorogold into the substantia nigra. This contrasted with the substantial induction of c-Fos in nonretrogradely labeled striatal neurons following administration of the selective D_2 receptor antagonist eticlopride. Torres and Rivier (1994) reported that cocaine induced c-Fos primarily in neurons containing the dopamine D_1 receptor-associated protein DARPP32. Using adjacent sections to determine the neuropeptides utilized by cocaine-responding neurons in the lateral margin of the caudate-putamen, Johansson et al. (1994) reported that acute cocaine treatment induced c-Fos in 96% of the substance P neurons, 32% of the nonsubstance P neurons, none of the proenkephalin neurons, and 84% of the nonproenkephalin neurons. Some of the neurokinin B cells were double-labeled, but none of the cholinergic or somatostatinergic interneurons were labeled, nor were neurons containing the adenosine A_2 receptor. This general pattern was confirmed using double *in situ* hybridization at different developmental ages (Kosofsky et al., 1995); nearly all the c-*fos*-positive neurons also expressed PPT mRNA, as early as P5 and continuing into adulthood. These authors also noted that "the majority of c-fos+ cells were distinct from PPE+ cells," and that the association between c-*fos*-positive cells and PPT-positive neurons was more obvious than was the association between c-*fos* and expression of the dopamine D_1 receptor, especially at early ages. Interestingly, c-*fos* mRNA appeared to be preferentially expressed in cells with high levels of PPT mRNA. Since PPD is coexpressed with PPT in striatal neurons (Anderson and Reiner, 1990), these results suggest that the predominant striatal phenotype in which IEGs are induced by acute cocaine treatment are the PPT/PPD neurons that project to the substantia nigra and that express the dopamine D1 receptor (Fig. 4A). However, the results of Cenci et al., (1992) and Johansson et

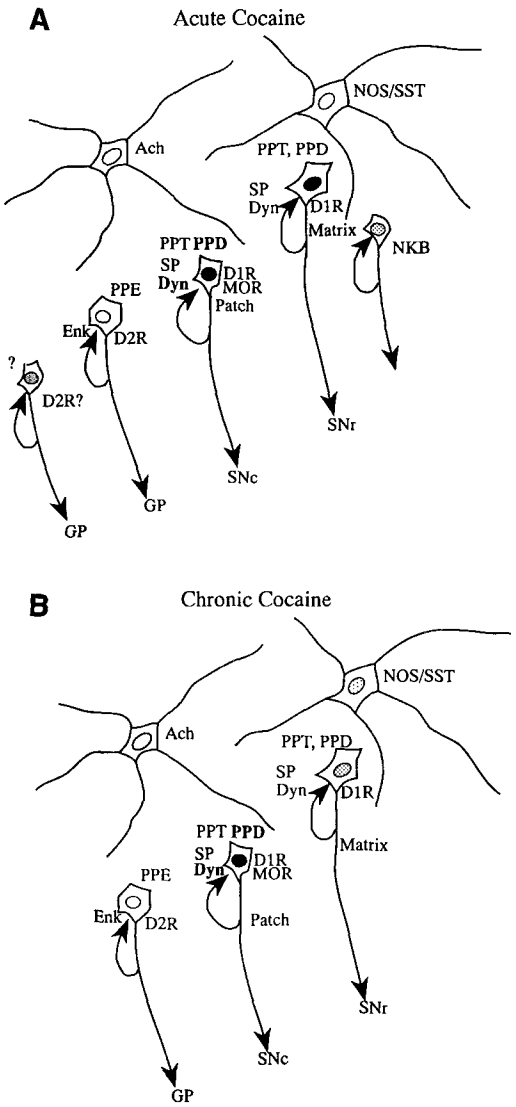

Fig. 4. Striatal phenotypes displaying a c-Fos response to acute cocaine **(A)** or displaying a JunB/Fra response to acute cocaine in rats previously given chronic cocaine **(B)**. The intensity of the nuclear shading correlates with the frequency of IEG expression in each phenotype. The major phenotypes activated by acute cocaine injection (A) were neurons expressing PPT and PPD, and projecting to the substantia nigra, although c-*fos* mRNA was expressed somewhat preferentially in neurons expressing high levels of PPD, i.e., those found in the patch compartment. Some NKB neurons contained c-Fos, as did a population of non-PPE neurons that projected to the globus pallidus. Expression of c-Fos in PPE neurons or interneurons was not detected. Acute injection of cocaine in rats receiving chronic cocaine treatment (B) reduced the IEG response in matrix neurons, thus restricting the response more to the patch compartment. The predominant activated phenotype continued to be the PPT/PPD neurons, although some interneurons containing nitric oxide synthase (NOS) and somatostatin (SST) were labeled.

al., (1994) suggest than an additional phenotype may express c-Fos in response to acute cocaine treatment, i.e., neurons that project to the globus pallidus, but express neither PPE nor PPT. We are not aware of any studies examining the phenotypes of cocaine-responsive neurons outside of the striatum.

Pharmacology of IEG Induction by Cocaine

The pharmacology of the induction of immediate-early genes by acute cocaine administration has been studied by several groups. A critical involvement of dopamine D_1 receptors has been demonstrated by antagonists of these receptors (Graybiel et al., 1990; Young et al., 1991; Bhat and Baraban, 1993; Couceyro et al., 1994; Steiner and Gerfen, 1995; Daunais and McGinty, 1996) and by the complete lack of an immediate-early gene response to cocaine in transgenic mice lacking the D_1 receptor (Moratalla et al., 1996; Drago et al., 1996). These results blend well with the observations that cocaine induces IEGs in striatal neurons expressing D_1 receptors. Interestingly, antagonists of D_1 receptors blocked the IEG response to cocaine in the cerebellum, as well as the striatum (Couceyro et al., 1994).

The degree of involvement of dopamine D_2 receptors in the IEG response to cocaine has been more difficult to address, since antagonists of D_2 receptors increase IEGs in the striatum. Graybiel et al., (1990) found that the D_2 receptor antagonist YM-09151 greatly reduced the cocaine-induced c-Fos response in the dorsal striatum, but the antagonist by itself also induced c-Fos somewhat in the dorsal striatum and strongly in the ventral striatum. Young et al., (1991) found that high doses of the D_2 receptor antagonist sulpiride reduced the c-Fos response to cocaine; however, they suggested that the dose used may have interacted with D_1 receptors. When administered alone, the D_2

receptor antagonist eticlopride greatly induced c-Fos in the striatum (Ruskin and Marshall, 1994); however, administration of eticlopride 30 min prior to cocaine reduced the c-Fos response to levels lower than that produced by either cocaine or eticlopride alone. Moreover, eticlopride induced c-Fos primarily in neurons that did not project to the substantia nigra, and blocked the cocaine-induced c-Fos response in striato-nigral neurons. Further evidence for involvement of D_2 receptors in cocaine-induced c-Fos expression was reported by Couceyro et al., (1994), who found that sulpiride did not induce c-Fos in the cerebellum, but partially blocked the c-Fos response to cocaine in this structure. Daunais and McGinty (1996) reported that the D_2-receptor antagonist sulpiride significantly reduced the cocaine-induced *zif/268* response in the dorsomedial, but not dorsolateral CPu. Taken together, these results indicate a vital role for dopamine D_1 receptors, and probably a partial role for D_2 receptors, in the IEG response to acute cocaine injection. The results comport with the finding that cocaine induced c-Fos in striatal grafts, but only in regions of the graft enriched in tyrosine hydroxylase (Liu et al., 1991), and that lesioning of striatal dopamine terminals with 6-hydroxydopamine (6-OHDA) blocked the IEG response to cocaine (Bhat and Baraban, 1993). The results also make sense from the view that one of the major targets of cocaine is the dopamine transporter (Kilty et al., 1991; Giros and Caron 1993), and that cocaine rapidly and transiently increased extracellular levels of dopamine in the CPu and Acb (e.g., Di Chiara and Imperato, 1988).

Evidence for neurotransmitters other than dopamine in the IEG response to acute cocaine has also been presented. Reasoning that a drug that is more selective for the dopamine transporter than is cocaine should mimic the effect of cocaine, Bhat and Baraban (1993) injected the dopamine transport blocker mazindole and examined the effect on expression of c-*fos* and *zif268* in the striatum. They reported that mazindole by itself had little effect on either IEG. However, when they combined mazindole with the serotonin (5-HT) transport blockers fluoxetine or citalopram, they found a robust IEG response in the striatum. Moreover, lesioning of 5-HT systems with *p*-chloroamphetamine significantly reduced, but did not eliminate, the IEG response to cocaine. Torres and Rivier (1994) reported that fenfluramine, a halogenated derivative of amphetamine that blocks reuptake of 5HT, induced c-Fos in a striatal pattern almost identical to that induced by cocaine. An involvement of norepinephrine (NE) in the IEG response to acute cocaine has been reported in the cerebral cortex, but not striatum (Bhat and Baraban, 1993). These results are consistent with the finding that cocaine blocks not only the dopamine transporter (Kilty et al., 1991; Giros and Caron 1993), but also the 5-HT and norepinephrine transporters (Jayanthi et al., 1993; Barker et al., 1994).

An involvement of glutamate receptors, especially of the NMDA type, in the IEG response to acute cocaine treatment has also been reported. Torres and Rivier (1993) reported a complete block of the c-Fos response to cocaine by pretreatment with ketamine, and a partial block by prior exposure to MK-801. Ketamine did not block the response in the piriform cortex, septum, or paraventricular nucleus of the thalamus. A partial block of the c-Fos response in the striatum by MK-801 has also been reported by Couceyro et al., (1994). An involvement of NMDA receptors in the IEG response to cocaine fits with the prevailing view of interactions between NMDA receptors and dopamine D_1 receptors in the striatum (e.g., Berretta et al., 1992; Konradi et al., 1996; Schwarzschild et al., 1997). Moreover, glutamate agonists have been reported to increase c-Fos in striatal neurons containing DARPP-32 (Berretta et al., 1992), as does cocaine (Torres and Rivier, 1994).

Neuropeptides expressed by striatal neurons have also been implicated in regulating the IEG response to cocaine. Steiner and Gerfen (1993) reported that systemic administration of the kappa opioid receptor agonist spiradoline blunted the c-Fos response to acute cocaine injection. In a following study, these authors

(Steiner and Gerfen, 1995) found that infusion of spiradoline into the CPu 10–15 min prior to systemic injection of cocaine blocked the c-*fos* and *zif268* responses in the area surrounding the cannula. Stimulation of kappa receptors with U-50,488 has also been reported to reduce the c-Fos response to a cocaine-associated chamber (Crawford et al., 1995). These results suggest that local release of dynorphin, the endogenous ligand for the kappa receptor, may decrease the IEG response to cocaine in the striatum. The results are consistent with the finding of an inverse correlation between expression of prodynorphin mRNA and induction of c-*fos* mRNA following chronic administration of cocaine (Steiner and Gerfen, 1995).

Alterations in IEG Patterns with Chronic Cocaine Treatment

Desensitization of the IEG response upon chronic treatment with cocaine has been reported by several groups. Hope et al., (1992) reported that chronic cocaine treatment (15 mg/kg twice daily for 14 d) blocked the c-*fos*, *c-jun*, and *zif268* responses and blunted the *fosB* and *junB* responses to acute challenge with cocaine. However, they found that AP-1 binding was elevated 18 h after cessation of the chronic treatment, with or without an acute challenge with cocaine. A shorter regimen of chronic cocaine treatment (15 mg/kg twice daily for 5 d) blunted, but did not eliminate, the *zif268* response to an acute challenge of cocaine given 8 h after the last injection (Bhat et al., 1992). However, Steiner and Gerfen (1993) reported a complete block of the c-*fos* response to acute cocaine by twice-daily injections of cocaine (15 mg/kg) for 3 d, with the challenge dose given approx 22 h after the last chronic dose. A higher dose of cocaine (30 mg/kg) continued to produce some elevation in c-*fos* mRNA levels, a finding consistent with that of Daunais and McGinty (1994), who found significant, but blunted, elevations of both *zif268* and c-*fos* mRNAs following 10 daily injections of cocaine (30 mg/kg, but not lower doses). However, Couceyro et al., (1994) reported complete desensitization of the c-*fos* mRNA response following 4 or more daily injections of cocaine. Interestingly, the desensitization was found in the striatum, but not in the cerebellum. Moratalla et al., (1996) noted that with different durations of chronic cocaine exposure, c-Fos was downregulated before JunB, and Fra was not decreased. Continuous exposure to cocaine via an osmotic pump produced no increase in c-Fos in the striatum or other brain regions (Torres and Rivier, 1992). In contrast, Ennulat et al. (1994) reported that three injections of cocaine on one day actually augmented the IEG response to a cocaine challenge the next day, and recently Torres and Horowitz (1996) reported no desensitization following 15 daily injections of 20 mg/kg cocaine. The desensitization of the c-*fos* response with chronic exposure to cocaine may underlie the lack of a c-*fos* response to self-administration of cocaine (Daunais et al., 1993; 1995), since the rats were trained for several days to self-administer cocaine prior to examination of the effects on c-*fos* expression. Although underlying mechanisms of this desensitization have not been explored thoroughly, Asin et al., (1994) found that daily administration of the D_1 receptor agonist A-77636 for four days blunted the c-Fos response to cocaine, in both the Acb and CPu. It is possible that chronic administration of cocaine downregulates expression of D_1 receptors, thus desensitizing the IEG response to acute administration of cocaine, as has been reported at certain time intervals after chronic exposure (Kleven et al., 1990), although this has not been a universal finding (Alburges et al., 1993; Unterwald et al., 1994).

Chronic administration of cocaine has also been reported to shift the pattern of immediate-early gene expression, both biochemically and anatomically. Whereas both acute and chronic administration of cocaine induced AP-1 binding in the striatum, the complex of proteins involved differed between acute and chronic treatment (Hope et al., 1994). Acutely, cocaine induced primarily c-Fos (58 kDa), FosB (45 kDa), and a 40-kDa Fra. Subsequent work demonstrated a complex of Fras induced by

acute cocaine administration, with different degrees of induction: C-Fos > Fra-1 > Fra2/FosB and ΔFosB (Chen et al., 1995). These acute proteins were rapidly induced and disappeared by 18 h after treatment (Hope et al., 1994). However, chronic treatment with cocaine induced Fras of 35–37 kDa, with little induction of higher molecular-weight proteins (Hope et al., 1994; Chen et al., 1995; Nye et al., 1995). These chronic Fras lasted considerably longer than did acute Fras, with detectable levels 7 d after cessation of chronic treatment with cocaine. The 35-37-kDa proteins appeared to be the truncated FosB protein, ΔFosB, or related proteins (Hope et al., 1994; Rosen et al., 1994). In addition, the AP-1-binding complex was either disrupted or supershifted by antibodies to JunD and JunB, but not c-Jun (Chen et al., 1995). The shift from higher to lower molecular-weight Fra proteins was dependent on the dose of cocaine, although induction of chronic Fras occurred at lower doses in the Acb than in the CPu (Nye et al., 1995). Several different intermittent cocaine treatments induced chronic Fras, whereas continuous infusion of cocaine induced neither acute nor chronic Fras (Nye et al., 1995). Self-administration of cocaine has been shown to increase levels of chronic Fras, but not c-*fos* (Daunais et al., 1993; 1995) in the prefrontal cortex, Acb and CPu, but not amygdala (Pich et al., 1997).

The pharmacology of chronic Fra induction by cocaine appeared to be somewhat different from the IEG response to acute cocaine injection. The D_1 dopamine-receptor antagonist SCH-23390 only partially blocked the induction of chronic Fras (Nye et al., 1995), whereas this agent completely blocked the IEG response to acute cocaine (Graybiel et al., 1990; Young et al., 1991; Bhat and Baraban, 1993; Couceyro et al., 1994; Steiner and Gerfen, 1995; Daunais and McGinty, 1996). Chronic treatment with the D_2-receptor antagonist eticlopride induced chronic Fras to a greater extent than did chronic cocaine administration, and did not attenuate the chronic Fra response to cocaine (Nye et al., 1995), as opposed to the decrease in the c-Fos response to acute cocaine produced by eticlopride (Ruskin and Marshall, 1994). These results suggest a predominant role of D_1 receptors, and little involvement of D_2 receptors, in the cocaine-induced production of chronic Fras. However, chronic administration of the selective D_1-receptor agonist SKF 81297 did not induce chronic Fras (Nye et al., 1995), whereas chronic treatment with the D_1-receptor agonist A-77636 blocked the c-Fos response to acute cocaine treatment (Asin et al., 1994). Thus, induction of chronic Fras and downregulation of acute Fras may be separable events. Further distinctions between the pharmacology of acute and chronic Fra induction are found in the actions of drugs inhibiting specific neurotransmitter transporters. Nye et al. (1995) found that chronic Fras were induced by chronic administration of the dopamine transport inhibitor GBR 12909, but not by selective inhibitors of the norepinephrine or serotonin transporters; however, Bhat and Baraban (1993) reported that acute administration of the dopamine-transport blocker mazindole did not induce c-*fos* acutely, but when combined with agents blocking serotonin transport, c-*fos* was induced.

Chronic cocaine exposure has also been reported to alter the anatomical distribution of IEG-responding neurons. Moratalla et al. (1996) reported that chronic administration of cocaine (twice daily injections of 25 mg/kg for 5, 7, or 14 d) downregulated the c-Fos and JunB responses to acute injection, with little change in the Fra response, determined immunocytochemically. However, the remaining response shifted from the fairly homogeneous response centered on the dorso-central CPu, to a clustered response similar to that obtained with acute injection of amphetamine. This shift resulted from a decrease in the response in matrix neurons and an increase in the response of the patch neurons, as determined by comparisons to calbindin and dynorphin immunoreactivity as patch-matrix markers. They also noted a shift in the phenotypes of responding neurons; whereas the vast majority of the JunB/Fra response continued to be restricted to dynorphin neurons,

there was a two- to fourfold increase in the number of NADPH diaphorase neurons displaying an IEG response after chronic cocaine and acute challenge (Fig. 4B). Increased levels of dynorphin immunoreactivity in the same patches was also noted with 2 wk of chronic cocaine treatment.

Significance of IEG Induction by Cocaine

Potential roles for IEG induction by acute cocaine treatment have been explored. For instance, infusion of a c-*fos* antisense oligonucleotide into the nucleus accumbens blocked the locomotor response to acute cocaine given 8 h later (Heilig et al., 1993). Several studies also have demonstrated that cocaine administration can alter expression of neuropeptide genes in the striatum. Acute cocaine injection has been reported to increase expression of the PPT gene in the striatum (Steiner and Gerfen, 1993; Drago et al., 1996), whereas an acute binge administration (Daunais and McGinty, 1996) or chronic administration (Steiner and Gerfen, 1993; Daunais and McGinty, 1994, 1995) increased expression of the PPD gene, with a patchy distribution in the CPu. Either no change (Steiner and Gerfen, 1993; Daunais et al., 1995) or an increase (Hurd and Herkenham, 1992) in PPE mRNA levels have been reported with acute cocaine administration, whereas no changes have been reported following chronic treatment (Steiner and Gerfen, 1993; Daunais et al., 1995). Whether these increases in PPT and PPD mRNA levels are related to alterations in IEG expression has not been demonstrated. Daunais et al., (1995) noted that self-administration of cocaine produced increased expression of the PPD gene in the CPu, with no associated increase in c-*fos* or *zif268* mRNAs, although self-administration of cocaine has been reported to induce chronic Fras (Pich et al., 1997). Steiner and Gerfen (1995) found a significant negative correlation between PPD mRNA levels and the peak induction of c-*fos* mRNA in the CPu following chronic cocaine administration. Thus, it is possible that cocaine activates IEG expression acutely in a population of striatal neurons expressing PPT and PPD, stimulating release of dynorphin from local circuits or recurrent collaterals, increasing expression of the PPD gene, and downregulating the c-Fos response to subsequent exposure to cocaine. A critical involvement of dopamine D_1-receptors in this process has also been demonstrated in transgenic mice lacking these receptors, in which chronic cocaine treatment had no effect on chronic Fras or on dynorphin immunoreactivity (Moratalla et al., 1996). Indeed, in these animals, baseline levels of striatal PPD (Moratalla et al., 1996) and PPT (Drago et al., 1996) mRNAs were much lower than in wild-type, suggesting a requirement of activation through D_1 receptors in maintenance of PPD and PPT expression. However, acute cocaine administration greatly increased PPT expression in the CPu, and in brain areas in which PPT mRNA is not normally increased after cocaine, in transgenic mice lacking the dopamine D_1 receptor, despite the lack of cocaine-induced c-*fos* or *zif268* mRNA expression (Drago et al., 1996). Thus, cocaine may normally increase expression of IEGs in striatal neurons expressing both PPT and PPD, but the IEG proteins may regulate expression of the PPD gene, without much control over the PPT gene. Nevertheless, striatal PPT mRNA levels were increased rapidly following acute cocaine injection (Steiner and Gerfen, 1993; Drago et al., 1996). It is possible that early-responding proteins other than those involved in the AP-1 complex regulate expression of the PPT gene. An involvement of CREB (cAMP response element binding protein) in cocaine-regulated gene expression has been emphasized (Hyman, 1996). Using differential display of PCR products, Douglass et al., (1995) cloned a novel product induced by acute administration of either cocaine or amphetamine. Fosnaugh et al., (1995) demonstrated a rapid and transient elevation in mRNA encoding *arc*, a novel effector immediate-early gene, in response to acute cocaine administration. The potential involvement of these early-responding genes in regulation of PPT gene expression in response to cocaine has not been explored.

Amphetamine

Neuroanatomical Pattern of IEG Induction by Amphetamine

Numerous studies have demonstrated that acute administration of amphetamine induced expression of several immediate-early genes, including c-*fos*, *fosB*, *JunB*, *c-jun*, *fra-1*, and *zif/268* Graybiel et al., 1990; Moratalla et al., 1992; Persico et al., 1993; Konradi et al., 1996). Anatomical studies utilizing *in situ* hybridization and immunocytochemistry have demonstrated a large induction of the c-*fos* gene in the rat cerebral cortex, including cingulate cortex (Graybiel et al., 1990), ventrolateral limbic cortex (e.g., insular cortex; Graybiel et al., 1990; Curran et al., 1996), sensory cortex (Wang et al., 1994a,b; Curran et al., 1996) and motor regions (Wang et al., 1994a,b; Curran et al., 1996). In sensory and motor regions, induction was observed primarily in layers IV and VI (Wang et al., 1994a,b, 1995; Johansson et al., 1994; Curran et al., 1996). Methamphetamine was reported to induce *zif/268* to a much greater extent in the cortex than did amphetamine (Wang et al., 1995); moreover the pattern within the cortex differed between the two drugs, with amphetamine producing a graded response in the following sequence: sensory cortex, upper limb > motor cortex >> sensory cortex, nose, whereas methamphetamine induced a different pattern: sensory cortex, nose > sensory cortex, upper limb = motor cortex. Several nonstriatal, subcortical regions have also been reported to demonstrate a c-*fos* response to acute amphetamine injection, including the septum (Graybiel et al., 1990), thalamus (Persico et al., 1993), hypothalamus (Persico et al., 1993), hippocampus (Persico et al., 1993), lateral habenula (Wirtshafter et al., 1994), globus pallidus (Labandeira-Garcia et al., 1994; Vargo et al., 1996), substantia nigra (Jaber et al., 1995), and cerebellum (Persico et al., 1993). In cynomolgus monkeys (*Macaca fascicularis*) acute amphetamine injection was found to increase c-Fos in the periventricular thalamus, lateral habenula, and supramamillary region (Asin et al., 1996).

The striatal response to acute amphetamine injection has been studied extensively. In the initial report on effects of amphetamine on c-Fos expression, an enrichment in the patch compartment in the dorsal striatum was noted, as determined by immunostaining with an antibody to calbindin D_{28k} on adjacent sections (Graybiel et al., 1990). The c-Fos response was not absent in the matrix, but was reduced relative to the response in the patch compartment. Indeed, the camera lucida drawings comparing the c-Fos response in patch vs matrix with amphetamine and cocaine (Graybiel et al., 1990), suggested that the number of labeled neurons in the patch compartment was similar following both drugs, but the number of labeled cells in the matrix compartment was reduced following amphetamine. The Fos-positive neurons were found primarily in the medial 2/3 of the CPu, with few detected in the dorsolateral region, except for the subcallosal streak. Thus, the enrichment in the patch compartment may correspond better to patches defined by relative presence of calbindin in the surrounding matrix than by expression of mu opiate receptors. This enrichment of immediate-early gene response to amphetamine in patch-like areas of the CPu has been reported by several labs (Nguyen et al., 1992; Hooper et al., 1994; Simpson et al., 1995; Paul et al., 1995; Curran et al., 1996), and for *zif/268* (Moratalla et al., 1992; Wang et al., 1995). Using adjacent sections processed for *zif/268* mRNA or for (^{3}H) naloxone binding to identify the mu opiate receptors of the patch compartment, Moratalla et al. (1992) reported that all dorsal striatal regions of amphetamine-induced *zif/268* mRNA corresponded to mu opiate receptor patches, although not all receptor patches corresponded to increased *zif/268* mRNA. A clustering of neurons expressing c-*fos* mRNA 1 h after injection of methamphetamine was also reported (Merchant et al., 1994), although no markers of the patch-matrix organization were used. As with amphetamine, the c-*fos* response to methamphetamine was more robust in the medial 2/3 of the CPu.

However, several labs have either not reported a patch-like distribution of IEGs in the dorsal striatum after acute amphetamine (Labandeira-Garcia et al., 1994; Wang et al., 1994a,b; Bjelke et al., 1994; Johansson et al., 1994; Jaber et al., 1995; Cadoni et al., 1995; Turgeon et al., 1996), or have reported such a pattern without performing studies with markers to define patch-matrix boundaries (Nguyen et al., 1992; Simpson et al., 1995; Wang et al., 1995; Curran et al., 1996; Moratalla et al., 1996). Moreover, increased expression of immediate-early genes in the subcallosal streak following acute amphetamine has not always been obvious from published photographs (Moratalla et al., 1992; Curran et al., 1996). However, in cynomolgus monkeys, a strong association between amphetamine-induced c-fos and calbindin-poor patches of the dorsal striatum was noted (Asin et al., 1996). Interestingly, in these animals, the calbindin-poor patches were restricted primarily to the medial portion of the caudate, whereas amphetamine-induced c-fos was noted in all regions of both the caudate and the putamen. Thus, it is likely that acute amphetamine induces IEGs to a greater extent in the patch compartment than in the matrix (Fig. 5), but the association may be more obvious in certain regions of the striatum, in both rats and monkeys. The striatal regions in which the amphetamine response is most enriched in the patch compartment may be those regions in which patches are defined by the presence of calbindin D_{28k} in the surrounding matrix. Thus, in rats, the association between patches and amphetamine-induced IEGs may be clearest in the ventral half of the dorsal striatum (Moratalla et al., 1992), while in monkeys, this association may be most obvious in the medial portion of the caudate nucleus (Asin et al., 1996). Because calbindin D_{28k} is enriched in those portions of the matrix compartment where amphetamine-induced IEG expression is reduced relative to the response in patches, it is possible that the intracellular calcium buffering actions of calbindin D_{28k} (e.g., Baimbridge et al., 1992) may contribute to the reduced IEG response to amphetamine.

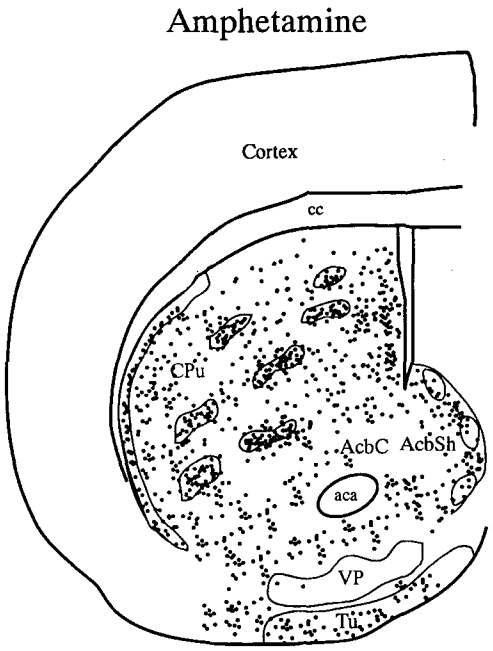

Fig. 5. Schematic diagram of the pattern of c-Fos induction in the striatum after acute injection of amphetamine. Note the enrichment of the response in the patch compartment compared to the matrix. The response in the AcbSh was somewhat greater than the response in the AcbC. Although responses were seen in the cortex and septum, these were not plotted, for the sake of clarity. Abbreviations as in Fig. 3.

Induction of immediate-early genes in the ventral striatum following acute amphetamine injection has been less consistently reported. A strong c-Fos (Graybiel et al., 1990) and *zif/268* (Moratalla et al., 1992) response to acute amphetamine has been reported in the olfactory tubercle, although other groups have not reported induction of c-Fos in this region (Dalia and Wallace, 1995). Within the Acb, some groups have reported a similar level of response between the core and the shell (Graybiel et al., 1990; Robertson and Jian, 1995), although other groups have noted a stronger response in the shell than in the core (Nguyen et al., 1992; Turgeon et al., 1996; Curran et al., 1996). Some groups have also reported no increase in IEG expression in the Acb following acute amphetamine injection (Wang et al.,

1994a,b; Simpson et al., 1995). Induction of c-Fos in the Acb, especially in the shell, was reported in monkeys and in one marmoset (Asin et al., 1996).

In a developmental study, Labandeira-Garcia et al., (1994) reported that amphetamine injected on the day of birth (P0) or on P7 did not induce c-Fos in either the CPu or the globus pallidus 2 h later. At P14, amphetamine augmented c-Fos expression in both the CPu, where most of response was in the medial half, and in the globus pallidus, where the response was strong (as opposed to a weak response in the adult). At P21, amphetamine induced an adult-like pattern in the CPu (except for the rostral pole, which had a reduced response), and in the globus pallidus, where the response was more intense than in the CPu. At P28, an adult pattern was found in both the CPu and the globus pallidus. It is interesting to note that the c-Fos response in the CPu, once it appeared at P14, was rather homogeneous and concentrated in the medial half, rather than enriched in patches. Because the patch compartment develops earlier than does the matrix compartment (van der Kooy and Fishell, 1987; Snyder-Keller, 1995; Song and Harlan, 1994), a relative restriction of the amphetamine-induced c-Fos response to the patch compartment would be expected at P14, unless other developmental considerations predominate, such as the ontogeny of striatal inputs, specific receptors, or intracellular signaling mechanisms.

Several groups have characterized the phenotypes of striatal neurons displaying an IEG response to acute amphetamine. Robertson and Jian (1995) noted that approximately equal numbers of Fos-positive neurons in the Acb following acute amphetamine injection projected to the ventral pallidum or to the substantia nigra; moreover, about half of the Fos-positive projecting neurons sent axons to both of these regions. These authors reported that approx 20–25% of projecting neurons in the Acb expressed c-Fos after amphetamine, although they did not provide information on the proportion of Fos-positive neurons that were retrogradely labeled. Ruskin and Marshall (1994) reported that dorsal striato-nigral neurons tended to form groups surrounding small regions containing fewer retrogradely-labeled cells; these regions relatively devoid of striato-nigral neurons corresponded to the patch compartment, as determined by immunostaining of calbindin D_{28k} in adjacent sections. Following acute amphetamine injection, approx 25% of the retrogradely-labeled neurons expressed c-Fos. However, no information was provided regarding the percentage of fos-positive neurons that were retrogradely labeled. In double-label studies following acute amphetamine injection, Johansson et al. (1994) found that 37% of the PPT neurons in the CPu expressed *c-fos* mRNA, as did 8% of the non-PPT neurons, 4% of the PPE neurons, and 27% of the non-PPE neurons. None of the neurons expressing ChAT, adenosine A_2 receptors, NKB, or somatostatin expressed *c-fos* mRNA. In a similar type of double-label study combining immunocytochemistry for Fos/Fra with *in situ* hybridization for neuropeptide genes, Jaber et al. (1995) reported that following acute amphetamine injection, 78% of the PPT neurons in the CPu had Fos/Fra, as did 33% of the PPE neurons. Thus, acute injection of amphetamine appears to increase c-Fos primarily, but not exclusively, in dorsal striatal neurons expressing the PPT gene and projecting to the substantia nigra (Fig. 6A). The phenotypes of Fos-positive neurons in the patch compartment have not been clearly identified, since the retrograde label studies of Ruskin and Marshall (1994) identified primarily matrix neurons. In the ventral striatum, neurons projecting to the ventral pallidum, substantia nigra, or both regions expressed c-Fos after amphetamine (Robertson and Jian, 1995).

The amphetamine derivative MDMA (3,4-methylenedioxy-methamphetamine; ecstasy) has been reported to increase dopamine release in the striatum (Yamamoto and Spanos, 1988). Dragunow et al. (1991) reported a large increase in Fos/Fra in the CPu, Acb, Tu, and piriform cortex 3 h after injection of MDMA. Within the CPu, the greatest response was seen in the dorsomedial and ventromedial portions

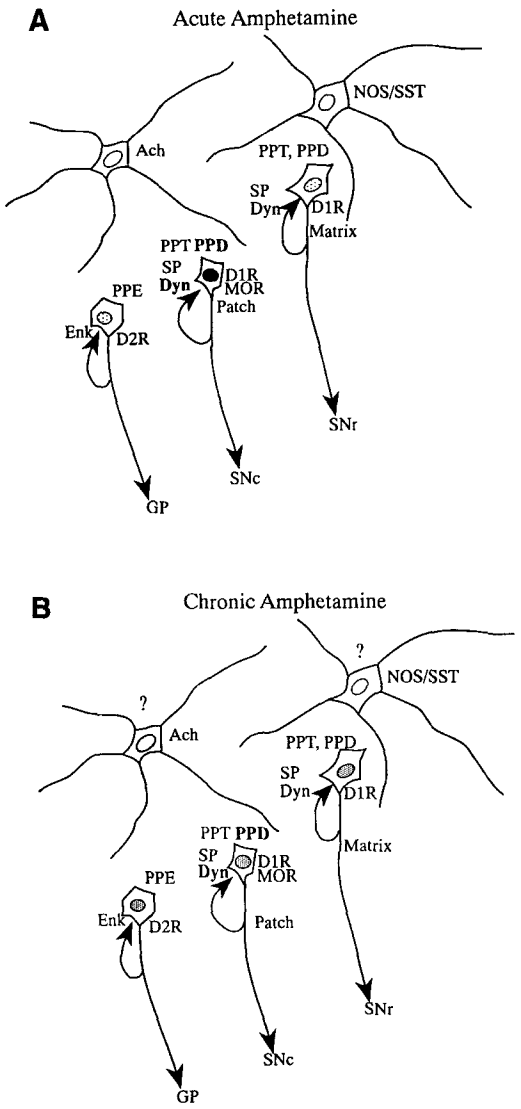

Fig. 6. Striatal phenotypes displaying a c-Fos response to acute amphetamine **(A)** or displaying a Fos/Fra response to acute amphetamine in rats given chronic amphetamine **(B)**. The intensity of the nuclear shading correlates with the frequency of IEG expression in each phenotype. Although the predominant activated phenotypes after acute amphetamine injection (A) were neurons expressing the PPT and PPD genes, the response was restricted somewhat to the patch compartment. In addition, estimates of the percentage of PPE neurons containing c-Fos after acute amphetamine injection ranged from 4–33% (*see* text for details). There was no expression of c-Fos in interneurons. In rats given chronic amphetamine treatment (B), the phenotypes displaying a Fos/Fra response were evenly divided among neurons expressing PPE and PPT/PPD. Expression of Fos/Fra in interneurons following chronic amphetamine has not been determined.

rostrally and in the dorsomedial region at caudal levels. However, the photomicrographs provided did not offer sufficient anatomical landmarks to provide detailed comparisons with other drugs of abuse. Double-labeling for calbindin showed some Fos-positive neurons contained calbindin, but others were found in the calbindin-poor patch compartment. None of the Fos-positive cells contained parvalbumin or neuropeptide Y.

Pharmacology of IEG Induction by Amphetamine

Pharmacological mechanisms underlying the amphetamine-induced expression of IEGs in the striatum have been investigated by several groups. A predominant involvement of dopamine D_1 receptors in the striatal IEG response to amphetamine has been demonstrated by the pharmacological antagonist SCH 23390 (Graybiel et al., 1990; Moratalla et al., 1992; Nguyen et al., 1992), by reduction of the response following unilateral destruction of ascending catecholaminergic systems with 6-OHDA (Cenci and Bjorklund, 1994; Paul et al., 1995), by a reduction of the response following depletion of catecholamines with α-methyl-*p*-tyrosine (Cadoni et al., 1995), and by absence of the response in transgenic mice lacking the dopamine D_1 receptor (Moratalla et al., 1996). Unilateral lesioning of catecholaminergic systems with 6-OHDA has also been reported to block the amphetamine-induced c-Fos response in the lateral habenula (Wirtshafter et al., 1994). These studies are consistent with the observation that amphetamine releases dopamine from terminals in several brain regions (e.g., Sharp et al., 1987; Butcher et al., 1988). Because depletion of catecholamines with 6-OHDA or α-methyl-*p*-tyrosine would affect both D_1- and D_2-receptor responses, the involvement of D_2 receptors in the normal rat cannot be eliminated. Indeed, pharmacological studies with D_2-receptor antagonists have

demonstrated a reduction in the c-Fos response to amphetamine (Graybiel et al., 1990; Ruskin and Marshall, 1994), although the antagonist-induced c-Fos expression makes interpretation of the results somewhat more difficult. Adenosine A_2 receptors have been colocalized to striatal neurons which express dopamine D_2 receptors (Fink et al., 1992; Gerfen et al., 1990) or in striatal PPE neurons (Schiffmann et al., 1991), and a functional antagonism between A_2 and D_2 receptors has been reported (Ferre et al., 1994; Ferre et al., 1991). Thus, stimulation of A_2 receptors would be expected to oppose events activated by D_2 receptors, such as activation of c-Fos in response to acute amphetamine. Indeed, Turgeon et al. (1996) reported that administration of the A_2 agonist, APEC, 30 min prior to injection of amphetamine inhibited the c-Fos response in the CPu and Acb.

Because amphetamine has been reported to release serotonin (e.g., Kuczenski et al., 1995), and because the D_1-receptor antagonist SCH 23390 also blocks 5-HT_2 receptors (Bischoff et al., 1986), an involvement of serotonin receptors has also been investigated. Graybiel et al. (1990) reported an inability to block the c-Fos response to amphetamine with the serotonin receptor antagonist metergoline or with the 5-HT synthesis inhibitor *p*-chlorophenylalanine. However, Bhat and Baraban (1993) reported that lesioning of 5-HT systems with PCA attenuated the *zif/268* response to acute amphetamine injection. Moreover, fenfluramine, a halogenated derivative of amphetamine which blocks reuptake of 5-HT, reportedly induced c-Fos in the CPu and Acb (Torres and Rivier, 1994). Striatal release of 5-HT by amphetamine might underlie the amphetamine-induced c-Fos response in both the intact and 6-OHDA-lesioned CPu (Bjelke et al., 1994), rather than, or in addition to, the proposed volume transmission of dopamine from the intact to the lesioned side. Moreover, since 5-HT is enriched in the patch compartment of the dorsal striatum, especially in ventral regions (Halliday et al., 1995), it is possible that synergy between dopamine and serotonin released by amphetamine augments the IEG response in portions of the patch compartment relative to the matrix. Because antagonism of dopamine D_1 receptors did not block the amphetamine-induced c-Fos response in the cerebral cortex (Graybiel et al., 1990), it is possible that serotonin, or other neurotransmitters released by amphetamine, is involved in the cortical response.

Evidence has also been reported on involvement of glutamate receptors in amphetamine-induced IEG expression in the striatum. Administration of the AMPA/KA-receptor antagonist, DNQX, 30 min prior to amphetamine blocked the increased expression of *zif/268* in the CPu, but not in the overlying cortex (Wang et al., 1994a). Similarly, administration of the NMDA-receptor antagonists, MK-801 or CPP, 30 min prior to amphetamine blocked the induction of *zif/268* mRNA, in both CPu and overlying cortex (Wang et al., 1994b). However, since the NMDA-receptor antagonists by themselves decreased baseline levels of *zif/268* in both the CPu and the cortex, another way of interpreting these data is that the decrease in baseline levels produced by NMDA-receptor antagonism was partially overcome by injection of amphetamine. A somewhat different pharmacology of the *zif/268* induction by methamphetamine was reported (Wang and McGinty, 1996); CPP reduced the methamphetamine induction of *zif/268* in both CPu and cortex, but DNQX did not. Konradi et al., (1996) reported that administration of MK-801 15 min prior to amphetamine partially blocked the *c-fos, JunB, c-jun,* and *zif/268* mRNA increases in the CPu. Ohno et al., (1994) reported that prior administration of MK-801 blocked the induction of c-Fos in the CPu following methamphetamine, whereas Dragunow et al., (1991) reported that MK-801 could block the c-Fos response to MDMA. In contrast to these findings, Dalia and Wallace (1995) reported that administration of MK-801 or the AMPA-receptor antagonist, NBQX, 10–20 min prior to injection of a low dose of amphetamine (1 mg/kg, rather than the more typical dose of 5 mg/kg) potentiated the c-Fos response in the Acb. An

involvement of glutamatergic cortical projections to the striatum in the IEG response to amphetamine has also been demonstrated (Cenci and Bjorklund, 1994). Lesioning of the medial agranular cortex also reportedly reduced amphetamine-induced c-Fos in the globus pallidus (Vargo et al., 1996).

Postreceptor signal-transduction mechanisms linking acute amphetamine injection to activation of IEG expression have also been investigated. Konradi et al., (1994) reported that amphetamine-induced c-*fos* expression in the striatum could be blunted by infusion of an antisense oligonucleotide against CREB. Whereas acute administration of amphetamine did not alter CREB levels (Konradi et al., 1994; Simpson et al., 1995), phospho-CREB was greatly increased within 20 min after amphetamine (Konradi et al., 1994; Simpson et al., 1995). However, the increase was not enriched in the patch compartment of the dorsal striatum (Konradi et al., 1994; Simpson et al., 1995). Moreover, acute amphetamine induced phosphorylation of CREB in the AcbC, but not in the AcbSh (Simpson et al., 1995), whereas this treatment has been reported to increase IEGs in both the core and the shell of the accumbens (Graybiel et al., 1990; Nguyen et al., 1992; Robertson and Jian, 1995; Turgeon et al., 1996; Curran et al., 1996). In addition, amphetamine did not induce binding to oligonucleotides corresponding to the cAMP- and Ca^{2+}-inducible element (CaRE) in the c-*fos* promoter, or to the consensus CREB-binding site called ATF, in gel-shift assays of the striatum (Konradi et al., 1994).

Alterations in the IEG Patterns with Chronic Amphetamine Treatment

Norman et al., (1993) reported that injection of amphetamine augmented the striatal c-Fos response to a second injection given 3 d later. However, the paradigm used in this study may have elicited a conditioned place preference that could have contributed to the enhanced response. A downregulation of the amphetamine-induced increases in several IEG mRNAs following chronic administration of amphetamine has also been reported (Persico et al., 1993; Cole et al., 1995; Konradi et al., 1996). An involvement of NMDA receptors in the downregulation of the IEG response was suggested by blockade of the desensitization by administration of MK-801 15 min prior to each injection of amphetamine (Konradi et al., 1996). The anatomical pattern of c-*fos* expression following chronic administration of amphetamine has been reported to become more restricted to the patch compartment (Simpson et al., 1995; Curran et al., 1996) or more concentrated in the medial portion of the CPu (Jaber et al., 1995). However, sensitization to amphetamine reportedly enhanced the c-*fos* response in the cerebral cortex (Curran et al., 1996). In addition, the phenotypic profiles of striatal Fos-positive neurons reportedly changed after chronic amphetamine (Fig. 6B); 56% of the Fos/Fra-positive neurons expressed PPT mRNA (as opposed to 78% following acute administration), and 52% expressed PPE mRNA (as opposed to 33% after acute injection) (Jaber et al., 1995). As with chronic cocaine administration, continued treatment with amphetamine has been reported to induce chronic Fras (Nye et al., 1995).

Significance of IEG Induction by Amphetamine

The potential functions of amphetamine-induced IEGs have been explored. Hooper et al. (1994) infused a c-*fos* antisense oligonucleotide into the CPu, then gave amphetamine 12 h later. Animals in which this infusion decreased the c-Fos response unilaterally, as determined by immunocytochemistry, displayed repeated turning away from the side of the highest c-Fos response, following amphetamine injection. In a somewhat similar, though more complicated, experiment, Cenci and Bjorklund (1994) performed unilateral 6-OHDA lesions, which are well known to produce amphetamine- or apomorphine-induced rotations. They then implanted a cell suspension from embryonic ventral midbrain into the deafferented CPu, and waited until the grafts had abolished the rotational response to amphetamine. Some of these rats then received

a lesion to cut corticostriatal afferents. Looking across all animals, they found a significant correlation between the number of rotations and the left-right difference in the number of Fos-positive neurons in the CPu following amphetamine injection. Dalia and Wallace (1995) also found that infusion of a *c-fos* antisense oligonucleotide into the Acb abolished the locomotor response to amphetamine when given five, but not 10 h later.

Nicotine

Several studies have reported that acute administration of nicotine induces expression of c-Fos in various brain regions. To our knowledge, the effect of nicotine on the expression of IEGs other than c-Fos has not been studied. The initial reports focused on responses in the hindbrain and midbrain, with nicotine-induced c-Fos in the superficial layers of the superior colliculus (Ren and Sagar, 1992; Pang et al., 1993), medial terminal nucleus of the accessory optic tract (Ren and Sagar, 1992; Pang et al., 1993; Salminen et al., 1996), interpeduncular nucleus (Ren and Sagar, 1992; Salminen et al., 1996), supramamillary nucleus (Pang et al., 1993), caudal linear nucleus (Pang et al., 1993), periaqueductal gray (Pang et al., 1993; Matta et al., 1993), nucleus of the solitary tract (Matta et al., 1993), and locus ceruleus (Matta et al., 1993). Forebrain regions reportedly displaying a c-Fos response to acute nicotine administration include the central nucleus of the amygdala (Matta et al., 1993), the bed nucleus of the stria terminalis (Kiba and Jayaraman, 1994), lateral septum (Kiba and Jayaraman, 1994), paraventricular and supraoptic nuclei of the hypothalamus (Matta et al., 1993; Salminen et al., 1996), medial habenula (Clegg et al., 1994), the granule cells of the dentate gyrus (Matta et al., 1993), portions of the cortex (Kiba and Jayaraman, 1994), and the striatum (Kiba and Jayaraman, 1994). Within the CPu, the response was primarily in the medial half, although clusters of labeled cells were also noted in the lateral striatum (Fig. 7). However, since no markers of the patch-matrix organization have been employed, it is not possible to determine whether these clusters of nicotine-responsive neurons follow this organizational scheme. A marked dose-responsiveness of the striatum was noted, with lower doses (e.g., 0.4 mg/kg) inducing c-Fos primarily in the medial and central portions of the CPu, and higher doses (1.0–1.4 mg/kg) producing a response in more lateral regions. The c-Fos response in the Acb was considerably smaller than in the dorsal striatum, with a slightly stronger response in the shell than in the core (Fig. 7). In the cerebral cortex, clusters of labeled cells were found in all regions, but especially in the cingulate and piriform cortex (Kiba and Jayaraman, 1994). One developmental study has been reported,

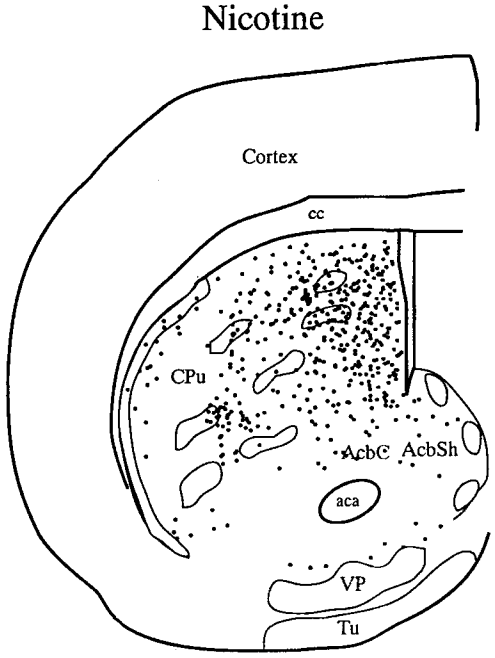

Fig. 7. Schematic diagram of the pattern of c-Fos induction in the striatum after acute injection of nicotine. Note the response primarily in the medial half of the CPu, with a smaller response in the Acb. Although clusters of labeled cells were noted, no markers were employed to determine patch-matrix boundaries. Responses were seen in the cortex and septum; however, these were not plotted, for the sake of clarity.

demonstrating that nicotine induced c-*fos* mRNA in the suprachiasmatic nucleus in embryonic and early postnatal brains, but this response was not present in rats older than P2 (Clegg et al., 1994). Self-administration of nicotine in trained rats resulted in increased Fra immunoreactivity in portions of the cerebral cortex, Acb, medial (but not lateral) CPu, and the superior colliculus (Pich et al., 1997). To our knowledge, no studies have determined the phenotypes of Fos-positive neurons following acute administration of nicotine, although the responding neurons of the ventral midbrain were not dopaminergic (Ren and Sagar, 1992).

The pharmacology and neural circuitry of nicotine-induced c-Fos expression has also been investigated. Several studies have demonstrated that the centrally-acting nicotine receptor antagonist mecamylamine blocked the c-Fos response to nicotine (Ren and Sagar, 1992; Pang et al., 1993, Kiba and Jayaraman, 1994; Clegg et al., 1994). Administration of the peripherally acting nicotine receptor antagonist hexamethonium did not block the c-Fos response in the superior colliculus, medial terminal nucleus of the accessory optic tract, or interpeduncular nucleus (Ren and Sagar, 1992). An involvement of dopamine D_1 receptors in nicotine-induced c-Fos expression in the striatum, but not the cortex, was demonstrated by preadministration of the antagonist SCH 23390 (Kiba and Jayaraman, 1994). This result is in concert with the reports that nicotine increased extracellular dopamine levels in the nucleus accumbens (e.g., Imperato et al., 1986; Di Chiara and Imperato, 1988). This increased release of dopamine in the Acb may be caused by a direct effect of nicotine in the VTA, since infusion of nicotine into this midbrain region induced locomotion and c-Fos expression in the Acb (Panagis et al., 1996). Whereas administration of the D_2-receptor antagonist YM-09151-2 induced c-Fos, primarily in the dorsolateral CPu, administration of this antagonist 30 min prior to nicotine produced a c-Fos pattern in the CPu that appeared to be the additive effect of both drugs. This contrasts with the reduction in the response to cocaine (Graybiel et al., 1990) or amphetamine (Graybiel et al., 1990; Ruskin and Marshall, 1994) induced by D_2-receptor antagonists. Moreover, nicotine-induced c-Fos expression in the striatum was blocked by clozapine (Kiba and Jayaraman, 1994), which has a high affinity for the dopamine D_4 receptor (e.g., Van Tol et al., 1991). To our knowledge, the ability of clozapine to alter the c-Fos response to cocaine or amphetamine has not been reported. As with both cocaine (Torres and Rivier, 1993) and amphetamine (Wang et al., 1994b), an involvement of NMDA receptors in nicotine-induced c-Fos expression in the striatum has also been demonstrated pharmacologically (Kiba and Jayaraman, 1994).

Alterations in IEGs following chronic exposure to nicotine have not been systematically studied. However, the self-administration studies of Pich et al. (1997) involved training rats over a 2-wk period, during which they were repeatedly exposed to nicotine. In these rats, AP-1-binding proteins included the 35–37-kDa chronic Fras that have been reported to be induced by chronic cocaine (Hope et al., 1994; Chen et al., 1994, Nye et al., 1994) or amphetamine (Nye et al., 1995). These results suggest that chronic exposure to nicotine may produce biochemical alterations in AP-1-binding proteins similar to those of other drugs of abuse.

Caffeine

Although caffeine is not usually included among drugs of abuse, it is by far the most commonly used psychostimulant in humans (reviewed in Nehlig et al., 1992), and addiction to caffeine has been observed frequently (reviewed in Griffiths and Woodson, 1988). A growing body of literature attests to the ability of caffeine to induce a strong IEG response in the striatum (Nakajima et al., 1989; Johansson et al., 1992, 1993, 1994; Sveningsson et al., 1995a,b, 1996). The IEGs induced by caffeine included c-*fos* (Nakajima et al., 1989; Le et al., 1992; Johansson et al., 1992, 1993, 1994; Sven-

ingsson et al., 1995a,b, 1996), c-*jun* (Sveningsson et al., 1995a), *junB* (Sveningsson et al., 1995a), NGFI-A (*zif/268* Sveningsson et al., 1995c; 1996), and NGFI-B (also known as *nur-77*; Sveningsson et al., 1995c), but not *junD* (Sveningsson et al., 1995a). The response was dose-dependent and biphasic; a low dose of caffeine (25 mg/kg) reduced baseline striatal levels of NGFI-A, NGFI-B and *junB* mRNAs, whereas higher doses (50 or 100 mg/kg) induced several IEGs quite robustly (Nakajima et al., 1989; Sveningsson et al., 1995a). Within the CPu, the strongest response for all of these IEGs was found in the dorsolateral region (Fig. 8), where the induction was widespread and rather homogeneous. Although no studies have been reported on the potential patch-matrix organization of the responding neurons, the published photographs suggest that this compartmentalization is not critical to the IEG response to caffeine. No statistically significant increase in c-*fos* mRNA was found in the Acb, although published photographs suggest a c-*fos* response in this region (Sveningsson et al., 1995a). Moreover, a significant increase in *junB*, *zif/268*, and NGFI-B mRNAs was detected in the Acb 1 and 4 h after caffeine injection (Sveningsson et al., 1995a,c). Although high, the dose of 100 mg/kg did not induce seizures; indeed, a larger dose (178 mg/kg) induced seizures and massive c-*fos* induction throughout the CPu and cortex (Nakajima et al., 1989). Low and moderate doses of caffeine have also been reported to increase IEG expression in the cingulate and parietal cortex (Sveningsson et al., 1995a,c), and high doses reportedly increased c-fos in the globus pallidus (Sveningsson et al., 1995a). The photos provided by Sveningsson et al. (1995a) also suggested increased expression in the bed nucleus of the stria terminalis and preoptic area, although no mention of these structures was made in this report.

The striatal phenotypes displaying an IEG response to a high dose of caffeine have been studied. Johansson et al. (1994) reported that 68% of PPT-positive, 62% of PPT-negative, 96% of PPE-positive, and 53% of PPE-negative neurons expressed c-*fos* mRNA following caffeine; indeed, the PPE-positive cells were heavily labeled for c-fos mRNA, whereas PPE-negative cells were barely labeled (Fig. 9). Moreover, all of the adenosine A_2-receptor-positive cells expressed c-fos mRNA and 80% of A_2-receptor-negative cells expressed this IEG. Reasonably similar percentages were found by Sveningsson et al. (1995a) for both c-*fos* and *junB* mRNAs, although the percentages were lower regarding c-*jun* expression; c-*jun* mRNA was reported in 27% of PPE-positive cells, 22% of PPE-negative cells, 27% of PPT-positive cells, and 21% of PPT-negative cells in the lateral CPu after a high dose of caffeine. A generalized NGFI-A and -B mRNA response to this dose of

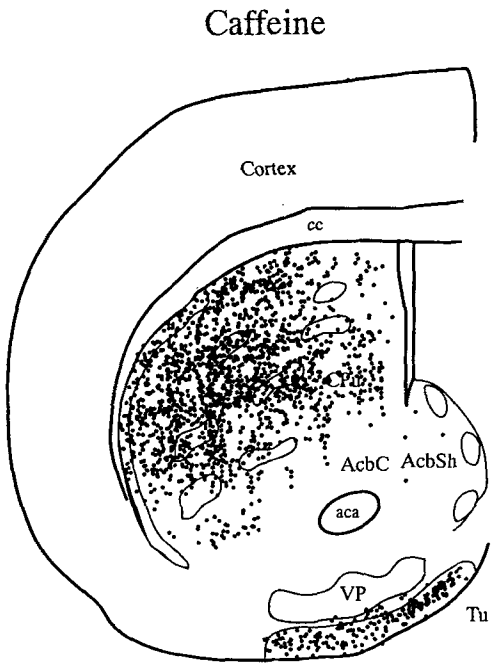

Fig. 8. Schematic diagram of the pattern of c-Fos induction in the striatum after acute injection of caffeine. Note the large response in the dorsolateral CPu and the Tu, and the smaller response in the Acb. Although no markers of patch-matrix boundaries were employed, the assumption is made that c-Fos was expressed in both compartments. Responses were seen in the cortex and septum; however, these were not plotted, for the sake of clarity.

caffeine was also reported in another study from this same group (Sveningsson et al., 1995c); approx 90% of neuron-like cells in the lateral CPu expressed each of these genes following caffeine. A low dose of caffeine (25 mg/kg) reduced the percentage of PPE-positive, but not PPT-positive, neurons expressing these genes, suggesting a preferential effect of caffeine on PPE-positive neurons, which fits with the somewhat higher percentage of c-fos-PPE neurons and the greater intensity of c-*fos* labeling in PPE neurons following a high dose of caffeine (Johansson et al., 1994).

The pharmacology of caffeine-induced IEG expression has been shown to be rather complicated. Caffeine is known to act as an adenosine-receptor antagonist (reviewed in Nehlig et al., 1992). Indeed, Nakajima et al. (1989) reported that the adenosine A_2-receptor agonist NECA, but not the A_1-receptor agonist CHA, blocked the c-*fos* induction produced by caffeine. However, Johansson et al. (1992) could not induce c-*fos* mRNA with DPCPX (A_1-receptor antagonist, 10,000 times more potent than caffeine) or CGS 15943 (A_2 antagonist 100 times more potent that caffeine). Moreover, they noted that the pattern of c-*fos* mRNA response to caffeine did not match the distribution of neurons with A_2 receptors, which are colocalized with PPE mRNA in a homogeneous distribution throughout the CPu (Schiffmann et al., 1991). Because caffeine is known to inhibit cyclic nucleotide phosphodiesterases (reviewed in Nehlig et al., 1992), Sveningsson et al. (1995b) tried to determine if other phosphodiesterase inhibitors would mimic the pattern of c-*fos* induction in the dorsolateral CPu; theophylline and IBMX induced similar patterns of c-*fos* mRNA, but several other phosphodiesterase inhibitors did not. Whereas a slight antagonism of caffeine at benzodiazepine receptors has been reported (reviewed in Nehlig et al., 1992), neither diazepam nor the benzodiazepine-receptor antagonist Ro 15-1788 blocked the c-*fos* induction by caffeine (Nakajima et al., 1989). Pretreatment with ethanol also did not block induction of c-*fos* mRNA by caffeine (Le et al., 1992), in contrast

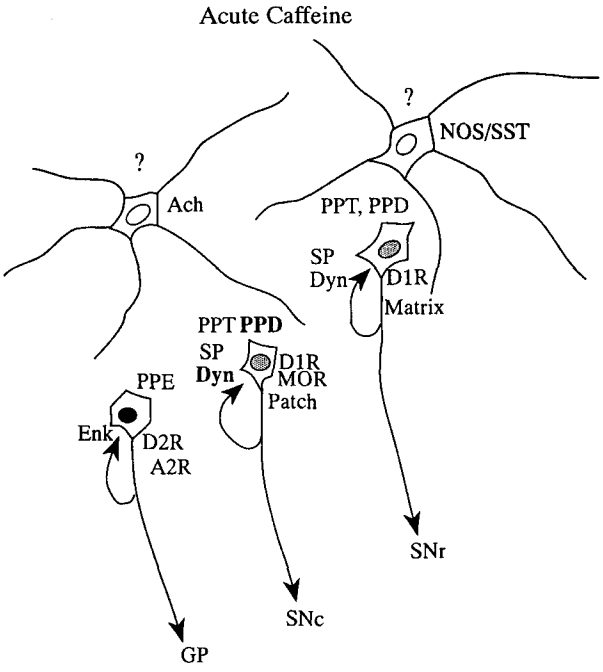

Fig. 9. Striatal phenotypes displaying a c-Fos response to acute caffeine injection. Expression of c-*fos* mRNA in PPE neurons was both more frequently observed and expressed at a higher level per neuron, compared with expression in PPT/PPD neurons. Expression of c-Fos in interneurons has not been determined.

to the inhibitory effect of alcohol on c-*fos* induction by NMDA (Le et al., 1992) or cocaine (Torres, 1994). Caffeine has been reported to decrease release of dopamine from mesolimbic and mesocortical terminals (reviewed in Nehlig et al., 1992). However, Johansson et al. (1992) reported that, in rats with a unilateral 6-OHDA lesion, caffeine (40 mg/kg) induced c-*fos* mRNA to a greater extent on the lesioned than on the unlesioned side. Because dopamine D_2 receptors have been reported to be colocalized with adenosine A_2 receptors and PPE mRNA (Le Moine et al., 1990; Schiffmann et al., 1991), the potential involvement of D_2 receptors in the decrease in striatal IEG expression following a low dose of caffeine (25 mg/kg) has also been studied (Svenningsson et al., 1995b). Whereas the D_2-receptor agonist quinpirole also decreased NGFI-A and -B

mRNAs in CPu, the effects of caffeine and quinpirole were not additive. Moreover, the D_2-receptor antagonist raclopride did not alter the expression of NGFI-A, NGFI-B, or *junB* genes by itself, and did not alter the decrease in expression caused by 25 mg/kg caffeine. An involvement of NMDA receptors in caffeine-induced IEG expression in the medial, but not in the lateral, CPu has also been reported (Sveningsson et al., 1996). In summary, since no one mechanism of action of caffeine apparently can account for the increased expression of IEGs following a high dose, it is likely that the multiple actions of this drug combine to induce several IEGs in the dorsolateral CPu.

Morphine

Neuroanatomical Pattern of IEG Induction by Morphine

Several studies have reported that acute administration of morphine increased expression of IEGs in the striatum of the rat brain. Indeed, morphine was the first drug of abuse demonstrated to increase expression of c-*fos* in the brain (Chang et al., 1988). Increased expression of the c-*fos* gene in the striatum has been detected by Northern blots (Chang et al., 1988; Hayward et al., 1990), *in situ* hybridization (Liu et al., 1994; Curran et al., 1996), immunocytochemistry (Liu et al., 1994; Garcia et al., 1995), AP-1 binding (Liu et al., 1994), and Western blotting (Nye and Nestler, 1996). Within the CPu, a consistent c-Fos response was noted in the dorsomedial region, extending throughout the entire rostral-caudal length of the striatum (Fig. 10; Liu et al., 1994; Garcia et al., 1995; Curran et al., 1996). In some rats, a response in distinct clusters of neurons was noted in the ventrolateral CPu (Liu et al., 1994; Garcia et al., 1995). In both regions of the CPu, there was no obvious relationship to the patch-matrix organization, as determined by adjacent sections immunostained for calbindin D_{28k} (Garcia et al., 1995). However, many of the labeled neurons were found in the extensive calbindin-poor zone of the dorsal CPu, where

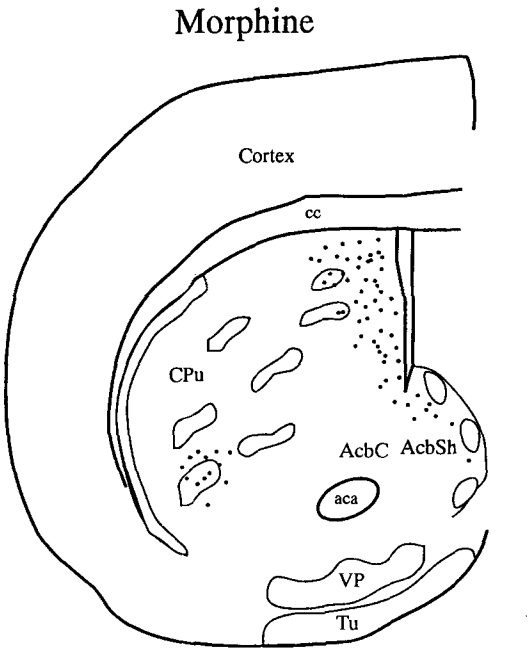

Fig. 10. Schematic diagram of the pattern of c-Fos induction in the striatum after acute injection of morphine. Note the response in the dorsomedial CPu and extending into the Acb, especially into the shell. Clusters of Fos-positive neurons were also found in the ventrolateral CPu. The great majority of Fos-positive neurons were found in the matrix, although some were found in the patch compartment, and some along the borders of patches.

the patch-matrix organization cannot be defined by calbindin immunostaining. In more recent work, Garcia et al. (1997) reported that the great majority of morphine-induced Fos-positive neurons were found in the matrix compartment of the striatum, although some were found on the borders of patches, and some clearly within patches, as determined by double immunocytochemistry for c-Fos and mu opiate receptors. A JunB response to acute morphine was noted in the dorsomedial CPu, with a pattern nearly identical to that of c-Fos, except that large numbers of labeled neurons were found under baseline conditions (Liu et al., 1994; Garcia et al., 1995). A considerably smaller induction of c-Jun was noted in the same part of the CPu, whereas no change in

JunD immunoreactivity was found (Garcia et al., 1995). Induction of c-Fos in the Acb has also been reported by several groups (Liu et al., 1994; Garcia et al., 1995; Nye and Nestler, 1996; Curran et al., 1996), and in the Tu (Garcia et al., 1995). Within the Acb, the shell responded somewhat more than the core (Garcia et al., 1995), and there was no obvious relationship with the distribution of mu opiate receptors (Garcia et al., 1997). No JunB , c-Jun, or JunD response to acute morphine injection was found in the Acb (Garcia et al., 1995). However, Nye and Nestler (1996) reported induction of a 33-kDa Fra in the Acb following acute morphine administration; they assumed this to be ΔFosB.

More variable IEG responses have been reported in the cerebral cortex and thalamus following acute injection of morphine. Although Hayward et al. (1990) reported no change in c-*fos* mRNA in whole cortex after acute morphine injection, Garcia et al. (1995) reported induction of c-Fos in layer VI of the parietal and granular insular cortex, with smaller responses in the cingulate and retrosplenial cortex. In some rats, clusters of labeled neurons in layer IV, and spreading into layers II/III and V of the parietal cortex were noted; in these rats, more labeled cells were also detected in lateral regions of the CPu. Although a JunB response to acute morphine injection was noted in these same regions of cortex, the response was generally smaller than the c-Fos response (Garcia et al., 1995). No c-Jun or JunD response was found in the cerebral cortex. Acute morphine injection also induced c-Fos, but not c-Jun or JunB, in several midline/intralaminar thalamic regions, including the paraventricular, centrolateral, paracentral, central medial, and rhomboid nuclei (Garcia et al., 1995). Interestingly, a decrease in JunD immunoreactivity in the paraventricular and central medial thalamic nuclei following acute morphine injection was also noted, suggesting that shifts in the sets of AP-1 heterodimers may be induced in these thalamic regions by morphine. It is also important to note that these thalamic regions express mu opiate receptors (e.g., Mansour et al., 1987), whereas the pattern of IEG induction in the striatum following acute morphine administration was not related to the distribution of mu opiate receptors (Garcia et al., 1995, 1997), despite high affinity of morphine for these receptors (e.g., Alexander and Peters, 1997). A consistent c-Fos response to acute morphine was also noted in the supracapsular portion of the bed nucleus of the stria terminalis, but not in other portions of the extended amygdala (Garcia et al., 1995).

Little information has been provided regarding phenotypic characterization of striatal or cortical neurons displaying a c-Fos response to acute morphine administration. Recently, Garcia et al. (1997) reported that Fos-positive neurons did not contain somatostatin or neuronal nitric oxide synthase in either the striatum or the cortex, but many contained the NMDAR1-receptor subunit, and the AMPA-receptor subunits GluR2/3 or GluR4, but not GluR1. In the CPu, some of the neurons contained the protein kinase C βII isoform, which is found in most medium spiny neurons, and a very small percentage contained the βI isoform, found in larger interneurons. However, the neuropeptide content of striatal neurons displaying a c-Fos response to morphine has not been determined.

Pharmacology of IEG Induction by Morphine

The pharmacology of the IEG response to acute morphine administration has been studied to some extent. Several groups have reported that the IEG responses were blocked by administration of the opiate-receptor antagonists naloxone or naltrexone (Chang et al., 1988; Liu et al., 1994; Garcia et al., 1995). Because both morphine and naloxone have higher affinities for mu than for delta or kappa opiate receptors (e.g., Alexander and Peters, 1997), these data suggest an action on mu opiate receptors. However, a distinction between Mu1 and Mu2 opiate receptors has not been made. As with other drugs of abuse, the c-Fos response in the striatum, but not the lateral

septum, was blocked by prior administration of the dopamine D_1 antagonist SCH 23390 (Liu et al., 1994). This result fits with the observations that acute morphine administration activated midbrain dopaminergic neurons (Gysling and Wang, 1983) and increased release of dopamine in the nucleus accumbens (e.g., Di Chiara and Imperato, 1988). An involvement of glutamate receptors has also been demonstrated. Liu et al., (1994) reported a complete blockade of the c-Fos response in the striatum with prior administration of the noncompetitive NMDA receptor antagonist MK-801, in female rats. However, results from Garcia and Harlan (1996) indicated a significant, but incomplete, reduction of the c-Fos response with MK-801 treatment in male rats. Additional studies with both competitive (NPC 17742) and noncompetitive (MK-801) NMDA receptor antagonists have demonstrated a complete blockade of the striatal c-Fos response in females, but an incomplete reduction in male rats (D'Souza et al., 1997). Administration of the AMPA-receptor antagonist GYKI 52466 also significantly decreased, but did not eliminate, the striatal c-Fos response to morphine (Garcia and Harlan, 1996). Unlike the striatum, there was no significant decrease in the c-Fos response in midline/intralaminar thalamic nuclei with either NMDA or AMPA receptor antagonists (Garcia and Harlan, 1996).

Alterations in IEG Patterns with Chronic Morphine Administration

As with other drugs of abuse, chronic administration of morphine has been shown to induce chronic Fras (Nye and Nestler, 1996). However, unlike the effects of chronic cocaine (Hope et al., 1994; Chen et al., 1995; Nye et al., 1995) or amphetamine (Persico et al., 1993; Cole et al., 1995; Konradi et al., 1996), chronic administration of morphine also induced acute Fras, including 58-kDa c-Fos, 45 kDa FosB, and 41 kDa Fra1/Fra2, in both the CPu and the Acb (Nye and Nestler, 1996). Consistent increases in chronic Fras were also reported in the substantia nigra and VTA, whereas a decrease was noted in the locus ceruleus. This decrease in the locus ceruleus was consistent with a reported decrease in 58 kDa c-Fos and *c-fos* mRNA in this region following chronic morphine treatment (Hayward et al., 1990). Acute administration of morphine to postdependent rats has also been studied. Curran et al. (1996) reported that injection of morphine in rats given daily injections of morphine for 14 d, followed by 14 d of abstinence, induced a greater *c-fos* response in parietal and insular cortex, the ventrolateral CPu (but with a decreased response in the central CPu), and in both the AcbSh and the AcbC. However, Frankel et al. (1997), using a different chronic morphine treatment protocol, noted a significant increase in the c-Fos response in the dorsolateral CPu following 7 d abstinence, but a significant reduction in the striatal response with 14 d abstinence.

IEG Induction by Withdrawal from Opiates

Several studies have reported large increases in IEG expression in numerous brain regions following naloxone-precipitated withdrawal from chronic morphine administration. Brain regions displaying increases in *c-fos* expression include the locus ceruleus (Hayward et al., 1990; Stornetta et al., 1993; Beckmann et al., 1995), nucleus of the solitary tract (Stornetta et al., 1993; Baraban et al., 1995), area postrema (Stornetta et al., 1993), rostral ventrolateral medulla (Stornetta et al., 1993; Baraban et al., 1995), raphe nuclei (Stornetta et al., 1993; Baraban et al., 1995), parabrachial nuclei (Stornetta et al., 1993), central gray (Stornetta et al., 1993; Chieng et al., 1995), cerebellum (Couceyro and Douglass, 1995), VTA (Hayward et al., 1990; Couceyro and Douglass, 1995), substantia nigra (Couceyro and Douglass, 1995), supraoptic and paraventricular hypothalamic nuclei (Stornetta et al., 1993; Couceyro and Douglass, 1995), CPu (Hayward et al., 1990; Couceyro and Douglass, 1995; Nye and Nestler, 1996), Acb (Hayward et al., 1990; Nye and Nestler, 1996), lateral septum (Couceyro and Douglass, 1995), hippocampus (Couceyro and Douglass, 1995), central nucleus of the amygdala (Hayward et al., 1990; Stornetta et al.,

1993; Couceyro and Douglass, 1995), and cerebral cortex (Hayward et al., 1990; Couceyro and Douglass, 1995; Beckmann et al., 1995). Within brain stem regions, many catecholaminergic neurons were Fos-positive after morphine withdrawal (Stornetta et al., 1993; Baraban et al., 1995). Several different IEGs have been shown to increase after precipitated withdrawal, including FosB (Nye and Nestler, 1996), Fra1/Fra2 (Nye and Nestler, 1996), c-*jun* (Hayward et al., 1990; Couceyro and Douglass, 1995; Beckmann et al., 1995), JunB (Couceyro and Douglass, 1995), and *zif268* (Beckmann et al., 1995), but not JunD (Couceyro and Douglass, 1995). Interestingly, acute Fras were elevated rapidly after precipitated withdrawal, and remained elevated for up to 72 h (for FosB), whereas chronic Fras were not elevated until 72 h after withdrawal (Nye and Nestler, 1996). However, because many of the brain regions displaying an IEG response to precipitated withdrawal are autonomic areas in which IEGs are induced by stress (e.g., Ceccatelli et al., 1989), the specific involvement of opiate withdrawal is difficult to ascertain. Indeed, many of the brain stem neurons displaying c-Fos after naloxone-precipitated withdrawal also contain tyrosine hydroxylase (Baraban et al., 1995), as do many brain stem neurons activated in response to stress (Ceccatelli et al., 1989).

Δ^9-Tetrahydrocannabinol (THC)

Although THC has been identified as a major psychoactive component of marijuana, few studies have examined effects of THC on IEG expression in the brain. Mailleux et al., (1994) reported that acute administration of THC increased levels of several IEG mRNAs 20 min later, as determined by *in situ* hybridization. Increased expression of c-*fos* and *zif/268* mRNAs was found in the cingulate and fronto-parietal cortex, as well as the CPu. Increased expression of c-*jun* mRNA was found in the cingulate, but not fronto-parietal cortex, or in the CPu. No change in *junD* mRNA levels was detected. Using immunocytochemistry, Miyamoto et al. (1996) reported that acute injection of THC induced c-Fos in the dorsomedial CPu, with the maximal response 2 hours after injection, but with continued significant elevation above baseline at 4 h. Increased numbers of c-Fos-positive neurons were also noted in the Acb, but not in the globus pallidus, hippocampus, or substantia nigra. Glass and Dragunow (1995) reported that administration of the cannabinoid-receptor agonist CP55,940 induced catalepsy in rats, accompanied by an induction of Krox 24 (Zif/268) protein in the patch compartment of the CPu, as determined by double labeling for calbindin D28k. They also reported weaker induction of c-Fos and JunB in the patch compartment. However, none of these studies displayed the regional pattern of striatal IEG induction in sufficient detail to provide comparison with other drugs of abuse. As with other drugs of abuse, prior administration of the dopamine D_1-receptor antagonist SCH 23390 blocked the c-Fos induction in the CPu, whereas administration of the D_2-receptor antagonist sulpiride did not (Miyamoto et al., 1996). These studies are consistent with reports that THC increased release of dopamine in forebrain regions (e.g., Sakurai-Yamashita et al., 1989; Chen et al., 1990). Interestingly, the dopamine-releasing action of THC was reportedly blocked by the opiate antagonist naloxone (Chen et al., 1990), a finding that may relate to the reported increased expression of IEGs in the patch compartment of the CPu (Glass and Dragunow, 1995).

Hallucinogens: PCP, PCA, and Ketamine

Hallucinogens such as phencyclidine (PCP) and *p*-chloroamphetamine (PCA) have been abused by humans in recent years. Administration of PCP has been reported to increase several IEG mRNAs in the fronto-parietal cortex, piriform cortex, dentate gyrus, CA1-3 of the hippocampus, diffusely in the thalamus, and in the CPu (Nakki et al., 1996b). However, no information was provided regarding the regional expression pattern in the CPu. In

addition, the photographs provided indicated a substantial induction of c-*fos* and other IEG mRNAs in the amygdala. A delayed increase of c-*fos* and NGFI-B mRNAs in the cingulate cortex and midline/intralaminar nuclei was also noted. Indeed, increased expression of several IEG mRNAs in the cingulate cortex was found 6 but not 24 h after injection. In mice, increased c-Fos protein was detected in the parietal cortex 1 wk after a single injection of PCP (Sugita et al., 1996). Increased expression of c-Fos in the inferior olive, clusters in the cerebellar granular layer, deep cerebellar nuclei, vestibular nuclei, nucleus of the solitary tract, gracile nucleus, and trigeminal nucleus were also reported in rats following acute administration of PCP (Nakki et al., 1996a). However, the tremors and convulsions elicited by PCP (Nakki et al., 1996a) make interpretation of IEG induction more problematic.

Acute administration of PCA has been reported to increase c-Fos in the frontal, cingulate and somatosensory cortex (esp layer Va), the claustrum, CPu, Acb, Tu, islands of Calleja, and the central nucleus of the amygdala (Moorman and Leslie, 1996). Within the CPu, the response was widespread, although there was no description of any regional variation, nor of differences between the core and shell of the Acb. The effect was attenuated by prior administration of the 5-$HT_{2A/2B}$-receptor antagonist ritanserin, suggesting an involvement of serotonergic systems. Moreover, the effect was essentially duplicated in the cortex, claustrum, Acb, and amygdala by the selective 5-$HT_{2A/2B}$-receptor agonist DOI. However, in the CPu, Tu, and islands of Calleja, the response to PCA was much greater than the response to DOI.

Recently, abuse of ketamine (also known as super K) has been reported, especially among teenagers. Ketamine has been shown to induce expression of c-Fos in several brain regions, including the cingulate cortex and midline/intralaminar thalamic nuclei (Nakao et al., 1993), although there was no mention of the striatum. The c-Fos induction in the cingulate cortex was attenuated by halothane or diazepam (Nakao et al., 1996).

Ethanol

Ethanol is consumed by large numbers of people throughout the world, and is clearly a drug of abuse. However, the effects on the central nervous system are quite different from those of many other drugs of abuse. These differences are borne out in studies of IEGs following ethanol exposure. To our knowledge, only three studies have reported that acute exposure to ethanol induces IEG expression in the brain. Morgan et al. (1992) found that exposure of rats to ethanol vapor initially produced a small increase in whole brain c-*fos* mRNA, followed by a reduction over control levels. Zoeller and Fletcher (1994) reported that acute injection of ethanol increased c-*fos* mRNA in the paraventricular nucleus (PVN) of the hypothalamus, while decreasing expression of c-*jun* mRNA in both the PVN and the hippocampus. In a more extensive study, Chang et al. (1995) reported that acute ethanol injection induced c-Fos protein in several brain regions, including the PVN, the supraoptic nucleus, the bed nucleus of the stria terminalis, the central nucleus of the amygdala, the Edinger-Westphal nucleus of the midbrain, the locus ceruleus, and the parabrachial nucleus. In unpublished results, we have also found that a large dose of ethanol (5 g/kg) induced substantial expression of c-Fos throughout the extended amygdala, in small clusters of neurons in the CPu, and in midline/intralaminar thalamic nuclei. However, because many of the hypothalamic and brain stem regions displaying an IEG response to ethanol have also been demonstrated to respond to stress and/or hydrodynamic alterations (e.g., Ceccatelli et al., 1989), it is possible that these ethanol-induced responses relate primarily to changes in osmotic stimuli or other visceral information. Chronic administration of ethanol followed by acute injection reportedly downregulated the c-Fos response in the PVN, central nucleus of the amygdala, and Edinger-Westphal nucleus (Chang et al., 1995).

Several studies have demonstrated that acute administration of alcohol attenuated

the IEG responses to other stimuli, including stress (Ryabinin et al., 1995), picrotoxin (Le et al., 1992), NMDA (Le et al., 1992), or cocaine (Torres, 1994; Torres and Horowitz, 1996), but not kainic acid or caffeine (Le et al., 1992). The ability of alcohol to inhibit cocaine-induced c-Fos was most marked in the central region of the CPu, with less effect in other portions of the striatum (Torres, 1994; Torres and Horowitz, 1996).

Studies have also demonstrated that withdrawal from chronic alcohol exposure induced expression of several IEGs in the brain. Although Dave et al. (1990) reported that mice withdrawing from chronic alcohol exposure did not display increased c-*fos* expression unless they underwent seizures, Morgan et al. (1992) found that in nonseizing rats withdrawing from ethanol, whole brain c-*fos* mRNA levels were greatly increased, with this increase found primarily in the piriform cortex and dentate gyrus, based on *in situ* hybridization. Increased expression of c-*fos* mRNA in the hippocampus, cerebellum, anterior cingulate cortex, globus pallidus, medial habenular nucleus, anterior thalamic complex, auditory cortex, locus ceruleus, and the para- and presubiculum following ethanol withdrawal has also been reported (Putzke et al., 1996). Interestingly, the anti-craving drug, acamprosate, significantly decreased withdrawal-induced c-*fos* mRNA in the hippocampus and cerebellum (Putzke et al., 1996).

Barbiturates and Benzodiazepines

We are not aware of any reports indicating that acute or chronic administration of benzodiazepines or barbiturates that are abused by humans (e.g., phenobarbital) induce IEG expression in the brain.

Combinations of Drugs of Abuse

Most abusers of addictive agents imbibe multiple drugs, often simultaneously. For instance, most users of cocaine also drink alcohol, as do many people who smoke cigarettes. However, very little information has been reported on effects of multiple drugs of abuse on expression of IEGs in the brain. Torres and Horowitz (1996) reported that chronic administration of ethanol greatly reduced the striatal c-Fos response to acute cocaine injection, although they noted that small groups of neurons still displayed a c-Fos response to cocaine. Although the relationship of these clusters to the patch-matrix organization of the CPu was not explored, it is possible that the anatomical pattern is similar to the clustering of Fos-positive neurons following chronic cocaine reported by Moratalla et al. (1996). Combined exposure of rats or humans to both ethanol and cocaine has been reported to result in the biological production of cocaethylene (Dean et al., 1992), which has dopamine-releasing actions similar to cocaine (Bradberry et al., 1993). Acute injection of cocaethylene reportedly induced c-Fos in the CPu and Acb (Torres and Horowitz, 1996). Because of the great prevalence of multiple drug use and abuse, we urge a greater research effort investigating the effects on IEG induction by common combinations of drugs of abuse. Basic questions remain unanswered, such as whether the neuroanatomical patterns of IEG induction by two different drugs would be additive, synergistic, or even subtractive, perhaps due to cross-talk of signal transduction mechanisms.

Commonalties Among Drugs of Abuse in Anatomical Localization and Pharmacological Mechanisms of IEG Induction in the Brain

A Common Neuroanatomical Substrate for Drugs of Abuse

One of the most effective uses of studies of IEG induction in the brain is the revelation of neuroanatomical patterns of individual neurons activated in response to a stimulus. This

Table 1
Common Brain Structures Displaying an IEG Response to Drugs of Abuse

Drug	CPu	Acb	Cg	Par	Pir	BST	Amy	Septum	Mid/Intra Thal
Cocaine	1	1	1	2	3		3	1	3
Amphetamine	1	1	1	4				1	5
Nicotine	6	6	6	6	6	6	7	6	
Caffeine	8	9	9	9		9			
Morphine	10	10	10	10		10		11	10
THC	12	13	12	12					
PCP	14		14	14	14		14		14
MDMA	15	15			15				
Ethanol	16	16	16	16		17	17		16

Citations listed are for the first or the most detailed report. Blank spaces indicate that no mention was made of the structure in any report. Citations: 1. Graybiel et al., 1990; 2. Johansson et al., 1994; 3. Brown et al., 1992; 4. Wang et al., 1995; 5. Persico et al., 1993; 6. Kiba and Jayaraman, 1994; 7. Matta et al., 1993; 8. Nakajima et al., 1989; 9. Svenningsson et al., 1995a; 10. Garcia and Harlan, 1995; 11. Liu et al., 1994; 12. Mallieux et al., 1994; 13. Miyamoto et al., 1996; 14. Nakki et al., 1996b; 15. Dragunow et al., 1991; 16. unpublished observations of Harlan and Garcia; 17. Chang et al., 1995.

Abbreviations: Acb, nucleus accumbens; Amy, amygdala; BST, bed nucleus of the stria terminalis; Cg, cingulate cortex; CPu, caudate-putamen; Mid/Intra Thal, midline/intralaminar thalamic nuclei; Par, parietal cortex; Pir, piriform cortex.

literature survey has revealed a common neural substrate for IEG induction by drugs of abuse. All of the drugs surveyed in this review have been reported to increase expression of IEGs in the striatum (Table 1), especially in the CPu, although differences in the regional expression patterns in the striatum are obvious (Figs. 3, 5, 7, 8, and 10). Although the nucleus accumbens has been a focus of research on rewarding behaviors and drugs of abuse (e.g. Koob, 1992), for most of the drugs surveyed here, the IEG response in the Acb has been less robust than that of the dorsal striatum. Camera lucida drawings indicated that the c-Fos response in the Acb was less than that in the dorsocentral CPu following cocaine administration (Graybiel et al., 1990), and less than that of the patch compartment of the CPu following injection of amphetamine (Graybiel et al., 1990). Caffeine produced a massive increase in c-Fos in the dorsolateral CPu, but no statistically significant increase in the Acb (Sveningsson et al., 1995a). Acute morphine injection induced a larger c-Fos response in the dorsomedial CPu than in the Acb, and there was no significant increase in JunB in the Acb, despite an increase in the dorsomedial CPu (Garcia et al., 1995). Comparisons between the CPu and the Acb for THC, ethanol, PCP, and MDMA were not specifically reported. However, these results suggest that drugs of abuse generally activate IEG expression in dorsal striatal neurons more robustly than in neurons of the ventral striatum.

Common nonstriatal brain regions have also been reported to display IEG responses after drugs of abuse. All the drugs discussed in this review also increased expression of one or more IEGs in the cerebral cortex, with the most frequent sites reported to be the cingulate, piriform, and somatosensory cortex. Most of the drugs surveyed here also increased expression of IEGs in portions of the extended amygdala, i.e., either the bed nucleus of the stria terminalis (nicotine; Kiba and Jayaraman, 1994; caffeine; Sveningsson et al., 1995a; morphine; Garcia et al., 1995; and ethanol; Chang et al., 1995; Harlan and Garcia, unpublished results) and/or the amygdala (cocaine; Graybiel et al., 1990; nicotine; Matta et al., 1993; PCP; Nakki et al., 1996b; and ethanol; Chang et al., 1995; Harlan and Garcia, unpublished results). Cocaine (Graybiel et al., 1990; Brown et al., 1992), amphetamine (Graybiel et al., 1990), nicotine (Kiba and Jayaraman, 1994, and morphine (Liu

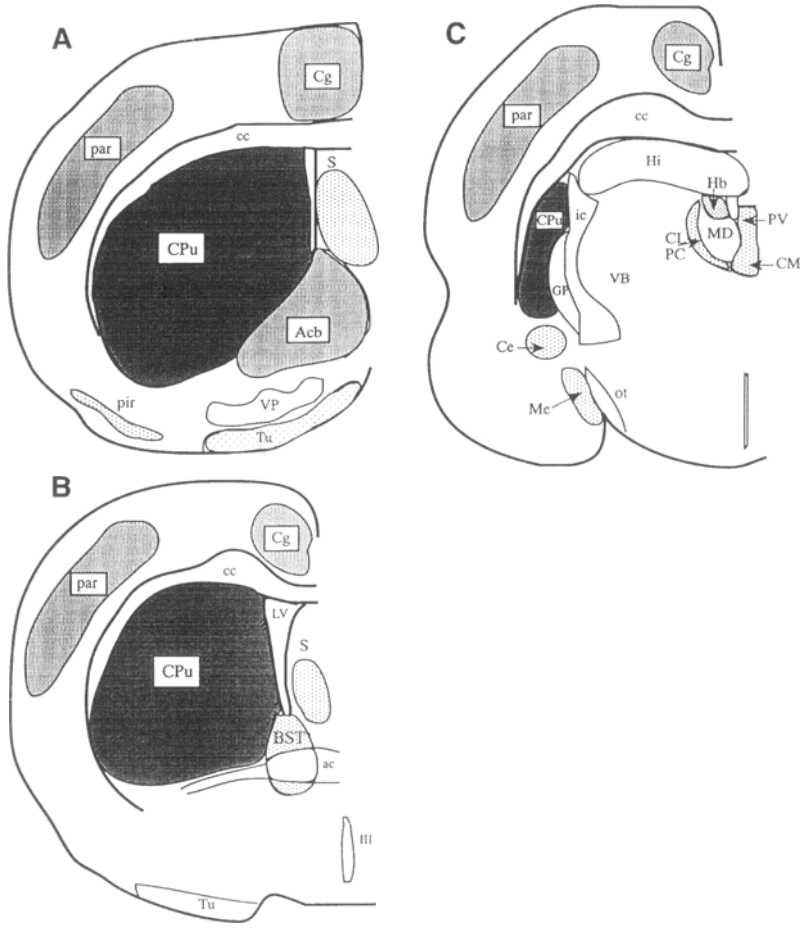

Fig. 11. Schematic diagrams of three sections through the forebrain showing regions that have been reported to display an IEG response to several drugs of abuse. The intensity of the shading corresponds to the frequency of activation by a variety of drugs. The CPu has been reported to respond to all the drugs surveyed here, whereas the Acb and cingulate (Cg) and parietal (par) cortex have been reported to respond to nearly all the drugs. The lateral septum (S), olfactory tubercle (Tu), piriform cortex (pir), bed nucleus of the stria terminalis (BST), central (Ce) and medial (Me) portions of the amygdala, habenula (Hb), and midline/intralaminar thalamic nuclei have all been reported to respond to about half of the drugs surveyed here. Abbreviations: ac, anterior commissure; cc, corpus callosum; CL, centrolateral nucleus; CM, central medial nucleus; GP, globus pallidus; Hi, hippocampus; ic, internal capsule; III, third ventricle; LV, lateral ventricle; MD, mediodorsal nucleus; ot, optic tract; PC, paracentral nucleus; PV, paraventricular thalamic nucleus; VP, ventral pallidum.

et al., 1994) reportedly increased expression of c-*fos* or other IEGs in the septum. All drugs surveyed in this review also increased expression of IEGs in midline/intralaminar thalamic nuclei, except for nicotine and caffeine. However, it must be emphasized that many reports have focused on the striatal response, and may have neglected to investigate potential responses in other brain regions, such as the extended amygdala and midline/intralaminar nuclei. Nevertheless, this comparative compilation suggests that drugs of abuse may activate common neural systems, including portions of the dorsal and ventral striatum, septum, extended amygdala, cortex, and midline/intralaminar nuclei (Fig. 11). As briefly

reviewed earlier (Subheading entitled "Developmental Insights into the Organization of the Forebrain"), all of these regions, except for the midline/intralaminar thalamus, can be seen in relatively close association as walls of the lateral ventricle during development. Thus, it is possible that this large-scale telencephalic ensemble that responds to drugs of abuse may relate to fundamental developmental processes.

A Common Pharmacology of Drugs of Abuse

The pharmacological mechanisms underlying the induction of IEG expression in the striatum appear to be similar for all drugs of abuse. A critical role for dopamine D_1 receptors in striatal induction of IEG expression has been reported following administration of cocaine (Graybiel et al., 1990; Young et al., 1991; Bhat and Baraban, 1993; Couceyro et al., 1994; Steiner and Gerfen, 1995; Moratalla et al., 1996; Drago et al., 1996), amphetamine (Graybiel et al., 1990; Moratalla et al., 1992; Nguyen et al., 1992; Moratalla et al., 1996), nicotine (Kiba and Jayaraman, 1994), morphine (Liu et al., 1994), and THC (Miyamoto et al., 1996). These results are consistent with the findings that all of these drugs increase concentrations of dopamine in the striatum (e.g., Di Chiara and Imperato, 1988; Self and Nestler, 1995). Involvement of D_2 receptors in the striatal IEG response to cocaine (Graybiel et al., 1990) and amphetamine (Graybiel et al., 1990; Ruskin and Marshall, 1994), but not for THC (Miyamoto et al., 1996) has been reported. The large induction of c-*fos* in the dorsolateral CPu in response to caffeine administration has not been conclusively linked to either activation or inhibition of either D_1 or D_2 receptors. The potential involvement of dopamine receptors in IEG induction by PCP, MDMA, and ethanol has not been studied.

An involvement of glutamate receptors, especially the NMDA receptor, in striatal induction of IEGs following drugs of abuse also appears to be a common mechanism. Blockade of NMDA receptors has been reported to attenuate, if not completely block, the striatal IEG response to cocaine (Torres and Rivier, 1993; Couceyro et al., 1994), amphetamine (Wang et al., 1994b; Konradi et al., 1996), nicotine (Kiba and Jayaraman, 1994), caffeine (Sveningsson et al., 1996), morphine (Liu et al., 1994; Garcia et al., 1996; D'Souza et al., 1997), and MDMA (Dragunow et al., 1991). An involvement of striatal AMPA receptors has also been reported for amphetamine (Wang et al., 1994a) and morphine (Garcia and Harlan, 1996). These results are consistent with the reported increased extracellular concentrations of glutamate following drugs of abuse (e.g., Smith et al., 1995; Labarca et al., 1995; Toth et al., 1993; Xue et al., 1996; reviewed by Kalivas, 1995).

The pharmacology of IEG responses in nonstriatal regions has not been well addressed. Unlike the striatal response, the cortical response to cocaine was reportedly mediated in part by norepinephrine (Bhat and Baraban 1993). Further evidence for differences between the cortical and the striatal responses were noted by the inability of dopamine D_1-receptor antagonists to block the cortical response to amphetamine (Graybiel et al., 1990) or nicotine (Kiba and Jayaraman, 1994), and by the inability to attenuate the cortical response to amphetamine by the AMPA/KA-receptor antagonist DNQX (Wang et al., 1994a). The midline/intralaminar thalamic IEG response to cocaine (Torres and Rivier, 1993) and morphine (Garcia and Harlan, 1996) was reported to be independent of glutamate-receptor activation.

A Common Response to Chronic Exposure to Drugs of Abuse

Studies on effects of chronic treatment with three drugs of abuse, cocaine, amphetamine and morphine, suggest that common alterations in AP-1 binding proteins may occur. For each of these drugs of abuse, acute administration resulted in activation of c-*fos*, *jun* family members, and acute Fras. Chronic treatment with cocaine (Torres and Rivier, 1992; Hope et

al., 1992; Bhat et al., 1992; Steiner and Gerfen 1993; Daunais and McGinty 1994; Moratalla et al., 1996), amphetamine (Persico et al., 1993; Cole et al., 1995; Konradi et al., 1996) or morphine (Hayward et al., 1990) resulted in decreased responses of most of these acute IEGs. Moreover, long-term induction of a common set of chronic Fras has been reported following chronic treatment with cocaine (Rosen et al., 1994; Hope et al., 1994; Chen et al., 1995; Nye et al., 1995), amphetamine (Nye et al., 1995), nicotine (Pich et al., 1997) and morphine (Nye and Nestler, 1996). In addition, chronic treatment with cocaine (Moratalla et al., 1996) or amphetamine (Simpson et al., 1995; Curran et al., 1996) has been reported to augment the chronic Fra response in the patch compartment relative to the matrix, whereas chronic treatment with morphine has been reported to produce more complicated regional alterations in IEG expression (Curran et al., 1996; Frankel et al., 1997). Shifts in striatal phenotypes expressing chronic Fras after chronic administration of cocaine (Moratalla et al., 1996) or amphetamine (Jaber et al., 1995) have also been reported.

Differing Patterns of IEG Response in the CPu Produced by Different Drugs of Abuse

Although all drugs of abuse surveyed in this chapter were reported to increase expression of IEGs in large regions of the forebrain, the anatomical distributions of the responses differed for each drug (Figs. 3, 5, 7, 8, and 10). Thus, each drug of abuse produces a unique signature consisting of a particular set of forebrain neurons activated biochemically. Within the CPu, these differing patterns of responses probably relate to the three organizational schemes of the CPu discussed in the subheading "Basic Organization of the Striatal-Pallidal Systems," i.e., the neuropeptide/projection delineation, the patch-matrix compartments, and the longitudinal organization of cortical and thalamic afferents. For all drugs of abuse that have been examined, c-fos is induced almost exclusively in medium spiny projection neurons, rather than in larger interneurons. The predominant, though not exclusive, striatal phenotype displaying a c-Fos response to acute injection of either cocaine (Cenci et al., 1992; Ruskin and Marshall, 1994; Johansson et al., 1994; Kosofsky et al., 1995) or amphetamine (Robertson and Jian, 1995; Ruskin and Marshall, 1994; Johansson et al., 1994; Jaber et al., 1995) was the medium spiny neuron expressing PPT and PPD, and projecting to the entopeduncular nucleus or substantia nigra. In contrast, both PPT/PPD neurons and PPE neurons projecting to the globus pallidus have been reported to be activated in response to caffeine (Johansson et al., 1994; Sveningsson et al., 1995a, 1995c). The neuropeptide phenotypes of striatal neurons responding to other drugs of abuse have not been characterized. However, the distribution patterns of these phenotypes within the CPu do not determine the distribution patterns of neurons expressing c-Fos in response to a particular drug of abuse. For instance, neurons expressing PPT/PPD have been reported to be expressed fairly homogeneously throughout the dorso-ventral and medial-lateral directions in the CPu, although a strong rostral-caudal gradient has been reported (Harlan et al., 1989). This homogenous distribution in the rostral CPu does not match the profile of neurons expressing IEGs after acute cocaine injection, since these neurons are concentrated in the dorsocentral region of the CPu (e.g., Graybiel et al., 1990), or following amphetamine, since the activated neurons were enriched in the patch compartment (e.g., Graybiel et al., 1990). Similarly, the neurons expressing the PPE gene are distributed homogeneously throughout the CPu (Harlan et al., 1987), but the PPE-expressing neurons displaying an IEG response to caffeine are located primarily in the dorsolateral CPu (Nakajima et al., 1989; Johansson et al., 1992, 1993, 1994; Sveningsson et al., 1995a,b, 1996).

Therefore, it is likely that additional organizational schemes of the striatum guide the pat-

terns of IEG response to drugs of abuse. For amphetamine (Graybiel et al., 1990; Nguyen et al., 1992; Hooper et al., 1994; Simpson et al., 1995; Paul et al., 1995; Curran et al., 1996; Moratalla et al., 1992; Wang et al., 1995) and THC (Glass and Dragunow, 1995), the IEG response has been reported to be more robust in the patch than in the matrix compartment. However, the opposite appears to be case for morphine (Garcia et al., 1997). The patch-matrix compartmental organization appears to contribute to, but does not dictate, the pattern of IEG-responding neurons, since some matrix neurons clearly responded to amphetamine (Graybiel et al., 1990), and some patch neurons clearly responded to morphine (Garcia et al., 1997). Underlying mechanisms for a more robust response in a particular compartment could include differences in binding of D_1- and D_2-selective ligands, expression of specific receptors (e.g., mu opiate receptors and certain glutamate receptors), cortical, thalamic, or dopaminergic innervation patterns, or expression of neuropeptides such as dynorphin.

However, neither the striatal phenotype nor the patch-matrix organization can fully explain the distribution patterns of IEG-responding neurons to different drugs of abuse. Thus, it is likely that the longitudinal arrays of cortical and thalamic inputs to the striatum also contribute to these patterns. Moreover, the glutamatergic nature of these striatal inputs may help to account for the involvement of AMPA and NMDA receptors in drug-induced expression of IEGs in the striatum, but not cortex. Stimulation of cerebral cortex (Fu and Beckstead, 1992; Parthasarathy and Graybiel, 1997) or midline/intralaminar thalamic nuclei (Erdtsieck-Ernste et al., 1995) has been reported to activate c-Fos expression in striatal neurons. Drugs that preferentially activate sensory-motor cortex (e.g., amphetamine and cocaine) may stimulate IEG expression through cortico-striatal connections that terminate in central and lateral portions of the CPu. Similarly, drugs, such as morphine, that activate more visceral regions of cortex, such as the cingulate and insular cortical regions, may enhance IEG expression in the dorsomedial CPu. Superimposed on the cortico-striatal afferent patterns are glutamatergic inputs from midline/intralaminar thalamic nuclei. For instance, the lateral region of the central medial thalamic nucleus projects to a dorsomedial portion of the CPu (Berendse and Groenewegen, 1990) which matches fairly well the distribution of Fos-positive neurons following systemic administration of morphine (Fig. 10). Thus, it is possible that morphine acts through mu opiate receptors found on midline/intralaminar thalamic neurons (Mansour et al., 1987) to augment glutamatergic input to the dorsomedial CPu, in turn facilitating expression of IEGs in this striatal region. Activated by different drugs of abuse, specific sets of thalamo- and cortico-striatal glutamatergic projections to certain arrays of striatal neurons may modulate the dopaminergic activation of intracellular mechanisms, some of which may differ between patch and matrix, to determine the degree of induction of IEGs in individual striatal phenotypes.

Questions and Future Directions

1. Do the common neuroanatomical patterns of IEG expression following acute administration of drugs of abuse delineate brain regions that subserve reinforced or motivated behaviors? To address this question, it may be useful to compare these patterns with the pattern activated in response to a different, presumably motivated, behavior, e.g., mating behavior. Several studies have indicated that mating increases expression of IEGs in both males and females, primarily in the extended amygdala, several hypothalamic nuclei, medial preoptic area, and olfactory bulb (Flanagan-Cato and McEwen, 1995; Baum and Everitt, 1992; Baum and Wersinger, 1993; Polston and Erskine, 1995; Erskine, 1993; Wood and Newman, 1993; Pfaus et al., 1993; Kollack-Walker and Newman, 1995). Other brain regions showing increased IEG expression after mating include the CPu (Pfaus et al., 1993; Polston and Erskine, 1995; Flanagan-Cato and McEwen, 1995), Acb (Pfaus et al., 1993), Tu

(Pfaus et al., 1993), claustrum (Pfaus et al., 1993), lateral septum (Pfaus et al., 1993; Wood and Newman, 1993; Polston and Erskine, 1995); midline/ intralaminar thalamic nuclei (Pfaus et al., 1993; Polston and Erskine, 1995); dentate gyrus (Pfaus et al., 1993; Polston and Erskine, 1995), lateral habenula (Pfaus et al., 1993; Polston and Erskine, 1995), zona incerta (Pfaus et al., 1993), periaqueductal gray (Polston and Erskine, 1995), peripeduncular region (Polston and Erskine, 1995), deep layers of superior colliculus (Polston and Erskine, 1995), central tegmental region of the midbrain (Polston and Erskine, 1995; Baum and Wersinger, 1993) and cerebral cortex (Pfaus et al., 1993). Within the CPu, the response was reported to be restricted primarily to the dorsal half, and occurred in distinct clusters of neurons (Polston and Erskine, 1995), although markers of the patch-matrix organization were not employed. The overall similarity of this neuroanatomical pattern compared to that activated by drugs of abuse is rather striking, and suggests that a large portion of the forebrain may be involved in reinforced behaviors. Activated forebrain regions include not only the classical dopamine-responsive regions of the ventral and dorsal striatum, but also the extended amygdala, midline/intralaminar thalamic nuclei, the lateral septum, and portions of the cerebral cortex (Fig. 11). These regions comprise an interconnecting network of neurons that are activated by all drugs of abuse, and which are involved in reinforced or rewarding behaviors.

2. Do the common anatomical and biochemical alterations in IEGs following chronic administration of drugs of abuse provide a deeper understanding of the nature of addiction? One of the most pernicious and intractable aspects of drug abuse is the phenomenon of drug craving. A recent theory of the neural basis underlying craving posits that repeated administration of drugs of abuse produce a progressive sensitization of neural systems, particularly those involving dopaminergic transmission in the forebrain, and that this sensitization, coupled with the psychological concept of incentive salience and the associative learning of environmental aspects of drug intake, produces craving (Robinson and Berridge, 1993). The common large regions of the forebrain demonstrating increased expression of IEGs following drugs of abuse, as well as the common biochemical and anatomical alterations in expression patterns, may help to designate the neural substrate for sensitization and the phenomenon of craving.
3. Is the induction of IEGs by drugs of abuse caused by behavioral arousal? All of the drugs of abuse reviewed here are capable of inducing locomotor ac tivity and/or stereotyped behavior, depending on the doses administered. However, it is unlikely that the augmentation of IEG expression by drugs of abuse is caused by the behavioral activation, since increases in IEG mRNA levels often precede behavioral effects, and since some drug doses (e.g., 10 mg/kg morphine or 5 g/kg ethanol) suppress locomotor activity, but induce IEG expression.
4. What are the functions of IEG proteins once they are induced by drugs of abuse? The immediate-early gene proteins discussed here all act as transcription factors, regulating expression of specific genes. For members of the AP-1-binding protein families (i.e., the *fos* and *jun* families), the particular genes regulated in response to binding to DNA regulatory elements probably varies greatly depending on the components of the binding complex, other transcription factors in the nucleus, and on the phenotype of the neuron. A consistent theme of much current research into the actions of drugs of abuse is the molecular plasticity that occurs following chronic exposure (Koob, 1996; Nestler, 1996; Hyman, 1996; Dani and Heinemann, 1996; Tabakoff and Hoffman, 1996). For instance, chronic treatment with morphine has been reported to increase expression of adenylate cyclase, protein kinase A, G-protein-receptor kinases, tyrosine hydroxylase, glutamate receptors, and extracellular signal-related kinases (ERKs) in different brain regions (reviewed in Nestler, 1996). Chronic exposure to nicotine has been shown to increase the number of nicotinic cholinergic receptors (reviewed in Dani and Heinemann, 1996). Increases in expression of particular NMDA-receptor subunits have also been reported following chronic administration of alcohol (reviewed in Tabakoff and Hoffman, 1996). Both acute and chronic treatment with cocaine or amphetamine have been reported to increase expression of the PPT and PPD genes (reviewed in Hyman, 1996). Moreover, evidence has been provided for an involvement of Fos and Jun in expression of the PPD gene in transfected cell lines (Naranjo et al., 1991), although demonstration of a role for IEG expression in

regulation of neuropeptide genes in vivo has been much more difficult to obtain. Increased expression of PPD, along with decreased expression of PPE and alterations in mu and kappa opiate-receptor binding have also been reported in the striatum of human cocaine abusers (Hurd and Herkenham, 1993). Increased production of dynorphin may contribute to the dysphoric aspects of addiction to psychostimulants such as cocaine and amphetamine. Moreover, it has been argued that the dysphoric aspects of drug abuse are a characteristic feature of drug dependence, leading abusers to seek additional drugs to relieve the dysphoria (Koob, 1996). A challenge in future work will be the determination of additional target genes regulated in response to activation of IEGs by drugs of abuse, and the potential roles of these proteins in addiction and drug craving.

Acknowledgments

Research reported in this review was supported by grant RD-A-29 from the Louisiana Education Quality Support Fund, to MMG.

References

Alburges M. E., Narang N., and Wamsley J. K. (1993) Alterations in the dopaminergic receptor system after chronic administration of cocaine. *Synapse* **14**, 314–323.

Alexander M. J., Miller M. A., Dorsa D. M., Bullock B. P., R.H. Melloni J., Dobner P. R., and Leeman S. E. (1989) Distribution of neurotensin/neuromedin N mRNA in rat forebrain: Unexpected abundance in hippocampus and subiculum. *Proc. Natl. Acad. Sci. USA* **86,** 5202–5206.

Alexander S. P. H. and Peters J. A. (1997) 1997 receptor and ion channel nomenclature supplement. *Trends Pharmacol. Sci.* **18 (Suppl),** 1–84.

Alheid G. F., Olmos J. S. de, and Beltramino C. A. (1995) Amygdala and extended amygdala, in *The Rat Nervous System* (Paxinos G. ed.) pp. 495–578.

Altman J. and Bayer S. A. (1995) *Atlas of Prenatal Rat Brain Development.* CRC Press, Boca Raton, FL.

Anderson K. D. and Reiner A. (1990) Extensive co-occurrence of substance P and dynorphin in striatal projection neurons: an evolutionarily conserved feature of basal ganglia organization. *J. Comp. Neurol.* **295,** 339–369.

Asin K. E., Nikkel A. L., and Wirtshafter D. (1994) Repeated D_1 receptor agonist treatment blocks cocaine-induced locomotor activity and c-*fos* expression. *Brain Res.* **637,** 342–344.

Asin K.E., Wirtshafter D., and Nikkel A. (1996) Amphetamine induces Fos-like immunoreactivity in the striatum of primates. *Brain Res.* **719,** 138–142.

Baimbridge K. G., Celio M. R., and Rogers J. H. (1992) Calcium-binding proteins in the nervous system. *Trends Neurosci.* **15,** 303–308.

Baraban S. C., Stornetta R. L., and Guyenet P. G. (1995) Effects of morphine and morphine withdrawal on adrenergic neurons of the rat rostral ventrolateral medulla. *Brain Res.* **676,** 245–257.

Barker E. L., Kimmel H. L., and Blakely R. D. (1994) Chimeric human and rat serotonin transporters reveal domains involved in recognition of transporter ligands. *Mol. Pharmacol.* **46,** 799–807.

Baum M. J. and Everitt B. J. (1992) Increased expression of *c-fos* in the medial preoptic area after mating in male rats: Role of afferent inputs from the medial amygdala and midbrain central tegmental field. *Neuroscience* **50,** 627–646.

Baum M. J. and Wersinger S. R. (1993) Equivalent levels of mating-induced neural c-*fos* immunoreactivity in castrated male rats given androgen, estrogen, or no steroid replacement. *Biol. Reprod.* **48,** 1341–1347.

Beckmann A. M., Matsumoto I., and Wilce P. A. (1995) Immediate early gene expression during morphine withdrawal., *Neuropharmacology* **34,** 1183–1189.

Berendse H. W. and Groenewegen H. J. (1990) Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J. Comp. Neurol.* **299,** 187–228.

Berendse H. W., Galais-de Graaf Y., and Groenewegen H. J. (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J. Comp. Neurol.* **316,** 314–347.

Berretta S., Robertson H. A., and Graybiel A. M. (1992) Dopamine and glutamate agonists stimulate neuron-specific expression of fos–like protein in the striatum. *J. Neurophys.* **68,** 767–777.

Bhat R. V., Cole A. J., and Baraban J. M. (1992) Chronic cocaine treatment suppresses basal expression of *zif268* in rat forebrain: *In situ* hybridization studies. *J. Pharm. Exp. Ther.* **263,** 343–349.

Bhat R. V. and Baraban J. M. (1993) Activation of transcription factor genes in striatum by cocaine:

Role of both serotonin and dopamine systems. *J. Pharm. Exp. Ther.* **267,** 496–505.

Bischoff S., Heinrich M., Sonntag J. M., and Krauss J. (1986) The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT_2) receptors. *Eur. J. Pharmacol.* **129,** 367–370.

Bjelke B., Stromberg I., O'Connor W. T., Andbjer B., Agnati L. F., and Fuxe K. (1994) Evidence for volume transmission in the dopamine denervated neostriatum of the rat after a unilateral nigral 6-OHDA microinjection. Studies with systemic D-amphetamine treatment. *Brain Res.* **662,** 11–24.

Bradberry C. W., Nobiletti J. B., Elsworth J. D., Murphy B., Jatlow P., and Roth R. H. (1993) Cocaine and cocaethylene: microdialysis comparison of brain drug levels and effects on dopamine and serotonin. *J. Neurochem.* **60,** 1429–1435.

Brown E. E., Robertson G. S., and Fibiger H. C. (1992) Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. *J. Neurosci.* **12,** 4112–4121.

Burgunder J. M. and Young W. S. (1989) Distribution, projection and dopaminergic regulation of the neurokinin B mRNA-containing neurons of the rat caudate-putamen. *Neuroscience* **32,** 323–335.

Butcher S. P., Fairbrother I. S., Kelly J. S., and Arbuthnott G. W. (1988) Amphetamine-induced dopamine release in the rat striatum: an in vivo microdialysis study. *J. Neurochem.* **50,** 346–355.

Cadoni C., Pinna A., Russi G., Consolo S., and Chiara G. D. (1995) Role of vesicular dopamine in the *in vivo* stimulation of striatal dopamine transmission by amphetamine: evidence from microdialysis and fos immunohistochemistry. *Neuroscience* **65,** 1027–1039.

Caine S. B., Heinrichs S. C., Coffin V. L., and Koob G. F. (1995) Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res.* **692,** 47–56.

Castner S. A. and Becker J. B. (1996) Sex differences in the effect of amphetamine on immediate early gene expression in the rat dorsal striatum. *Brain Res.* **712,** 245–257.

Ceccatelli S., Vilar M. J., Goldstein M., and Hokfelt T. (1989) Expression of c-Fos immunoreactivity in transmitter-characterized neurons after stress. *Proc. Natl. Acad. Sci. USA* **86,** 9569–9573.

Cenci M. A., Campbell K., Wictorin K., and Bjorklund 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.

Cenci M. A. and Bjorklund A. (1994) Transection of corticostriatal afferents abolishes the hyperexpression of Fos and counteracts the development of rotational overcompensation induced by intrastriatal dopamine-rich grafts when challenged with amphetamine. *Brain Res* **665,** 167–174.

Chang S. L., Squinto S. P., and Harlan R. E. (1988) Morphine activation of c-Fos expression in rat brain. *Biochem. Biophys. Res. Comm.* **157,** 698–704.

Chang S. L. and Harlan R. E. (1990) The fos proto-oncogene protein: regulation by morphine in the rat hypothalamus. *Life Sciences* **46,** 1825–1832.

Chang S. L., Patel N. A., and Romero A. A. (1995) Activation and desensitization of Fos immunoreactivity in the rat brain following ethanol administration. *Brain Res.* **679,** 89–98.

Chen J., Paredes W., J. Li D. S., Lowinson J., and Gardner E. L. (1990) Δ^9-Tetrahydrocannabinol produces a naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of freely moving rats. *Psychopharmacology* **102,** 156–162.

Chen J., Nye H. E., Kelz M. B., Hiroi N., Nakabeppu Y., Hope B. T., and Nestler E. J. (1995) Regulation of ΔFosB and FosB-like proteins by electroconvulsive seizure and cocaine treatments. *Mol. Pharmacol.* **48,** 880–889.

Chen Q. and Reiner A. (1996) Cellular distribution of the NMDA receptor NR2A/2B subunits in the rat striatum. *Brain Res.* **743,** 346–352.

Chesselet M.-F. and Delfs J. M. (1996) Basal ganglia and movement disorders: an update. *Trends Neurosci.* **19,** 417–422.

Chieng B., Keay K. A., and Christie M. J. (1995) Increased fos-like immunoreactivity in the periaqueductal gray of anaesthetised rats during opiate withdrawal., *Neurosci. Lett.* **183,** 79–82.

Clark M., Post R. M., Weiss S. R. B., and Nakajima, T. (1992) Expression of c-fos mRNA in acute and kindled cocaine seizures in rats. *Brain Res.* **582,** 101–106.

Clegg D. A., O'Hara B. F., Heller H. C., and Kilduff T. S. (1995) Nicotine administration differentially affects gene expression in the maternal and fetal circadian clock. *Dev. Brain Res.* **84,** 46–54.

Cole R. L., Konradi C., Douglass J., and Hyman S. E. (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* **14,** 813–823.

Couceyro P., Pollock K. M., Drews K., and Douglas J. (1994) Cocaine differentially regulates activator protein-1 mRNA levels and DNA-binding complexes in the rat striatum and cerebellum. *Mol. Pharm.* **46,** 667–676.

Couceyro P. and Douglass J. (1995) Precipitated morphine withdrawal stimulates multiple activator protein-1 signaling pathways in rat brain. *Mol. Pharmacology* **47,** 29–39.

Crawford C. A., McDougall S. A., Bolanos C. A., Hall S., and Berger S. P. (1995) The effects of the kappa agonist U-50,488 on cocaine-induced conditioned and unconditioned behaviors and Fos immunoreactivity. *Psychopharmacology* **120,** 392–399.

Curran E. J., Akil H. and Watson S. J. (1996) Psychomotor stimulant- and opiate-induced c-*fos* mRNA expression patterns in the rat forebrain: Comparisons between acute drug treatment and a drug challenge in sensitized animals. *Neurochem. Res.* **21,** 1425–1435.

D'Souza D. N., Harlan R. E., and Garcia M. M. (1997) Sexual dimorphism in the response to competitive and non-competitive NMDA receptor antagonists and morphine on c-Fos induction in the rat brain. *Abst. Soc. Neurosci.*

Dalia A. and Wallace L. J. (1995) Amphetamine induction of c-*fos* in the nucleus accumbens is not inhibited by glutamate antagonists. *Brain Res.* **694,** 299–307.

Dani J. A. and Heinemann S. (1996) Molecular and cellular aspects of nicotine abuse. *Neuron* **16,** 905–908.

Daunais J. B., Roberts D. C. S., and McGinty J. F. (1993) Cocaine self-administration increases preprodynorphin, but not *c-fos*, mRNA in rat striatum. *NeuroReport* **4,** 543–546.

Daunais J. B. and McGinty J. F. (1994) Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. *Synapse* **18,** 35–45.

Daunais J. B. and McGinty J. F. (1995) Cocaine binges differentially alter striatal preprodynorphin and *zif268* mRNAs. *Mol. Brain Res.* **29,** 201–210.

Daunais J. B., Roberts D. C. S., and McGinty J. F. (1995) Short-term cocaine self administration alters striatal gene expression. *Brain Res. Bull.* **1995,** 523–527.

Daunais J. B. and McGinty J. F. (1996) The effects of D1 or D2 dopamine receptor blockade on *zif/268* and preprodynorphin gene expression in rat forebrain following a short-term cocaine binge. *Mol. Brain Res.* **35,** 237–248.

Dave J. R., Tabakoff B., and Hoffman P. L. (1990) Ethanol withdrawal seizures produce increased c-*fos* mRNA in mouse brain. *Mol. Pharmacol.* **37,** 367–371.

Deacon T. W., Pakzaban P., and Isacson O. (1994) The lateral ganglionic eminence is the origin of cells committed to striatal phenotypes: neural transplantation and developmental evidence. *Brain Res.* **668,** 211–219.

Dean R. A., Harper E. T., Dumaual N., Stoeckel D. A., and Bosron W. F. (1992) Effects of ethanol on cocaine metabolism: formation of cocaethylene and norcocaethylene. *Toxicol. Appl. Pharmacol.* **117,** 1–8.

Di Chiara G. and Imperato A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *J. Pharmacol. Exp. Ther.* **244,** 1067–1080.

Douglass J., McKinzie A. A., and Couceyro P. (1995) PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J. Neurosci.* **15,** 2471–2481.

Drago J., Gerfen C. R., Westphal H., and 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., Logan B., and Laverty R. (1991) 3,4-Methlenedioxymethamphetamine induces Fos-like proteins in rat basal ganglia: reversal with MK 801. *Eur. J. Pharmacol.* **206,** 255–258.

Ennulat D. J., Babb S. M., and Cohen B. M. (1994) Persistent reduction of immediate early gene mRNA in rat forebrain following single or multiple doses of cocaine. *Mol. Brain Res.* **26,** 106–112.

Erdtsieck-Ernste E. B. H. W., Feenstra M. G. P., Botterblom M. H. A., Uum H. F. M. V., Sluiter A. A., and Heinsbroek R. P. W. (1995) C-Fos expression in the rat brain after pharmacological stimulation of the rat "mediodorsal" thalamus by means of microdialysis. *Neuroscience* **66,** 115–131.

Erskine M. S. (1993) Mating-induced increases in fos protein in preoptic area and medial amygdala of cycling female rats. *Brain Res. Bull.* **32,** 447–451.

Ferre S., Euler G. V., Johansson B., Fredholm B. B., and Fuxe K. (1991) Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes. *Proc. Natl. Acad. Sci. USA* **88,** 7238–7241.

Ferre S., O'Connor W. T., Snaprud P., Ungerstedt U., and Fuxe K. (1994) Antagonistic interactions between adenosine A_{2A} receptors and dopamine D_2 receptors in the ventral striatopallidal system. Implications for the treatment of schizophrenia. *Neuroscience* **63,** 765–773.

Fink J. S., Weaver D. R., Rivkees S. A., Peterfreund R. A., Pollack A. E., Adler E. M., and Reppert S. M. (1992) Molecular cloning of the rat A_2 adenosine receptor: selective co-expression with D_2 dopamine receptors in rat striatum. *Mol. Brain Res.* **14,** 186–195.

Flanagan-Cato L. M. and McEwen B. S. (1995) Pattern of fos and jun expression in the female rat forebrain after sexual behavior. *Brain Res.* **673,** 53–60.

Fosnaugh J. S., Bhat R. V., Yamagata K., Worley P. F., and Baraban J. M. (1995) Activation of *arc*, a putative "effector" immediate early gene, by cocaine in rat brain. *J. Neurochem.* **64,** 2377–2380.

Frankel P. S., Harlan R. E., and Garcia. M. M. (1997) Alterations in c-Fos response in the caudate-putamen and thalamus following morphine administration in post-dependent rats. *Abst. Soc. Neurosci.*

Fu L. and Beckstead R. M. (1992) Cortical stimulation induces fos expression in striatal neurons. *Neuroscience* **46,** 329–334.

Garcia M. M. and Harlan R. E. (1993) Chronic morphine increases calbindin D28k in rat striatum via NMDA receptor activation. *NeuroReport* **5,** 65–68.

Garcia M. M., Brown H. E., and Harlan R. E. (1995) Alterations in immediate-early gene proteins in the rat forebrain induced by acute morphine injection. *Brain Res.* **692,** 23–40.

Garcia M. M. and Harlan. R. E. (1996) Morphine induces c-fos expression in rat forebrain through glutamate receptor-dependent and -independent mechanisms. *Abst. Intern. Narcotics Res. Fdn.*

Garcia M. M., Guillot M., and Harlan R. E. (1997) Neurochemical characterization of cortical and striatal neurons expressing c-Fos protein after acute morphine administration. *Abst. Soc. Neurosci.*

Gerfen C. R. (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature* **311,** 461–464.

Gerfen C. R. (1985) The neostriatal mosaic: I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. *J. Comp. Neurol.* **236,** 454–476.

Gerfen C. R. (1992a) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. *Ann. Rev. Neurosci.* **15,** 285–320.

Gerfen C. R. (1992b) The neostriatal mosaic: multiple levels of compartmental organization. *Trends Neurosci* **15,** 133–139.

Gerfen C. R. and Young W. S. (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 C. R., Engber T. M., Mahan L. C., Susel Z., Chase T. N., Jr., F. J. M., and Sibley D. R. (1990) D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250,** 1429–1432.

Giros B. and Caron M. G. (1993) Molecular characterization of the dopamine transporter. *Trends Pharmacol. Sci.* **14,** 43–49.

Glass M. and Dragunow M. (1995) Induction of the Krox 24 transcription factor in striosomes by a cannabinoid agonist. *NeuroReport* **6,** 241–244.

Graybiel A. M. (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends, Neurosci.* **13,** 244–254.

Graybiel A. M., Moratalla R., and Robertson H. A. (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.

Gysling K. and Wang R. Y. (1983) Morphine-induced activation of A10 dopaminergic neurons in the rat. *Brain Res.* **277,** 119–127.

Halliday G., Harding A., and Paxinos G. (1995) Serotonin and tachykinin systems, in *The Rat Nervous System, 2nd ed.* (Paxinos G. ed.) 929–974.

Harlan R. E., Shivers B. D., Romano G. J., Howells R. D., and Pfaff. D. W. (1987) Localization of preproenkephalin mRNA in the rat brain and spinal cord by in situ hybridization. *J. Comp. Neurol.* **258,** 159–184.

Harlan R. E., Garcia M. M., and Krause J. E. (1989) Cellular localization of substance P- and neurokinin A-encoding preprotachykinin mRNA in the female rat brain. *J. Comp. Neurol.* **287,** 179–212.

Harlan R. E. and Garcia M. M. (1995) Charting of Jun family member proteins in the rat forebrain and midbrain: Immunocytochemical evidence for a new Jun-related antigen. *Brain Res.* **692,** 1–22.

Hayward M. D., Duman R. S., and Nestler E. J. (1990) Induction of the c-*fos* proto-oncogene during opiate withdrawal in the locus ceruleus and other regions of rat brain. *Brain Res.* **525,** 256–266.

Heilig M., Engel J. A., and Soderpalm B. (1993) C-*fos* antisense in the nucleus accumbens blocks the locomotor stimulant action of cocaine. *Eur. J. Pharmacology* **236,** 339–340.

Heimer L., Zahm D. S., and Alheid G. F. (1995) Basal ganglia, in *The Rat Nervous System, 2nd ed.* (Paxinos G. ed.) 579–628.

Heimer L., Harlan R. E., Alheid G. F., Garcia M., and Olmos. J. D. (1997a) Substantia innominata: a notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. *Neuroscience* **76,** 957–1006.

Heimer L., Alheid G., Olmos J. d., Groenewegen H., Haber S., Harlan R. E., and Zahm S. (1997b) The accumbens: beyond the core-shell dichotomy. *J. Neuropsychiatry,* in press

Helton T. E., Daunais J. B. and McGinty J. F. (1993) Convulsant doses of cocaine alter immediate early gene and opioid peptide expression in rat limbic forebrain. *Mol. Brain Res.* **20,** 285–288.

Herdegen T., Kovary K., Buhl A., Bravo R., Zimmermann M., and Gass P. (1995) Basal expression of the inducible transcription factors c-Jun, JunB, JunD, c-Fos, FosB, and Krox-24 in the adult rat brain. *J. Comp. Neurol.* **354,** 39–56.

Hollmann M. and Heinemann S. (1994) Cloned glutamate receptors. *Annu. Rev. Neurosci.* **17,** 31–108.

Hooper M. L., Chiasson B. J., and Robertson H. A. (1994) Infusion into the brain of an antisense oligonucleotide to the immediate-early gene *c-fos* suppresses production of fos and produces a behavioral effect. *Neuroscience* **63,** 917–924.

Hope B., Kosofsky B., Hyman S. E., and Nestler E. J. (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 B. T., Nye H. E., Kelz M. B., Self D. W., Iadarola M. J., Nakabeppu Y., Duman R. S., and Nestler E. J. (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.

Hughes P. and Dragunow M. (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmacol. Rev.* **47,** 133–178.

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

Hurd Y. L. and Herkenham M. (1993) Molecular alterations in the neostriatum of human cocaine addicts. *Synapse* **13,** 357–369.

Hyman S. E. (1996) Addiction to cocaine and amphetamine. *Neuron* **16,** 901–904.

Iadarola M. J., Chuang E. J., Yeung C.-L., Hoo Y., Silverthron M., Gu J. and Draisci G. (1993) Induction and suppression of proto-oncogenes in rat striatum after single or multiple treatments with cocaine or GBR-12909. *NIDA Res. Monogr.* **125,** 181–211.

Imperato A., Mulas A., and Di Chiara G. (1986) Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur. J. Pharm.* **132,** 337–338.

Jaber M., Cador M., Dumartin B., Normand E., Stinus L., and Bloch B. (1995) Acute and chronic amphetamine treatments differentially regulate neuropeptide messenger RNA levels and Fos immunoreactivity in rat striatal neurons. *Neuroscience* **65,** 1041–1050.

Jayanthi L. D., Prasad P. D., Ramamoorthy S., Mahesh V. B., Leibach F. H., and Ganapathy V. (1993) Sodium- and chloride-dependent, cocaine-sensitive, high-affinity binding of nisoxetine to the human placental norepinephrine transporter. *Biochemistry* **32,** 12178–12185.

Johansson B., Svenningsson P., Aden U., Lindstrom K., and Fredholm B. B. (1992) Evidence that the increase in striatal c-*fos* following acute high-dose caffeine is not due to direct interaction with striatal adenosine receptors. *Acta. Physiol. Scand.* **146,** 539–541.

Johansson B., Herrera-Marschitz M., Svenningson P., and Fredholm B. B. (1993) Caffeine-induced expression of the proto-oncogene c-*fos* in rat striatum is increased after dopamine denervation. *Acta Physiol. Scand.* **149,** 527–529.

Johansson B., Lindstrom K., and Fredholm B. B. (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 P.W. (1995) Interactions between dopamine and excitatory amino acids in behavioral sensiti-

zation to psychostimulants. *Drug Alcohol Depend.* **37**, 95–100.

Kawaguchi Y., Wislon C.J., Augood S.J., and Emson P.C. (1995) Striatal interneurons: chemical, physiological and morphological characterization. *Trends Neurosci.* **18**, 527–535.

Kiba H. and Jayaraman A. (1994) Nicotine induced c-*fos* expression in the striatum is mediated mostly by dopaminergic D_1 receptor and is dependent on NMDA stimulation. *Mol. Brain Res.* **23**, 1–13.

Kilty J. E., Lorang D., and Amara S. G. (1991) Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science* **254**, 578–579.

Kleven M.S., Perry B.D., Woolverton W.L., and Seiden L.S. Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. *Brain Res.* **532**, 265–270.

Kollack-Walker S., and Newman S. W. (1995) Mating and agonistic behavior produce different patterns of fos immunolabeling in the male Syrian hamster brain. *Neuroscience* **66**, 721–736.

Konradi C., Cole R. L., Heckers S., and Hyman S. E. (1994) Amphetamine regulates gene expression in rat striatum via transcription factor CREB. *J. Neuroscience* **14**, 5623–5634.

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

Koob G. F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* **13**, 177–184.

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

Kosofsky B. E., Genova L. M., and Hyman S. E. (1995a) Postnatal age defines specificity of immediate-early gene induction by cocaine in developing rat brain. *J. Comp. Neurol.* **351**, 27–40.

Kosofsky B. E., Genova L. M., and Hyman S. E. (1995b) Substance P phenotype defines specificity of c-fos induction by cocaine in developing rat striatum. *J. Comp. Neurol.* **1995**, 41–50.

Kuczenski R., Segal D. S., Cho A. K., and Melega W. (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J. Neurosci.* **15**, 1308–1317.

Labandeira-Garcia J. L., Tobio J. P., and Guerra M. J. (1994) Comparison between normal developing striatum and developing striatal grafts using drug-induced fos expression and neuron-specific enolase immunohistochemistry. *Neuroscience* **60**, 399–415.

Labarca R., Gajardo M. I., Sequel M., Silva H., Jerez S., Ruiz A., and Bustos G. (1995) Effects of D-amphetamine administration on the release of endogenous excitatory amino acids in the rat nucleus accumbens. *Prog. Neuropsychopharmacol Biol. Psychiatry* **19**, 467–473.

Le F., Wilce P., Cassady I., Hume D., and Shanley B. (1990) Acute administration of ethanol suppresses pentylenetetrazole-induced c-*fos* expression in rat brain. *Neurosci. Lett.* **120**, 271–274.

Le F., Wilce P. A., Hume D. A., and Shanley B. C. (1992) Involvement of γ-aminobutyric acid and *N*-methyl-D-aspartate receptors in the inhibitory effect of ethanol on pentylenetetrazole-induced c-*fos* expression in rat brain. *J. Neurochem.* **59**, 1309–1315.

Le Moine C., Normand E., Guitteny A. F., Fouque B., Teoule R., and Bloch B. (1990)
Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proc. Natl. Acad. Sci. USA* **87**, 230–234.

Liu F.-C., Dunnett S. B., Robertson H. A., and Graybiel A. M. (1991) Intrastriatal grafts derived from fetal striatal primordia. III. Induction of modular patterns of Fos-like immunoreactivity by cocaine. *Exp. Brain Res.* **85**, 501–506.

Liu J., Nickolenko J. and Sharp F. R. (1994) Morphine induces c-*fos* and *junB* in striatum and nucleus accumbens via D_1 and N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* **91**, 8537–8541.

Lucas L. R., Hurley D. L., Krause J. E., and Harlan R. E. (1992) Localization of the tachykinin neurokinin-B precursor peptide in the rat brain by immunocytochemistry and *in situ* hybridization. *Neuroscience* **51**, 317–345.

Mailleux P., Verslype M., Predu'homme X., and Vanderhaeghen J.-J. (1994) Activation of multiple transcription factor genes by tetrahydrocannabinol in rat forebrain. *NeuroReport* **5**, 1265–1268.

Mailleux P., Zhang F., and Vanderhaeghen J.-J. (1992) The dopamine D1 receptor antagonist SCH-23390 decreases the mRNA levels of the transcription factor zif268 (krox24) in adult rat intact striatum—an in situ hybridization study. *Neurosci. Lett.* **147**, 182-184.

Mansour A., Khachaturian H., Lewis M. F., Akil H., and Watson S. J. (1987) Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. *J. Neurosci.* **7**, 2445–2464.

Matta S. G., Foster C. A., and Sharp B. M. (1993) Nicotine stimulates the expression of cFos protein in the parvocellular paraventricular nucleus and brainstem catecholaminergic regions. *Endocrinology* **132,** 2149–2156.

McGeorge A. J., and Faull R. L. M. (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience* **29,** 503–537.

McGregor A. and Roberts D. C. S. (1993) Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules or reinforcement. *Brain Res.* **624,** 245–252.

Merchant K. M., Hanson G. R., and Dorsa D. M. (1994) Induction of neurotensin and c-*fos* mRNA in distinct subregions of rat neostriatum after acute methamphetamine: comparison with acute haloperidol. *J. Pharm. Exp. Ther.* **269,** 806–812.

Middleton F. A. and Strick P. L. (1996) New concepts regarding the organization of basal ganglia and cerebellar output in *Integrative and Molecular Approach to Brain Function,* (Ito M. and Miyashita Y. eds.) Elsevier, pp. 253–269.

Miyamoto A., Yamamoto T., Ohno M., Watanabe S., Tanaka H., Morimoto S., and Shoyama Y. (1996) Roles of dopamine D_1 receptors in Δ^9-tetrahydrocannabinol-induced expression of Fos protein in the rat brain. *Brain Res.* **710,** 234–240.

Moorman J. M. and Leslie R. A. (1996) *P*-Chloroamphetamine induces c-*fos* in rat brain: a study of serotonin$_{2A/2C}$ receptor function. *Neuroscience* **72,** 129–139.

Moratalla R., Robertson H. A., and Graybiel A. M. (1992) Dynamic regulation of NGFI-A (*zif*268, *egr*1) gene expression in the striatum. *J. Neuroscience* **12,** 2609–2622.

Moratalla R., Viockers E. A., Robertson H. A., Cochran B. H., and Graybiel A. M. (1993) Coordinate expression of c-*fos* and *jun B* is induced in the rat striatum by cocaine. *J. Neurosci.* **13,** 423–433.

Moratalla R., Elibol B., Vallejo M., and Graybiel A. M. (1996a) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal., *Neuron* **17,** 147–156.

Moratalla R., Xu M., Tonegawa S., and Graybiel A. M. (1996b) 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 P. F., Nadi N. S., Karanian J., and Linnoila M. (1992) Mapping rat brain structures activated during ethanol withdrawal: role of glutamate and NMDA receptors. *Eur. J. Pharmacol.* **225,** 217–223.

Nakajima T., Daval J.-L., Morgan P. F., Post R. M., and Marangos P. J. (1989) Adenosinergic modulation of caffeine-induced c-fos mRNA expression in mouse brain. *Brain Res.* **501,** 307–314.

Nakao S.-I., Arai T., Mori K., Yasuhara O., Tooyama I., and Kimura H. (1993) High-dose ketamine does not induce c-Fos protein expression in rat hippocampus. *Neurosci. Lett.* **151,** 33–36.

Nakao S.-I., Adachi T., Murakawa M., Shinomura T., Kurata J., Shichino T., Shibata M., Tooyama I., Kimura H., and Mori K. (1996) Halothane and diazepam inhibit ketamine-induced c-fos expression in the rat cingulate cortex. *Anesthesiology* **85,** 874–882.

Nakki R., Sharp F. R. and Sagar S. M. (1996a) FOS expression in the brainstem and cerebellum following phencyclidine and MK801. *J. Neurosci. Res.* **43,** 203–212.

Nakki R., Sharp F. R., Sagar S. M., and Honkaniemi J. (1996b) Effects of phencyclidine on immediate early gene expression in the brain. *J. Neurosci. Res.* **45,** 13–27.

Napier T. C., Mitrovic I., Churchill L., Klitenick M. A., Lu X. Y., and Kalivas P. W. (1995) Substance P in the ventral pallidum: projection from the ventral striatum, and electrophysiological and behavioral consequences of pallidal substance P. *Neuroscience* **69,** 59–70.

Naranjo J. R., Mellstrom B., Achaval M., and Sassone-Corsi P. (1991) Molecular pathways of pain: *fos/jun*-mediated activation of a noncanonical AP-1 site in the prodynorphin gene. *Neuron* **6,** 607–617.

Nehlig A., Daval J.-L., and Debry G. (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Reviews* **17,** 139–170.

Nestler E. J. (1996) Under siege: the brain on opiates. *Neuron* **16,** 897–900.

Nguyen T. V., Kosofsky B. E., Cohen B. M., and Hyman S. E. (1992) Differential expression of c-Fos and Zif268 in rat striatum after haloperidol, clozapine, and amphetamine. *Proc. Natl. Acad. Sci. USA* **89,** 4270–4274.

Norman A. B., Lu. S. Y., Klug J. M., and Norgren R. B. (1993) Sensitization of c-fos expression in rat striatum following multiple challenges with D-amphetamine. *Brain Res.* **603,** 125–128.

Nye H. E., Hope B. T., Kelz M. B., Iadarola M., and Nestler E. J. (1995) Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. *J. Pharm. Exp. Ther.* **275,** 1671–1680.

Nye H. E. and Nestler E. J. (1996) Induction of chronic fos-related antigens in rat brain by chronic morphine administration. *Mol. Pharmacology* **49,** 636–645.

Ohno M., Yoshida H., and Watanabe S. (1994) NMDA receptor-mediated expression of Fos protein in the rat striatum following methamphetamine administration: relation to behavioral sensitization. *Brain Res.* **665,** 135–140.

Pakzaban Deacon P. T. W., Burns L. H., and Isacson O. (1993) Increased proportion of acetylcholinesterase-rich zones and improved morphological integration in host striatum of fetal grafts derived from the lateral but not the medial ganglionic eminence. *Exp. Brain Res.* **97,** 13–22.

Panagis G., Nisell M., Nomikos G. G., Chergui K., and Svensson T. H. (1996) Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. *Brain Res.* **730,** 133–142.

Pang Y., Kiba H., and Jayaraman A. (1993) Acute nicotine injections induce c-*fos* mostly in non-dopaminergic neurons of the midbrain of the rat. *Mol. Brain Res.* **20,** 162–170.

Parthasarathy H. B. and Graybiel A. M. (1997) Cortically-driven immediate-early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. *J. Neurosci.* **17,** 2477–2491.

Paul M. L., Currie R. W., and Robertson H. A. (1995) Priming of a D1 dopamine receptor behavioral response is dissociated from striatal immediate-early gene activity. *Neuroscience* **66,** 347–359.

Persico A. M., Schindler C. W., O'Hara B. F., Brannock M. T., and Uhl G. R. (1993) Brain transcription factor expression: effects of acute and chronic amphetamine and injection stress. *Mol. Brain Res.* **20,** 91–100.

Petraglia R. S. and Wenthold R. J. (1992) Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *J. Comp. Neurol.* **318,** 329–354.

Pfaus J. G., Kleopoulos S. P., Mobbs C. V., Gibbs R. B., and Pfaff D. W. (1993) Sexual stimulation activates c-*fos* within estrogen-concentrating regions of the female rat forebrain. *Brain Res.* **624,** 253–267.

Pich E. M., Pagliusi S. R., Tessari M., Talabot-Ayer D., Huijsduijnen R. H. v., and Chiamulera C. (1997) Common neuronal substrates for the addictive properties of nicotine and cocaine. *Science* **275,** 83–86.

Polston E. K. and Erskine M. S. (1995) Patterns of induction of the immediate-early genes c-*fos* and *egr*-1 in the female rat brain following differential amounts of mating stimulation. *Neuroendocrinology* **62,** 370–384.

Putzke J., Spanagel R., Tolle T. R., and Zieglgansberger W. (1996) The anti-craving drug acamprosate reduces c-*fos* expression in rats undergoing ethanol withdrawal., *Eur. J. Pharmacol.* **317,** 39–48.

Ragsdale C. W. J. and Graybiel A. M. (1988) Fibers from the basolateral nucleus of the amygdala selectively innervate striosomes in the caudate nucleus of the cat. *J. Comp. Neurol.* **269,** 506–522.

Ren T. and Sagar S. M. (1992) Induction of c-*fos* immunostaining in the rat brain after the system administration of nicotine. *Brain Res. Bull.* **29,** 589–597.

Robertson G. S. and Jian M. (1995) D1 and D2 dopamine receptors differentially increase fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. *Neuroscience* **64,** 1019–1034.

Robinson T. E. and Berridge K. C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Reviews* **18,** 247–291.

Robledo P. and Koob G. F. (1993) Two discrete nucleus accumbens projection areas differentially mediate cocaine self-administration in the rat. *Behav. Brain Res.* **55,** 159–166.

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

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

Ryabinin A. E., Melia K. R., Cole M., Bloom F. E., and Wilson M. C. (1995) Alcohol selectively attenuates stress-induced c-*fos* expression in rat hippocampus. *J. Neurosci.* **15,** 721–730.

Sakurai-Yamashita Y., Kataoka Y., Fujiwara M., Mine K., and Ueki S. (1989) Δ^9-Tetrahydrocannabinol facilitates striatal dopaminergic transmission. *Pharmacol. Biochem. Behav.* **33,** 397–400.

Salminen O., Lahtinen S., and Ahtee L. (1996) Expression of fos protein in various rat brain areas following acute nicotine and diazepam. *Pharm. Biochem. Behav.* **54,** 241–248.

Schiffmann S. N., Jacobs O., and Vanderhaeghen J.-J. (1991) Striatal restricted adenosine A_2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. *J. Neurochem.* **57,** 1062–1067.

Schwarzschild M.A., Cole R.L., and Hyman S.E. (1997) Glutamate, but not dopamine, stimulates stress-activated protein kinase and AP-1-mediated transcription in striatal neurons. *J. Neurosci.* **17,** 3455–3466.

Self D. S. and Nestler E. J. (1995) Molecular mechanisms of drug reinforcement and addiction. *Annu. Rev. Neurosci.* **18,** 463–495.

Sharp T., Zetterstrom T., Ljungberg T., and Ungerstedt U. (1987) A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. *Brain Res.* **401,** 322–330.

Simpson J. N., Wang J. Q., and McGinty J. F. (1995) Repeated amphetamine administration induces a prolonged augmentation of phosphorylated cyclase response element-binding protein and fos-related antigen immunoreactivity in rat striatum. *Neuroscience* **69,** 441–457.

Smith J. A., Mo Q., Guo H., Kunko P. M., and Robinson S. E. (1995) Cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens. *Brain Res.* **683,** 264–269.

Snyder-Keller A. M. (1995) The development of striatal patch/matrix organization after prenatal methylazoxymethanol: a combined immunocytochemical and bromo-deoxy-uridine birthdating study. *Neuroscience* **68,** 751–763.

Song D. D. and Harlan R. E. (1994a) Genesis and migration patterns of neurons forming the patch and matrix compartments of the rat striatum. *Dev. Brain Res* **83,** 233–246.

Song D. D. and Harlan R. E. (1994b) The development of enkephalin and substance P neurons in the basal ganglia: insights into neostriatal compartments and the extended amygdala. *Dev. Brain Res.* **83,** 247–261.

Steiner H. and Gerfen C. R. (1993) Cocaine-induced c-*fos* messenger RNA is inversely related to dynorphin expression in striatum. *J. Neurosci.* **13,** 5066–5081.

Steiner H. and Gerfen C. R. (1995) Dynorphin opioid inhibition of cocaine-induced, D1 dopamine receptor-mediated immediate-early gene expression in the striatum. *J. Comp. Neurol.* **353,** 200–212.

Stornetta R. L., Norton F. E., and Guyenet P. G. (1993) Autonomic areas of rat brain exhibit increased Fos-like immunoreactivity during opiate withdrawal in rats. *Brain Res.* **624,** 19–28.

Sugita S., Namima M., Nabeshima T., Okamoto K., Furukawa H., and Watanabe Y. (1996) Phencyclidine-induced expression of c-Fos-like immunoreactivity in mouse brain regions. *Neurochem. Int.* **28,** 545–550.

Sukhatme V. P., Cao X., Chang L. C., Tsai-Morris C.-H., Stamenkovich D., Ferreira P. C. P., Cohen D. R., Edwards S. A., Shows T. B., Curran T., Beau M. M. L., and Adamson E. D. (1988) A zinc finger-encoding gene coregulated with c-*fos* during growth and differentiation, and after cellular depolarization. *Cell* **53,** 37–43.

Svenningsson P., Johansson B., and Fredholm B. B. (1995a) Effect of different xanthines and phosphodiesterase inhibitors on c-*fos* expression in rat striatum. *Acta Physiol. Scand.* **154,** 17–24.

Svenningsson P., Nomikos G. G., and Fredholm B. B. (1995b) Biphasic changes in locomotor behavior and in expression of mRNA for NGFI-a and NGFI-B in rat striatum following acute caffeine administration. *J. Neurosci.* **15,** 7612–7624.

Svenningsson P., Strom A., Johansson B., and Fredholm B. B. (1995c) Increased expression of c-*jun*, *jun*B, AP-1, and preproenkephalin mRNA in rat striatum following a single injection of caffeine. *J. Neurosci.* **15,** 3583–3593.

Svenningsson P., Johansson B., and Fredholm B. B. (1996) Caffeine-induced expression of c-*fos* mRNA and NGFI-A mRNA in caudate putamen and in nucleus accumbens are differentially affected by the *N*-methyl-D-aspartate receptor antagonist MK-801. *Mol. Brain Res.* **35,** 183–189.

Swanson L. W. (1995) Mapping the human brain: past, present, and future. *Trends. Neurosci.* **18,** 471–474.

Tabakoff B. and Hoffman P. L. (1996) Alcohol addiction: an enigma among us. *Neuron* **16,** 909–912.

Torres G. (1994) Acute administration of alcohol blocks cocaine-induced striatal *c-fos* immunoreactivity protein in the rat. *Synapse* **1994,** 161–167.

Torres G. and Rivier C. (1992) Differential effects of intermittent or continuous exposure to cocaine on the hypothalamic-pituitary-adrenal axis and c-*fos* expression. *Brain Res.* **571,** 204–211.

Torres G. and Rivier C. (1993) Cocaine-induced expression of striatal c-fos in the rat is inhibited

by NMDA receptor antagonists. *Brain Re. Bull.* **30,** 173–176.

Torres G. and Rivier C. (1994) Induction of c-*fos* in rat brain by acute cocaine and fenfluramine exposure: a comparison study. *Brain Research* **647,** 1–9.

Torres G. and Horowitz J. M. (1996) Combined effects of ethanol and cocaine on FOS-like protein and cocaethylene biosynthesis in the rat. *Psychopharmacology* **128,** 105–114.

Toth E., Vizi E. S., and Lajtha A. (1993) Effect of nicotine on levels of extracellular amino acids in regions of the rat brain in vivo. *Neuropharmacology* **32,** 827–832.

Turgeon S. M., Pollack A. E., Schusheim L., and Fink J. S. (1996) Effects of selective adenosine A1 and A2a agonists on amphetamine-induced locomotion and c-Fos in striatum and nucleus accumbens. *Brain Res* **707,** 75–80.

Unterwald E.M., Ho A., Rubenfeld J.M., and Kreek M.J. (1994) Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. *J. Pharmacol. Exp. Ther.* **270,** 1387–1396.

van der Kooy D. and Fishell G. (1987) Neuronal birthdate underlies the development of striatal compartments. *Brain Res.* **401,** 155–161.

Van Tol J. H. M., Bunzow J. R., Guan H. C., Sunahara R. K., Seeman P., Niznik H. B., and Civelli O. (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* **350,** 610–614.

Vargo J. M., Estrick M. S., and Marshall J. F. (1996) Amphetamine-induced Fos expression in globus pallidus is altered by frontal cortex injury. *Brain Res.* **716,** 207–212.

Wang J. G., Daunais J. B. and McGinty J. F. (1994a) NMDA receptors mediate amphetamine-induced upregulation of *zif/268* and preprodynorphin mRNA expression in rat striatum. *Synapse* **18,** 343–353.

Wang J. Q., Daunais J. B., and McGinty J. F. (1994b) Role of kainate/AMPA receptors in induction of striatal *zif/268* and preprodynorphin mRNA by a single injection of amphetamine. *Mol. Brain Res.* **27,** 118–126.

Wang J. Q., Smith A. J. W., and McGinty J. F. (1995) A single injection of amphetamine or methamphetamine induces dynamic alterations in *c-fos, zif/268* and preprodynorphin messenger RNA expression in rat forebrain. *Neuroscience* **68,** 83–95.

Wang J.Q. and McGinty J.F. (1996) Acute methamphetamine-induced zif/268, preprodynorphin, and preproenkephalin mRNA expression in rat striatum depends on activation of NMDA and kainate/AMPA receptors *Brain Res. Bull.* **39,** 349–357.

Warden M. K. and III, W. S. Y. (1988) Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. *J. Comp. Neurol.* **272,** 90–113.

Wirtshafter D., Asin K. E., and Pitzer M. R. (1994) Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula. *Brain Res.* **633,** 21–26.

Wood R. I. and Newman S. W. (1993) Mating activates androgen receptor-containing neurons in chemosensory pathways of the male Syrian hamster brain. *Brain Res.* **614,** 65–77.

Worley P. F., Christy B. A., Nakabeppu Y., Bhat R. V., Cole A. J., and Baraban J. M. (1991) Constitutive expression of *zif268* in neocortex is regulated by synaptic activity. *Proc. Natl. Acad. Sci. USA* **88,** 5106–5110.

Xue C.J., Ng J.P., Li Y., and Wolf M.E. (1996) Acute and repeated systemic amphetamine administration: effects on extracellular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. *J. Neurochem.* **67,** 352–363.

Yamamoto B. K. and Spanos L. J. (1988) The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur. J. Pharmacol.* **148,** 195–203.

Young S. T., Porrino L. J., and Iadarola M. J. (1991) Cocaine induces striatal c-Fos-immunoreactive proteins via dopaminergic D1 receptors. *Proc. Natl. Acad. Sci. USA* **88,** 1291–1295.

Zoeller R. T. and Fletcher D. L. (1994) A single administration of ethanol simultaneously increases c-*fos* mRNA and reduces c-*jun* mRNA in the hypothalamus and hippocampus. *Mol. Brain Res.* **24,** 185–191.