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Dopaminergic Regulation of Immediate-early gene Expression in the Central Nervous System

by

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A thesis submitted in conformity with the
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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

to my parents

ABSTRACT

Alterations in dopaminergic neurotransmission have profound effects on neuronal expression of the putative activity marker, Fos, in both the dorsal and ventral striatum. Stimulants such as *d*-amphetamine and cocaine increase striatal Fos-like immunoreactivity by enhancing the activation of D1-like dopamine receptors. In contrast, neuroleptics such as haloperidol and raclopride increase Fos-like immunoreactivity in the striatum by blocking striatal D2-like dopamine receptors. In the dorsal striatum, D1-like receptor agonists elevates Fos-like immunoreactivity predominantly in neurons projecting to the midbrain (substantia nigra), whereas D2-like receptor antagonist enhances Fos-like immunoreactivity principally in neurons projecting to the pallidum (globus pallidus). These finds are consistent with the proposal that D1 receptors are located chiefly on striatonigral neurons, whereas D2 receptors reside mainly on striatopallidal neurons. Since the nucleus accumbens (largest component of the ventral striatum) also sends projections to the midbrain (ventral tegmental area and substantia nigra) and pallidum (ventral pallidum), the present study utilized retrograde tract tracing techniques to determine if there was a similar segregation of D1-like receptor agonist- and D2-like receptor antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity in these accumbal projections. In addition, we examined whether these relationships were the same in the core and shell regions of the nucleus accumbens.

Like in the dorsal striatum, D1-like receptor agonists (*d*-amphetamine and CY 208-243), but not D2-like receptor antagonists (haloperidol and clozapine), increased $Fos_{2\text{-}16}$-like immunoreactivity in accumbal neurons projecting to the midbrain (ventral tegmental

area and substantia nigra). Also like in the dorsal striatum, D2-like receptor antagonist-induced Fos_{2-16}-like immunoreactivity was located preferentially in accumbal neurons projecting to the pallidum (ventral pallidum). However, unlike in the dorsal striatum where the vast majority of neurons which display D1-like receptor agonist-induced Fos_{2-16}-like immunoreactivity project to the midbrain, nearly 50% of those neurons in the nucleus accumbens which were Fos_{2-16} immunoreactive after *d*-amphetamine or CY 208-243 administration projected to the ventral pallidum. Thus, a similar number of accumbal neurons which expressed D1-like receptor agonist-induced Fos_{2-16}-like immunoreactivity were retrogradely labelled from the midbrain and ventral pallidum. Accumbal projections to the midbrain and ventral pallidum were retrogradely labelled with different retrograde tracers in order to determine the degree of collateralization between these pathways. Approximately 20% of retrogradely labelled neurons displayed both tracers indicating that collateralization and damage to fibres of passage could not account for all of those cases in which D1-like receptor agonist-induced Fos_{2-16}-like immunoreactivity was detected in accumbal neurons projecting to the ventral pallidum. All results of the present study were the same for both the shell and core regions of the nucleus accumbens. Our findings suggest that in contrast to the projection from the dorsal striatum to the globus pallidus which is composed primarily of neurons that express the D2 receptor, both D1 and D2 receptors are located on a comparable number of accumbal neurons that project to the ventral pallidum. Given that dopamine receptors in the nucleus accumbens are thought to play an important role in mediating the rewarding effects of stimulants and the antipsychotic actions of neuroleptics, our results may have significant implications for the

anatomical bases of these processes.

Destruction of the nigrostriatal pathway produces a long-lasting and widespread increase in Fos-like immunoreactivity in the denervated striatum detected with a rabbit polyclonal antibody raised against amino acids 128-152 of the Fos molecule. In order to determine the connectional nature of neurons that express $Fos_{128-152}$-like immunoreactivity after dopaminergic denervation, striatonigral or striatopallidal projections were retrogradely labelled with Fluoro-Gold. In the striatum, $Fos_{128-152}$-like immunoreactivity detected 6-weeks postlesion was frequently located in retrogradely labelled striatopallidal neurons, but seldom in retrogradely labelled striatonigral neurons. These results are consistent with studies suggesting that dopamine tonically inhibits striatopallidal neurons which become more active in its absence.

In the forebrain, D1 dopamine receptors are located in the caudate-putamen, the nucleus accumbens, olfactory tubercle, amygdala, lateral septum and cortical areas. Consistent with distribution, administration of D1-like receptor agonist-CY 208-243 to rats with unilateral lesions of the mesotelencephalic dopamine pathway induced Fos_{2-16}-like immunoreactivity in the ipsilateral parietal cortex, striatum, nucleus accumbens, amygdala, lateral septal nucleus and olfactory tubercle. The close topographical relationship between D1-like receptor agonist-induced Fos_{2-16}-like immunoreactivity and D1 receptors suggests that D1-like receptor agonist-induced Fos_{2-16}-like immunoreactivity is located in neurons that express the D1 receptor. That these increases were in fact mediated by activation of D1-like dopamine receptors was confirmed by the ability of the selective D1-like receptor antagonist SCH 23390 to block all CY 208-243-induced increases in Fos_{2-16}-like immunoreactivity.

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LIST OF ABBREVIATIONS

AP	anterior-posterior
CaRE	calcium responsive element
CNS	central nerves system
CRB	Cambridge Research Biochemicals
CRE	cAMP-responsive elements
DA	dopamine
DAB	diaminobenzidine
D1	DA receptor positively coupled to adenylate cyclase
D2	DA receptor negatively coupled to adenylate cyclase
DV	dorso-ventral
FG	Fluoro-Gold
GABA	γ-aminobutyric acid
GP	globus pallidus
hr	hour
i.p.	intraperitoneal
IEG	immediate-early gene
kg	kilogram
min	minute
MFB	medial forebrain bundle
ML	medio-lateral
PBS	phosphate-buffered saline

6-OHDA	6-hydroxydopamine
RLB	rhodamine labelled latex beads
s.c.	subcutaneous
SNC	substantia nigra pars compacta
SNR	substantia nigra pars reticulata
VP	ventral pallidum

LIST OF PUBLICATIONS AND PRESENTATION

Robertson, G.S. and M. Jian. (1994). D1 and D2 dopamine receptors differentially increase Fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. *Neurosci.*, in press.

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Jian, M., W.A. Staines, M.J. Iadarola and G.S. Robertson. (1993). Destruction of nigrostriatal pathway increases Fos-like immunoreactivity predominantly in striatopallidal neurons. *Mol. Brain Res.* **19**:156-160.

INTRODUCTION

1. Historical Background

A. Expression of *c-fos* Protein as a Metabolic Marker

In 1987, Hunt and colleagues reported that the *c-fos* protein (Fos) was induced dramatically in the dorsal horn of the spinal cord by painful cutaneous stimulation (Hunt et al., 1987). In the same year, Dragunow and Robertson reported that partial seizures elicited by unilateral electrical stimulation of the amygdala, elevated Fos-like immunoreactivity in the ipsilateral amygdala and piriform cortex (Dragunow and Robertson HA, 1987). However, generalized seizures, which invade the entire neocortex and hippocampus as well as the contralateral amygdala and piriform cortex resulted in the appearance of Fos-like immunoreactivity in all of these areas (Dragunow and Robertson HA, 1987). Thus, Fos immunocytochemistry identified brain regions known to be activated during seizures. These discoveries also provided an early indication that Fos, a transcriptional regulating factor, might play an important role in the establishment of prolonged functional changes in the central nervous system (CNS). Since then a wide variety of pharmacological and physiological treatments have been found to increase Fos-like immunoreactivity in regions of the CNS that are thought to be excited by such manipulations. This has led to the suggestion that it might be possible to use Fos immunohistochemistry to map functional pathways in the brain. Since this initial proposal, a large number of different stimuli have been shown to produce anatomically

relevant increases in *c-fos* expression in the CNS (Sagar et al., 1988). However, because the majority of antibodies used to detect Fos also recognize a variety of other proteins referred to as Fos-related antigens, the term Fos-like immunoreactivity is used rather than Fos immunoreactivity.

B. Discovery of *c-fos*

i. Proto-oncogenes

Oncogenes were first described as the genetic information responsible for the induction of tumours by RNA viruses of the class Retroviridae. In 1989 the Nobel Prize was awarded to Michael J. Bishop and Harold Varmus for their discovery of the cellular origin of retroviral oncogenes (Stehelin et al., 1976). The transforming genes of retroviruses are copies of genes found in normal cells that play important roles in the regulation of growth and differentiation. However, unlike their normal cellular counterparts, they are often mutated resulting in deregulated expression. Cellular transformation occurs when these genes are expressed inappropriately, outside of natural constraints. The normal cellular genes from which retroviral oncogenes were derived are most correctly referred to as "proto-oncogenes", meaning that they represent the progenitors of oncogenes (Bishop, 1985). Over the last five to ten years, the study of oncogenes has revealed that as a class, proto-oncogenes function in several aspects of signal transduction processes. That is, they encode proteins which participate in the transmission of information between and within

cells. For instance, a variety of extracelluar polypeptide messengers, cell-surface receptors, protein kinases, G-proteins, and transcriptional regulating factors are known to be proto-oncogene products.

The *c-fos* gene is the normal cellular counterpart of the viral oncogene *v-fos* which causes osteogenic sarcoma (Curran et al., 1983; for review, see Curran and Morgan, 1987). The name *fos* is derived from the first letter of the closely related mouse viruses (FBJ and FBR) which produce osteogenic sarcoma, the tumour from which the last two letters of *fos* are derived (Curran and Teich, 1982). Viral oncogenes (cancer causing genes) are copies of genes that are a normal constituent of the cellular genome. The cellular genes (or proto-oncogenes) from which viral oncogenes arose seem to be involved in the regulation of cell growth. This is based on the observations that deregulated expression of *c-fos* in fibroblasts causes unrestricted cell proliferation (Miller et al., 1984) and that a variety of polypeptide growth factors will increase the expression of *c-fos* and its 55 kilodalton phosphoprotein product called Fos (Morgan and Curran, 1986; Ran et al., 1986). After translation in the cytoplasm, Fos enters the nucleus where it undergoes extensive posttranslational modification (Curran et al., 1984) before complexing with several cellular proteins (Curran et al., 1985) and binding to DNA (Sambucetti and Curran, 1986). Once bound to DNA, Fos is thought to participate in a macromolecular complex which results in transcription of the target gene.

ii. Induction of IEG Expression

In most cells, basal expression of *c-fos* mRNA and protein are low. However, many types of stimuli, which promote differentiation or neuronal excitation, elicit a rapid and transient induction of *c-fos* mRNA and protein (see below). The time-course of the induction process is identical in most circumstances. Transcriptional activation occurs within 5 minutes and continues for 15-20 minutes (Greenberg and Ziff, 1984; Greenberg et al., 1985). *c-fos* mRNA accumulates and reaches peak values at 30-45 minutes post-stimulation (Muller et al., 1984); thereafter it declines with a relatively short half-life of about 12 minutes. Synthesis of Fos follows *c-fos* mRNA expression and is turned over with a half-life of about 2 hours (Muller et al., 1984; Curran et al., 1984). The induction of *c-fos* transcription occurring in the presence of protein synthesis inhibitors suggests that the proteins required for expression are present in unstimulated cells and that they are activated by post-translational modification. This feature led to the classification of *fos* and other rapidly induced genes as cellular immediate-early genes (IEGs) by analogy to viral IEGs.

Several regulatory elements located in the 5' untranslated region of *c-fos* have been demonstrated to play a role in controlling its expression. The first regulatory element to be defined operationally was the serum response element (SRE) (Treisman, 1985; Gilman et al., 1986; Prywes and Roeder, 1987) which contributes to basal expression in unstimulated cells as well as the mediation of response to many different types of cell stimuli. These include induction by protein kinase C, epidermal growth factor and nerve

growth factor (Morgan and Curran, 1991). Another regulatory element at -60 also functions as a basal and inducible regulatory element (Gilman et al., 1986; Fisch et al., 1987; Sheng et al., 1988; Berkowitz et al., 1989). This site is similar to the cAMP-responsive elements (CRE) that is present in several neuropeptide genes (Comb et al., 1986; Montminy et al., 1986) and also acts as a calcium responsive element (CaRE) in mediating inducibility of *c-fos* by depolarizing stimuli in PC 12 cells (Sheng et al., 1990). Expression of *c-fos* in PC12 cells can also be induced by voltage-gated calcium influxes (Morgan and Curran, 1986), which appears to act through this CRE/CaRE sequence.

2. *c-fos* Protein and Differential Regulation of IEGs

Fos cannot act alone, in order to bind to DNA it must first form a heterodimer with Jun, the 39 kDa product of the immediate-early gene *c-jun*. Antibodies directed against the DNA binding region of Fos (amino acids 128-152) precipitate a set of Fos-related antigens (Fras) as well as several other Fos-associated proteins (Fap) from stimulated cells (Franza et al., 1987). These proteins bind to DNA and are induced by many of the agents and conditions previously shown to increase Fos expression (Miller et al., 1984; Franza et al., 1987; Cohen and Curran, 1988). Several Fos-related genes have been cloned and sequenced, they include *fosB*, *fra-1* and *fra-2* (Cohen and Curran 1988; Cohen et al., 1989; Zerial et al., 1989; Matsui et al., 1990; Nishina et al., 1990). These Fos-related antigens share several regions of homology with Fos, including the DNA-binding domain and the leucine zipper region which mediates dimerization with members of the *jun*

family. The *jun* family consists of c-*jun*, *jun*-B (Ryder et al., 1988) and *jun*-D (Ryder et al., 1989). All members of the *jun* family form complexes with each member of the *fos* family (Nakabeppu et al., 1988; Cohen et al., 1989; Zerial et al., 1989). For example, Fos dimerizes with Jun to form the Fos/Jun complex that binds specifically to the AP-1 binding site which has the consensus sequence -TGACTCA-.

Fos can either stimulate or repress transcription, depending on which member of the Jun family it complexes with. In addition, there is also the possibility of Jun/Jun homodimers with unknown potentials. Recent evidence suggests that other members of these two families may participate in regulation of gene expression. For example, the truncated form of FosB inhibits the transcriptional activation of Fos/Jun heterodimers (Nakabeppu and Nathans, 1991). Similarly, Fra-1 can combine with Jun and bind to the AP-1-recognition element, raising the possibility that this Fos family member might play a role in regulation at the AP-1 site (Cohen et al., 1989). Defining a precise role for the Fos/Jun transcriptional factor is complicated by the fact that several members of the *fos* and *jun* family are activated in concert. Indeed, IEGs are activated as part of a complex cascade with different members of the *fos* and *jun* families interacting with one another and with other second messengers at multiple transcriptional regulating sites to alter gene expression. However, it is becoming clear that these genes probably play a role in postmitotic tissue, one of the best examples being neurons of the CNS.

3. Dopaminergic Regulation of Neuronal Gene Expression

Dopamine is a catecholamine neurotransmitter that plays a pivotal role in the central regulation of functions as varied as motor control, cognition and drug addiction. It has been appreciated for some time that dopamine plays an important role in regulating neuropeptide concentrations in the basal ganglia. Blockade of dopaminergic neurotransmission, either by dopamine receptor antagonists or by lesioning the nigrostriatal pathway with 6-hydroxydopamine (6-OHDA), increases both enkephalin (Hong et al., 1978a) and mRNA encoding its precursor, proenkephalin (Sabol et al., 1983; Tacquet et al., 1983). Conversely, 6-OHDA lesions decrease the tachykinin neuropeptides substance P and substance K (Hong et al., 1978b) and the mRNAs for these neuropeptides in the striatum (Bannon et al., 1986). Although there is a general consensus that a decrease in striatal dopamine receptor stimulation is responsible for these changes, the mechanism by which these alterations in gene expression occur has remained obscure.

Recently, it has been demonstrated that dopaminergic stimulants and antagonists rapidly increase *c-fos* expression in the basal ganglia. The fact that *c-fos* encodes a transcriptional regulating factor suggests that it may mediate alterations in neuropeptide gene expression produced by changes in dopamine receptor stimulation. If this is the case, then *c-fos* induction produced by dopaminergic agonists and antagonists should occur in same neurons which undergo profound alterations in neuropeptide gene expression. However, before discussing these relationships it is first necessary to describe the prominent neuroanatomical features of the basal ganglia.

4. Anatomical Consideration

The striatum which is the major component of the basal ganglia can be broadly divided into dorsal and ventral sectors on the grounds of a variety of anatomical and behavioral criteria. The dorsal striatum (caudate-putamen) plays a major role in the regulation of sensorimotor processes, whereas the ventral striatum has been implicated in the control of affective and motivational aspects of behaviour (Delong, 1990; Koob and Bloom, 1988; Le Moal and Simon, 1991). The ventral striatum consists of the nucleus accumbens and olfactory tubercle. To simplify the terminology, the term striatum will be used when referring to the dorsal striatum.

A. Striatal Cytoarchitecture

Numerous studies indicate that the striatum plays a major role in the control of motor behaviour and is the primary projection site for dopaminergic neurons located in the substantia nigra (Groves, 1983; Marsden, 1982). In man, the striatum is composed of two major nuclei called the caudate nucleus and putamen which are separated by the fibres of the internal capsule (Brodal, 1981). However, in rodents the caudate and putamen are fused and the fibres of the internal capsule pass through the striatum. The vast majority of striatal cells are spiny type I which have been estimated to comprise approximately 95% of the striatal neuronal population (Pasik et al., 1979; Groves, 1983). Morphologically distinct neurons account for the remaining 5% of the striatal neuronal

population (Pasik et al., 1979).

On the basis of immunohistochemical and pharmacological findings, spiny I neurons are thought to use the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Pasik and Holstein, 1986). The spiny type I cell is of medium size with 4-7 dendrites (250 μm in length) that are heavily coated with dendritic spines, hence the synonymous term "medium spiny neuron" (Groves, 1983). Electron microscopic measurements of Golgi impregnated spiny I cells have indicated that the density of dendritic spines may reach 40 spines per 10μm of dendritic length (Pasik et al., 1979). The large number of dendritic spines on these neurons permits the convergence of considerable afferent input. For instance, afferents from the cortex, thalamus and substantia nigra as well as intrinsic inputs converge on the medium spiny neuron (Groves, 1983; Semba et al., 1987).

B. Striatal Outputs

The primary outputs of the striatum are targeted at the substantia nigra pars reticulata (SNR), globus pallidus (GP) and entopeduncular nucleus (EPN) (Fonnum, 1978; Brodal, 1981). In primates, the GP is composed of the internal (medial) and external (lateral) segment (Brodal, 1981). The internal segment of the GP in primates is equivalent to the entopeduncular nucleus in rodents. Retrograde tracing studies with horseradish peroxidase have demonstrated that medium spiny neurons compose the striatal projections to the SNR and GP. Neurons projecting to the SNR comprise the striatonigral pathway (Kawaguchi et al., 1990), whereas those projecting to the GP consist of the striatopallidal pathway,

that sends a major projection to the subthalamic nucleus which in turn projects to the SNR. A projection from the GP to the striatum has also been identified. In addition, the GP sends a projection to the SNR. Thus, the GP may influence SNR activity either directly or indirectly via the subthalamic nucleus. Neurochemical and electrophysiological studies have demonstrated that the pallidal projection to the subthalamic nucleus is inhibitory, whereas subthalamic efferents utilize the excitatory neurotransmitter glutamate and tonically stimulate SNR neurons (Smith and Bolam, 1990; Smith et al., 1990; Kita and Kitai, 1987; Nakanishi et al., 1987).

Striatonigral and striatopallidal pathways are composed principally of different populations of striatal neurons (Beckstead and Cruz, 1986). Although both of these pathways utilize the inhibitory transmitter γ-aminobutyric acid, they contain different neuropeptides (Beckstead and Kersey, 1985; Anderson and Reiner, 1990). Striatopallidal neurons are thought to contain enkephalin, whereas striatonigral neurons are considered to utilize both substance P and dynorphin as neurotransmitters (Beckstead and Kersey, 1985; Anderson and Reiner, 1990; Gerfen and Young, 1988; Gerfen et al., 1990; Staines et al., 1980; Vincent et al., 1982).

C. Striatal Inputs

The major striatal inputs arise from the cortex, thalamus and substantia nigra pars compacta (SNC) (Fig. 1).

Figure 1. Neurochemical anatomy of the basal ganglia in human brain. NAC = nucleus accumbens; ST = striatum; GP_E = external globus pallidus; GP_I = internal globus pallidus; SC = superior colliculus; SNC = substantia nigra pars compacta; SNR = substantia nigra pars reticulata; SUB = subthalamic nucleus; RN = raphe nucleus; RF = reticular formation; VP = ventral pallidum; TN = thalamic nucleus; DA = dopamine; GABA = γ-aminobutyric acid; 5-HT = 5-hydroxytryptamine (serotonin).

Neurochemical Anatomy of the Basal Ganglia in Human Brain

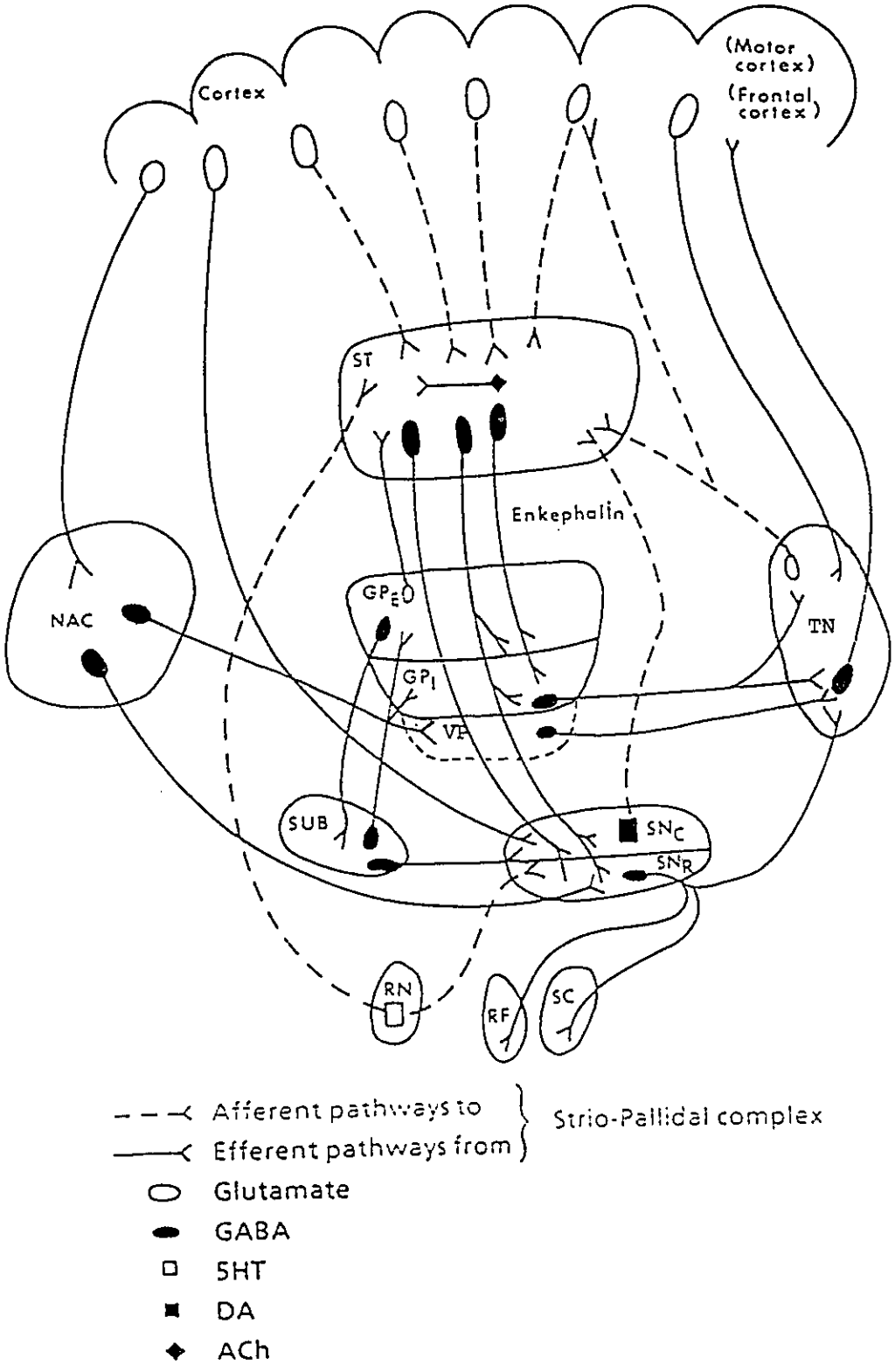

Figure 1.

i. Corticostriatal pathway

Corticostriatal pathway compose the major input to the striatum and utilize the excitatory amino acid transmitter glutamate (Kim et al., 1977; McGeer et al., 1997). Almost all major cortical areas project upon the striatum. The corticostriatal pathway is topographically organized. The frontal lobe, including the sensorimotor areas, projects to the anterior striatum, the parietal cortex to the midportion of the striatum, and the occipital cortex to the most posterior regions of the striatum (Webster, 1961; Kemp and Powell, 1970; Garcia-Rill et al., 1979).

ii. Thalamic projection

The thalamic projection to the striatum is excitatory and glutamatergic in nature (Semba et al., 1987). The striatum receives a major afferent projection from the intralaminar complex of the thalamus (Jones and Leavitt, 1974). Stimulation of the centromedian-parafascicular complex, the major source of thalamostriatal projection (Jones and Leavitt, 1974), produces excitation of caudate neurons (Purpura and Malliani, 1967). Thalamostriatal neurons also send collaterals to the neocortex (Jones and Leavitt, 1974). Neurons which project to the striatum from the thalamus have been shown to contain substance P, vasoactive intestinal peptide, cholecystokinin and neurotensin (Sugimoto et al., 1985).

iii. Nigrostriatal pathway

Afferents from the substantia nigra utilize dopamine as a neurotransmitter and arise from cell bodies located in the SNC (Bjorklund and Hokfelt, 1984). Using tyrosine hydroxylase (TH)-immunoreactivity as a marker for dopaminergic fibres (Pickel et al., 1981), TH-positive terminals have been observed to make contact with three morphologically distinct neurons in the striatum. The majority of striatal neurons which receive TH-immunoreactive terminals conform to the medium spiny I neuronal classification of Pasik et al. (1979). TH-immunoreactivity terminals have also been reported to form synaptic contact with short-axoned medium spiny neurons (Yohiyuki et al., 1987) that are believed to be striatal interneurons (Groves, 1983). The third type of cell that receives TH-positive terminals is a large neuron (Yohiyuki et al., 1987) that is similar to the spiny II type neuron described by Pasik et al., (1979).

D. Striatal Neurochemical Heterogeneity

Based on neurochemical grounds, the striatum has two interdigitating compartments (Gerfen, 1992; Pert et al., 1976). The patch compartment is defined by low levels of acetylcholinesterase immunoreactivity and high levels of μ opiate binding or compartments of high met-enkephalin-, dynorphin-B- and substance P-like immunoreactivity (Graybiel et al., 1981). The second compartment, the matrix, displays high acetylcholinesterase immunoreactivity and low binding or immunoreactivity for

opiate peptides (Herkenham and Pert, 1981). Anatomical studies have determined that the input-output connectivity of the striatum respects patch/matrix boundaries. Afferents from the sensory and motor cortices and the intralaminar thalamus project to the matrix compartment, whereas afferents from the frontal cortex, basolateral amygdala, and midline thalamus project to the patch (Herkenham et al., 1981; Donoghue and Herkenham, 1986). These arrangements implicate the matrix compartment in functions related to sensorimotor processing and the patch compartment in affective components of behaviour. Calbindin immunoreactivity can also be used to define the patch (calbindin-poor) and matrix (calbindin-rich) compartments of the striatum. Deutch et al. (1992) have reported that haloperidol produces a comparable elevation of Fos-like immunoreactivity in both these striatal compartments. Consequently, because of its close association with motor function, induction of Fos-like immunoreactivity in the matrix by haloperidol might contribute to the development of extrapyramidal side effects. Consistent with this proposal, clozapine, an antipsychotic with a low propensity for producing extrapyramidal side effects, increases Fos-like immunoreactivity primarily in the patch compartment (Deutch et al., 1992).

E. Ventral Striatum

i. Ventral striatum

As mentioned earlier, the striatum can be broadly divided into dorsal and ventral

sectors on the grounds of a variety of anatomical and behavioral criteria. The ventral striatum has been implicated in the control of affective and motivational aspects of behaviour and is composed of the nucleus accumbens and olfactory tubercle. The nucleus accumbens, which refers to an anterior, ventromedial part of the striatum that surrounds the bottom of the anterior horn of the lateral ventricle and extends dorsally into the lateral part of the septum, has been proposed as an interface between the "limbic" system and the "extrapyramidal" motor system (Mogenson et al., 1980; Mogenson and Yim, 1981; Nauta and Domesick, 1984; Nauta, 1986). A large number of studies suggest that the nucleus accumbens, the major structure of the ventral striatum, plays a key role in motivational behaviour (Mogenson, 1987; Wise, 1987). Considering these important functional and clinical connotations, the accumbens is becoming a widely known part of the mammalian basal forebrain. Despite much effort, however, the exact functional role of the nucleus accumbens remains elusive.

Historically, there has been some uncertainty as to whether the nucleus accumbens belongs to the septal system or the basal ganglia, however, most investigators now consider it a specialized part of the striatum (Chronister and DeFrance, 1981; White, 1981). The special status of the nucleus accumbens within the striatal complex is evident in many ways. For example, like the rest of the striatum, the accumbens is also characterized by a "patch-matrix" compartmentation (Herkenham et al., 1984; Voorn et al., 1989; Zahm and Heimer, 1988).

Based on the distribution of a number of neuropeptides and other neuroactive substances, including cholecystokinin, angiotensin II, and neurotensin, the nucleus

accumbens can be subdivided into shell and core regions. Like the dorsal striatum, both the core and shell are composed primarily of medium spiny neurons, which use GABA as a transmitter.

The entire accumbens, like the rest of the striatum, receives afferents from the cerebral cortex, mesencephalic dopamine neurons and intralaminar thalamic nuclei. The most prominent projections of the accumbens are directed at the pallidum (ventral pallidum) and midbrain (substantial nigra-ventral tegmental area). The core projects to the dorsolateral part of ventral pallidum (VP), whereas the shell region projects to the ventromedial part of VP (Heimer et al., 1991). However, the shell region, in addition to projecting to the VP and midbrain (substantia nigra-ventral tegmental area), also projects to the lateral hypothalamus and the sublenticular part of the extended amygdala, bed nucleus of stria terminalis and centromedial amygdala (Heimer et al., 1991). Furthermore, studies by Meredith et al. (1992) suggest that core neurons have a greater potential for collecting synaptic information than have shell neurons, because striatomesencephalic neurons in the shell have fewer dendritic arbours and fewer terminal segments than those in the core.

ii. Dorsal vrs ventral striatum

The ventral striatum can be distinguished from the dorsal striatum on the basis of differences in their efferent and afferent connections. For instance, unlike the dorsal striatum, the nucleus accumbens receives telencephalic connections from the

hippocampus, amygdala, medial prefrontal and entorhinal cortices (Groenewegan et al., 1987; Kelley and Domesick, 1982; McGeorge and Fuall, 1989). Furthermore, dopaminergic neurons which innervate the nucleus accumbens originate largely in the ventral tegmental area, whereas those which project to the dorsal striatum are located principally in the SNC (Ungerstedt, 1971). Lastly, although both the dorsal striatum and nucleus accumbens send projections to the pallidum and midbrain, the nucleus accumbens also projects to a variety of limbic nuclei which include the medial amygdala, bed nucleus of stria terminalis, sublenticular substantial innominata and lateral hypothalamus (Conrad and Pfaff, 1976; Nauta et al., 1978; Swanson and Cowan, 1975).

5. Dopaminergic Regulation of Basal Ganglia Function

A. Dopamine Receptor Subtypes

Initially, it was proposed that there were two distinct types of dopamine receptors in order to explain the opposite effects of dopamine on adenylate cyclase activity in the parathyroid and pituitary glands (Kebabian and Calne, 1979). The parathyroid gland was considered to contain just the D1 dopamine receptor while the pituitary was proposed to contain only the D2 receptor. Since dopamine activated adenylate cyclase activity in the parathyroid, the D1 receptor was said to be linked positively to adenylate cyclase activity. In contrast, dopamine inhibited adenylate cyclase activity in the pituitary suggesting that the D2 receptor was linked negatively to this enzyme. This theory was strengthened

considerably by the development of selective compounds for the D1 and D2 receptor. The benzazepine derivative SCH 23390 is a selective D1-like receptor antagonist (D1-like antagonist) while SKF 38393 and CY 208-243 preferential D1-like receptor agonists (D1-like agonists). Raclopride is a selective D2-like receptor antagonist (D2-like antagonist) while quinpirole is a selective D2-like receptor agonist (D2-like agonist) (Paul et al., 1992). Using radiolabelled versions of these compounds it has been possible to study the distribution of D1 and D2 receptors in the brain by autoradiography.

The development of molecular cloning techniques has revealed the existence of 5 distinct dopamine receptor subtypes termed D1, D2, D3, D4 and D5. On the basis of structure and pharmacological similarities these five receptors have been categorized as either D1- or D2-like receptors. The D1 and D5 receptors are called D1-like receptors because of their similar amino acid composition and high affinity for the benzazepine analog SCH 23390. The D2, D3 and D4 receptors are termed D2-like receptors because they are structural alike and all have high affinity for antipsychotic drugs.

B. Localization of D1 and D2 Receptors in the Basal Ganglia

Initially, the distribution of D1 and D2 receptor binding sites in the forebrain was mapped autoradiographically using radiolabelled ligands selective for the D1 and D2 receptor. These studies have demonstrated that D1 receptor binding sites are concentrated in the striatum, nucleus accumbens, olfactory tubercle, and substantia nigra pars reticulata while lower levels are present in the amygdala, cortex and thalamus. D2 receptor binding

sites are found in nearly all areas of the brain that receive dopaminergic innervation including dopamine neurons themselves. Although there is considerable overlap between the topographical distribution of D1 and D2 receptors in the basal ganglia some differences also exist. For instance, in striatum, D2 binding decreases more than D1 receptor binding in the rostrocaudal direction (Altar et al., 1985; Boyson et al., 1986). In addition, D2 receptor binding shows a greater increase in the medial-to-lateral axis of the striatum than D1 binding (Boyson et al., 1986). In the substantia nigra, there is also a remarkable divergence in the densities of the dopamine receptor subtypes. In the pars compacta, the density of D1 receptors is as high as in the striatum while the density of D2 receptors is approximately 30% of that in the striatum, yielding a D1/D2 receptor ratio of 11:1. In the pars reticulata of the substantia nigra, the density of D1 receptors is similar to that in the compacta, but the density of D2 receptors is very low, yielding a ratio of D1 to D2 receptors of 36:1 (Boyson et al., 1986). Taken together, these results suggest that while D1 and D2 receptors are located in the same regions they may not always be located on the same neurons.

The advent of molecular cloning techniques have permitted the cellular localization of mRNA encoding D1 and D2 receptors by *in situ* hybridization histochemistry. Unlike receptor autoradiography which cannot distinguish between binding sites located on the cell bodies and terminals of neurons, *in situ* hybridization detects mRNA that is generally confined to the cell body. Thus, it has been possible to determine not only the distribution but also the degree of overlap between D1 and D2 receptors in the basal ganglia. For example, D1 receptor mRNA is located mainly in striatonigral neurons

(Gerfen and Young, 1988; Gerfen et al., 1990) whereas D2 receptor mRNA is found predominantly in striatopallidal neurons (Gerfen and Young, 1988; Le Moine et al., 1990; Gerfen et al., 1990). The majority of dopaminergic cell groups express D2 but not D1 mRNA suggesting that D2 receptors likely function as presynaptic receptors on most dopaminergic neurons (David et al., 1991). In the globus pallidus, endopeduncular nucleus and substantia nigra only D2 mRNA was observed. These results suggest that D1 receptors found in the substantia nigra by receptor autoradiography are located on the terminals of the striatonigral pathway. The location of D1 and D2 dopamine receptors in the basal ganglia is shown in Fig. 2.

C. Regulation of Basal Ganglia Activity by D1 and D2 Dopamine Receptors

Using selective dopamine agonists, it has been established that D1-, but not D2-like agonists increase Fos-like immunoreactivity in the 6-OHDA-denervated striatum (Robertson GS et al., 1989). D1-like agonists have minor effects on Fos-like immunoreactivity in the striatum of intact animals, however, produce large increases in Fos-like immunoreactivity after dopaminergic denervation due to the development of D1 receptor supersensitivity (Robertson GS et al., 1989; Robertson HA et al., 1989). In contrast, dopaminergic stimulants, such as cocaine and *d*-amphetamine, increase expression of *c-fos* and other IEGs in both striata of normal animals and in the intact striatum of animals with unilateral 6-OHDA lesions (Moratalla et al., 1992; Robertson HA, 1989; Cole et al., 1992). Although selective D2-like agonists fail to increase *c-fos*

Figure 2. Schematic representation of the anatomical localization of D1 and D2 receptors in the rat nigrostriatal system. (a) Although controversial, D2 receptors appear to be located on the terminals of glutamatergic fibers of the corticostriatal pathway. (b) D2 receptors appear to be located presynaptically on the dopaminergic fibers of the nigrostriatal pathway. (c), (d) D1 and D2 receptors are found on distinct populations of intrinsic striatal neurons. (e) D2 receptors are located on the cell bodies of the dopaminergic neurons of the nigrostriatal pathway. (f) The majority of D1 receptors in the SNR are located on the terminals of the striatonigral pathway. (g) In the GP, D2 receptors are also located on the neuron terminals of the striatopallidal pathway.

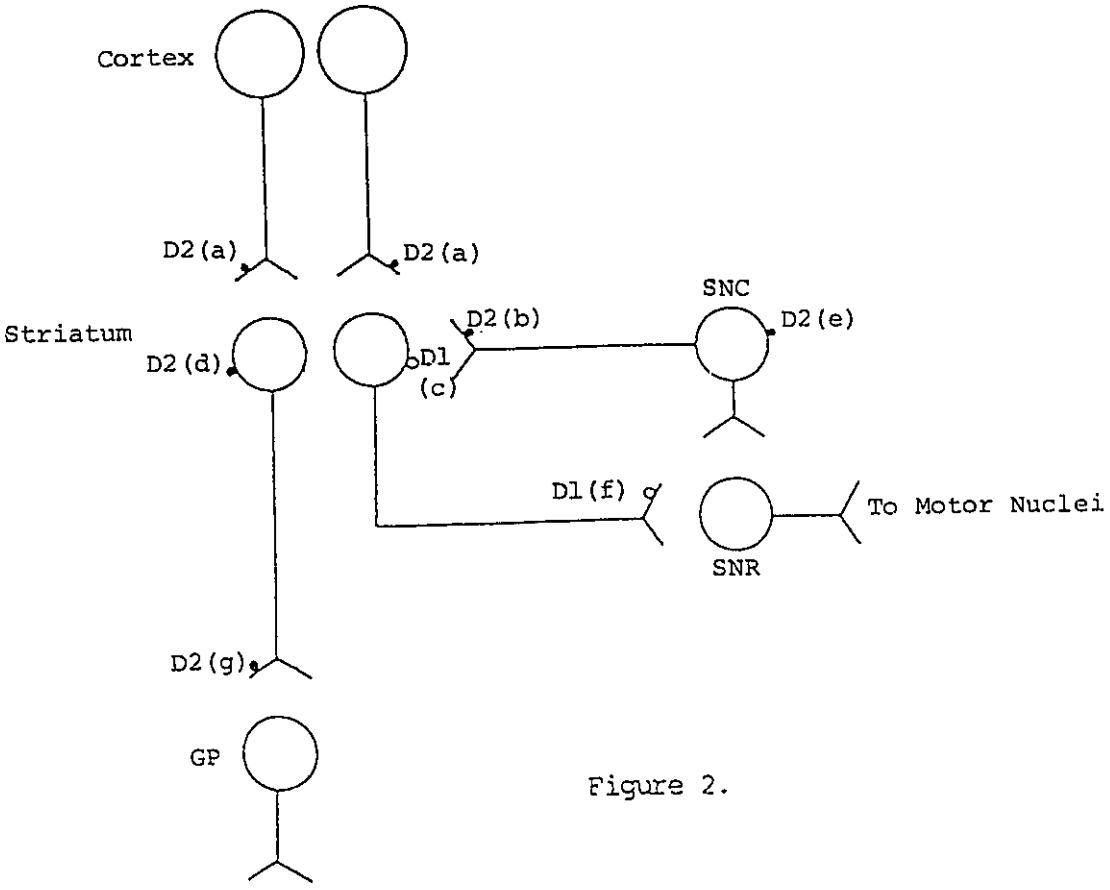
Cortex
D2(a)
D2(a)
SNC
Striatum
D2(b)
D2(e)
D2(d)
D1
(c)
D1(f)
To Motor Nuclei
SNR
D2(g)
GP

Figure 2.

expression in the striatum, coadministration of D1- and D2-like agonists produces a synergistic induction of *c-fos* (Paul et al., 1992).

D1-like agonist-induced *c-fos* expression appears to be confined to neurons projecting to the substantia nigra (Robertson GS et al., 1990; Robertson GS et al., 1992). D2-like agonists that have no effect on *c-fos* expression in the striatum but produce some activation of this IEG in the globus pallidus (Paul et al., 1992; Robertson GS et al., 1992). The distinct effects of D1- and D2-like agonists on *c-fos* expression in the striatum and globus pallidus suggests that these compounds may act on separate populations of striatal neurons. Indeed, the notion was originally proposed by Herrera-Marschitz and Ungerstedt (1984) that D1- and D2-like agonists elicit effects via different efferent pathways leading from the striatum. That is, D1-like agonists facilitate the striatonigral pathway, whereas D2-like agonists inhibit the striatopallidal pathway (Herrera-Marschitz and Ungerstedt, 1984).

The proposal that D1 receptors reside principally on striatonigral neurons and D2 receptors are located predominantly on striatopallidal neurons was based on the results of lesion studies (Beckstead, 1988; Harrison et al., 1990). Since then, *in situ* hybridization histochemistry studies have confirmed this arrangement by demonstrating that D1 receptor and substance P mRNAs are coexpressed by striatonigral neurons, whereas D2 receptor and enkephalin mRNAs are coexpressed by striatopallidal neurons (Gerfen et al., 1990; Le Moine et al., 1990; Le Moine et al., 1991). This organization suggests that D1-like dopamine receptor agonists should elevate *c-fos* expression, in the 6-OHDA-denervated striatum, largely in striatonigral neurons. Similarly, D2-like receptor

antagonists would be expected to increase Fos-like immunoreactivity predominantly in striatopallidal neurons. In order to test these predictions, striatonigral and striatopallidal neurons were labelled by injection of the retrograde tracer Fluoro-Gold (FG) into the substantia nigra and GP, respectively (Robertson GS et al., 1990; Robertson GS et al., 1992). Consistent with the largely separate localization of D1 and D2 receptors in the striatum, D1-like agonists preferentially increased Fos-like immunoreactivity in striatonigral neurons retrogradely labelled from the substantia nigra, whereas haloperidol elevated Fos-like immunoreactivity principally in striatopallidal neurons (Robertson GS et al., 1990; Robertson GS et al., 1992). To further verify that D1-like agonists and D2-like antagonists increase Fos-like immunoreactivity in different striatal efferents, Fos immunohistochemistry was combined with *in situ* hybridization histochemistry. D2-like antagonists frequently increased Fos-like immunoreactivity in striatal neurons labelled with an oligonucleotide probe complementary to mRNA encoding enkephalin (Robertson GS et al., 1992). In contrast, the D1-like agonist CY 208-243, did not elevate Fos-like immunoreactivity in neurons of the 6-OHDA-denervated striatum labelled with the enkephalin probe (Robertson GS et al., 1992). Taken together, these results suggest that the differential regulation of striatonigral and striatopallidal neurons by dopamine is mediated by the separate localization of D1 and D2 receptors on these outputs.

Dopamine-depletion can alter the levels of peptide mRNAs expressed by striatal output neurons. Dopamine depletion in the striatum results in increased enkephalin mRNA expression in striatopallidal neurons and decreased expression of dynorphin and substance P mRNA in striatonigral neurons (Gerfen et al., 1990; Young et al., 1986). Subsequent

to dopamine-depleting lesions, agonist treatment that is selective for the D2 receptor reverses the lesion-induced increase in enkephalin in striatopallidal neurons, whereas D1-like receptor agonists reverse the lesion-induced reduction of substance P and dynorphin mRNA levels in striatonigral neurons (Gerfen et al., 1990). These results suggest that the opposite effects of dopamine on neuropeptide gene expression in striatonigral and striatopallidal neurons is mediated by the largely separate localization of D1 and D2 receptors on these outputs. In agreement with these neurochemical findings, electrophysiological studies indicate that, after 6-OHDA lesions of the nigrostriatal pathway, striatopallidal neurons become more active, while striatonigral neurons are less active, which parallels the increases and decreases in gene expression in striatonigral and striatopallidal neurons (Pan et al., 1985; Pan and Walters, 1988).

6. Dopaminergic Regulation of *c-fos* Expression

Alterations in dopaminergic neurotransmitters produce long term changes in behaviour. For example, enduring changes in behaviour occur following exposure to psychostimulant drugs such as cocaine and amphetamine, which affect dopaminergic and other monoaminergic systems. These behavioral changes can occur after only a few exposures to the drug and can persist for many years (Koob and Bloom, 1988). How does dopamine exert such long-term effects? A possible answer to this question comes from the discovery that dopaminergic stimulants induce IEG expression in forebrain neurons thought to mediate the behavioral effects of these drugs (Robertson GS et al., 1989;

Robertson HA et al., 1989). These IEGs encode transcriptional activating (or inhibiting) factors that then alter the expression of other so called later response genes. Thus, by altering neuronal gene expression, IEGs may contribute to those changes which mediate the long lasting behavioral effects of cocaine and *d*-amphetamine.

A. Activation of *c-fos* by Direct- and Indirect-Dopaminergic Agonists

As mentioned above, directly acting dopamine agonists have minor effects on *c-fos* expression in the intact animal. However, destruction of the nigrostriatal pathway by injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) which results in supersensitive dopamine receptors endows direct agonists such as apomorphine with the ability to dramatically enhance Fos-like immunoreactivity in the denervated striatum. Using agonists selective for either D1- or D2- like receptors, it has been demonstrated that only D1-like agonists elevate Fos-like immunoreactivity in the deafferentated striatum. In 6-OHDA lesioned rats, L-Dopa and the D1-like selective agonist CY 208-243 produce a rapid increase in the production of Fos-like immunoreactivity in medium-sized neurons throughout the caudate-putamen (Robertson GS et al., 1989; Robertson HA et al., 1989). Fos-like immunostaining is seen after as little as 30 minutes and persists for 6-10 hours. In many systems, the Fos protein is only present for 2-4 hour; whereas Fos-related antigens have longer induction kinetics. Fos-related antigens of undetermined functions may therefore account for the immunostaining seen at longer survival times.

There is also a clear relationship between the degree of receptor supersensitivity and *c-fos* activation (Robertson GS et al., 1989). Unilateral destruction of the nigrostriatal pathway with 6-OHDA results in a postural asymmetry that is exaggerated into circling by the administration of dopamine agonists. This rotational behaviour can be used to assess the degree of supersensitivity of the dopamine receptors. Animals with incomplete lesions do not turn as rapidly as those with complete lesions. Similarly, animals with incomplete lesions show little or no Fos-like immunostaining in the denervated striatum while those which turn rapidly exhibit dramatic staining for Fos-like immunoreactivity. Infusion of D1-like agonists directly into the striatum of normal rats has little effect on *c-fos* expression. However, if the animals have been depleted of striatal dopamine by 6-OHDA lesions, such direct infusions of D1-like agonists produce robust *c-fos* activation (Robertson HA et al., 1990; Robertson GS et al., 1992).

Indirect dopamine agonists such as *d*-amphetamine and cocaine, which act to increase levels of endogenous dopamine, also activate the *c-fos* gene. However, indirect dopamine agonists can only elevate *c-fos* expression if there is an intact nigrostriatal pathway. Destruction of the nigrostriatal pathway dramatically reduces the ability of *d*-amphetamine and cocaine to elevate Fos-like immunoreactivity in the denervated striatum. This is in contrast to directly acting dopamine agonists which require the development of postsynaptic supersensitivity to elevate Fos-like immunoreactivity in the striatum (Robertson HA et al., 1989; Johnson et al., 1989; Young et al., 1989; Graybiel et al., 1990).

B. Activation of *c-fos* by Dopaminergic Antagonists

The initial observation that the dopamine precursor L-dopa increased *c-fos* expression in the striatum ipsilateral to a 6-OHDA lesion of the nigrostriatal pathway led to the exploration of the effects of dopaminergic antagonists on Fos-like immunoreactivity in the basal ganglia. A single injection of the prototypical antipsychotic haloperidol was first shown to dramatically elevated Fos-like immunoreactivity in the striatum by Dragunow et al. (1990). Several lines of evidence indicated that this increase was mediated by the blockade of dopamine-D2-like receptors. First, selective D2-, but not D1-like antagonists mimicked haloperidol's ability to increase *c-fos* expression in the striatum (Robertson GS et al., 1992; Dragunow et al., 1990; Robertson GS and Fibiger, 1992). Second, the selective D1-like antagonist SCH-23390 was unable to reduce haloperidol-induced Fos-like immunoreactivity in the striatum. This is particularly important because it is well known that haloperidol increases striatal dopamine release by antagonizing inhibitory D2-like receptors on nigrostriatal neurons (Westerink and De Vries, 1989). Consequently, the failure of SCH 23390 to block haloperidol-induced Fos-like immunoreactivity rules out the possibility that haloperidol increased striatal *c-fos* expression indirectly by enhancing the stimulation of D1-like receptors. Third, related studies have shown that administration of the D2-like agonist quinpirole attenuates the increase in striatal *c-fos* expression produced by haloperidol (Miller, 1990). Fourth, depletion of striatal dopamine, by destruction of the nigrostriatal pathway, abolishes haloperidol-induced Fos-like immunoreactivity (Robertson GS et al., 1992). This

indicates that haloperidol-induced striatal *c-fos* expression is dopamine dependent. Lastly, direct application of haloperidol into the striatum, via a microdialysis membrane, increased Fos-like immunoreactivity in the vicinity of the probe (Robertson GS et al., 1992). Hence, the blockade of local D2-like receptors in the striatum is at least partly responsible for the ability of haloperidol to elevate Fos-like immunoreactivity in the striatum.

7. Research Objectives

A. Distribution of D1-Like Agonist-Induced Fos_{2-16}-Like Immunoreactivity in the Forebrain of 6-OHDA-Lesioned Animals

Expression of D1 receptors have been examined in the rat brain by receptor autoradiography, immunohistochemistry and *in situ* hybridization. The main areas in the forebrain which contain D1 receptors are the prefrontal, piriform and cingulate cortices and subcortically, the caudate-putamen, nucleus accumbens, amygdala, septal area and olfactory tubercle. In the present experiment, using the selective D1-like agonist CY 208-243 to induce Fos_{2-16}-like immunoreactivity, the distribution of D1-like agonist-induced Fos_{2-16}-like immunoreactivity was examined in the forebrain of 6-OHDA-lesioned rats. Mediation by D1-like dopamine receptors was established by pretreatment with the D1-like receptor antagonist SCH 23390.

B. Localization of $Fos_{128-152}$-Like Immunoreactivity in the 6-OHDA Denervated Striatum

It has been shown that cocaine and *d*-amphetamine, stimulants which enhance extracelluar concentrations of dopamine, increase Fos-like immunoreactivity in striatonigral neurons, whereas antipsychotic drugs such as haloperidol-induce Fos-like immunoreactivity principally in striatopallidal neurons. Destruction of the nigrostriatal pathway has recently been reported to produce a long-lasting and widespread increase in the expression of striatal $Fos_{128-152}$-like immunoreactivity detected with an antibody raised against the DNA binding region of the Fos molecule (amino acids 128-152), a region which is highly conserved among members of the *fos* family (Cohen and Curran, 1988; Nishina et al., 1990; Dragunow et al., 1991). Since neurochemical as well as electrophysiological studies have indicated that striatopallidal neurons become more active while striatonigral neurons are less active after 6-OHDA lesions of the nigrostriatal pathway (Pan and Walters, 1988), $Fos_{128-152}$-like immunoreactivity produced by dopaminergic denervation would be expected to be expressed principally by striatopallidal neurons as a consequence of the persistent increase in excitatory transmembrane signalling which occurs in these neurons after dopamine depletion. Hence, to verifying this hypothesis, the present study used the fluorescent retrograde tracer FG to examine the nature of striatal neurons which express $Fos_{128-152}$-like immunoreactivity, 6 weeks after a 6-OHDA lesion of the nigrostriatal pathway. Striatonigral and striatopallidal neurons were retrogradely labelled by injection of FG into the SNR and GP, respectively, ipsilateral to a 6-OHDA lesion of the MFB. Then, by observing the overlaps between

$Fos_{128-152}$-like immunoreactivity nuclei and retrogradely labelled striatonigral or striatopallidal neurons, the nature of striatal neurons which express $Fos_{128-152}$-like immunoreactivity was determined.

C. Localization of Dopamine Agonist- and Antagonist-Induced Fos_{2-16}-Like Immunoreactivity in the Nucleus Accumbens

It has been shown that alterations in dopaminergic neurotransmission dramatically increase neuronal expression of Fos in the nucleus accumbens and dorsal striatum (Carney et al., 1991; Dragunow et al., 1991; Dragunow et al., 1990; Graybiel et al., 1990; Robertson HA et al., 1989). For example, dopaminergic stimulants, Cocaine and *d*-amphetamine, elevate striatal Fos-like immunoreactivity by increasing D1-like dopamine receptor activation (Bahat and Baraban, 1993; Berretta et al., 1992; Cole et al., 1992; Graybiel et al., 1990; Young et al., 1991). In contrast, antipsychotics such as haloperidol and raclopride elevate *c-fos* mRNA and Fos-like immunoreactivity in the striatum by blocking D2-like dopamine receptors (Deutch et al., 1992; Hope et al., 1992; Nguyen et al., 1992; Robertson GS and Fibiger, 1992; Roger et al., 1993). Retrograde tracing studies have revealed that dopaminergic stimulants and antipsychotics elevate Fos-like immunoreactivity in different populations of neurons in the dorsal striatum. Cocaine and *d*-amphetamine increase Fos-like immunoreactivity predominantly in striatonigral neurons, whereas haloperidol-induced Fos-like immunoreactivity is located principally in striatopallidal neurons (Cenci et al., 1992; Robertson GS et al., 1992). These results are

consistent with studies indicating that D1 receptors are situated chiefly on striatonigral neurons while D2 receptors are located primarily on striatopallidal neurons (Beckstead, 1988; Gerfen et al., 1990; Harrison et al., 1990; Harrison et al., 1992; Le Moine et al., 1990; Le Moine et al., 1991). Furthermore, they suggest that the ability of dopamine to facilitate the activity of striatonigral but inhibit striatopallidal neurons is mediated by the largely separate localization of D1 and D2 receptors on these outputs (Robertson GS et al., 1992; Weick and Walters, 1987).

Similar to the dorsal striatum, the nucleus accumbens shell and core also send projections to the pallidum and midbrain (Berendse et al., 1992; Zahm and Heimer, 1990, Heimer et al., 1991). However, it has not yet been established whether, like the dorsal striatum (Beckstead and Cruz, 1986; Kawaguchi et al., 1990), these projections arise largely from separate populations of accumbal neurons. Moreover, it is not known if there is a differential regulation of Fos expression in these accumbal projections by D1 and D2 dopamine receptors. Accordingly, we have utilized retrograde tracing methods to determine: (A) the degree of collaterlization between accumbal outputs to the midbrain and ventral pallidum; (B) if D1-like agonist-induced Fos-like immunoreactivity is located predominantly in accumbal neurons that project to the midbrain (ventral tegmental area and substantial nigra) and D2-like antagonist-induced Fos-like immunoreactivity is situated preferentially in projections to the ventral pallidum; and (C) whether these relationships were the same in the nucleus accumbens core and shell.

MATERIALS AND METHODS

1. Animals

Adult male Wistar rats, weighing 275-375 g (Charles Rivers, Montréal), were used in all the experiments. Animals were kept in a temperature-controlled environment with 12 hr light/12 hr dark cycle and were given free access to water and laboratory chow.

2. Drugs and Immunoreagents

Drugs and immunoreagents used in this thesis are listed below with the respective source, vehicle and the solution used to dilute each immunoreagent:

DRUG OR NEUROTOXIN	SUPPLIER	VEHICLE
d-Amphetamine	Du Pont, ONT.,	0.9% saline
Apomorphine-HCL	Sigma Chemical Co.	0.9% saline
Clozapine	Sandoz, Doval, P.Q.	distilled water containing 0.8mg/ml acetic acid
CY 208-243	Sandoz, Doval, P.Q.	0.9% saline containing 0.8mg/ml acetic acid
Desmethylimipramine	Sigma Chemical Co.	0.9% saline
Haloperidol	Research Biochemicals Incorporated	distilled water containing 0.8mg/ml acetic acid
6-Hydroxydopamine hydrogen bromide	Sigma, St. Louis, MO.	0.9% saline containing 0.5 mg/ml ascorbic acid
Pentobarbital	MTC Pharmaceuticals, Cambridge, Ontario.	sodium salt in aqueous propylene glycol base

IMMUNOREAGENT OR TRACER	SUPPLIER	DILUTING SOLUTION
Anti-rabbit Ig, fluorescein linked whole antibody	Amersham, U.K.	0.01M sodium phosphate
Biotin-SP-conjugated AffiniPure F(ab')2 Fragment Donkey Anti-sheep IgG(H+L)	Jackson Immuno Research.	0.01M sodium phosphate
Biotin-SP-Conjugated AffiniPure Donkey Anti-rabbit IgG	Jackson Immuno Research.	0.01M sodium phosphate
Fluorescein-conjugated Affinipure F(ab')2 Fragment donkey-anti-sheep IgG(H+L)	Jackson Immuno Research.	0.01M sodium phosphate
Fluoro-Gold	Fluorochrome Inc.	0.9% saline
Rabbit anti-$Fos_{128-152}$ (Polyclonal)	M.J. Iadarola (NIH, Bethesda, MD)	0.01M sodium phosphate
Normal Donkey Serum	Jackson Immuno Research.	0.01M sodium phosphate
Rabbit Anti-Fluoro-Gold (Polyclonal)	Chemicon International Inc.	0.01M sodium phosphate
Rhodamine-labelled latex beads	Lumafluor Inc., USA	0.9% saline
Sheep anti-Fos_{2-16} (Polyclonal)	Serotec, England	distilled water
Sheep anti-Fos_{2-16} (Polyclonal)	Cambridge, Ontario.	0.01M sodium phosphate
Streptavidin	Amersham Life Science	0.01M sodium phosphate

3. Stereotaxic Surgery

All of the animal surgery was completed with the aid of a stereotaxic apparatus (Kopf Instruments). Stereotaxic surgery was performed with the incisor bar positioned 4.0 mm below the interaural line according to the flat skull coordinate system of Paxinos and Watson (1986). The flat skull coordinate system allowed stereotaxic and histological comparison to the atlas of Paxinos and Watson (1986). For injections into the VP, GP and MFB, the bregmoidal intersection was used as the stereotaxic reference point, whereas interaural zero was used for injections into such midbrain structures as the SNR, ventral tegmental area and SNC.

4. 6-Hydroxydopamine Lesioning Procedure

The nigrostriatal pathway was lesioned by injection of the catecholamine neurotoxin 6-hydroxydopamine into the MFB. In order to protect norepinephrine neurons, desmethylimipramine (25 mg/kg, i.p., Sigma) was given 30 minutes prior to pentobarbital anaesthesia (50 mg/kg, i.p.). The injection needle (30 gauge) was connected with polyethylene (PE 20) tubing to a 10 µl Hamilton syringe that was controlled by a microinjection pump (Harvard Apparatus, Canadian Standards Association). The microinjection pump was programmed to deliver 11.4 µg of 6-hydroxydopamine HBr (Sigma) dissolved in 4 µl of saline containing 0.05% ascorbic acid over a 10 min period. The injection cannula was left in place for an addition 3 min to reduce movement of the toxin up the cannula tract after withdrawal of the 30 gauge needle. The 6-OHDA solution

was kept on ice. The solution was injected into right MFB at the coordinates AP 4.0, ML -1.5 and DV -8.4 from bregma. The animals were allowed a three week recovery period before further treatment.

5. Behavioral Assessment

Behavioral screening began 3 weeks after surgery. On the test day, rats were habituated to the rotation buckets for 20 minutes prior to the injection of apomorphine (0.1 mg/kg, s.c.). Turning usually began 5 min after the apomorphine injection. The rotation rates were recorded by automated rotometers. Animals rotating at least 9 times /minute were used in subsequent studies. Previous studies have shown that such animals have sustained a striatal dopamine depletion greater than 95% relative to the intact side (Hefti et al., 1980).

6. Retrograde Labelling Procedure

In order to determine the nature of striatal neurons that contained Fos-like immunoreactivity, medium sized neurons in the caudate-putamen and nucleus accumbens were retrogradely labelled by injection of fluorescent tracers into the pallidum and midbrain. FG (Fluorochrome Inc.) was used in most of these studies. However, when it was necessary to perform dual tracing experiments, both FG and rhodamine labelled latex beads (RLB) were used. In all cases, a 0.4 µl volume was injected over 5 minutes

using a 5 µl Hamilton syringe.

7. Localization of $Fos_{128-152}$-Like Immunoreactivity in the 6-OHDA Denervated Striatum

A. Retrograde Labelling with Fluoro-Gold

6-OHDA-lesioned rats weighing 325-375 g were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame. Striatonigral neurons were retrogradely labelled by pressure injections of FG into the SNR, whereas striatopallidal neurons were retrogradely labelled by pressure injections of FG into the GP. FG was dissolved in sterile saline (4 mg/100 µl) and injected into the right SNR of four 6-OHDA-lesioned animals using a 5 µl Hamilton syringe at the coordinates (in mm): AP 3.2, ML -2.2, DV 1.7 from interaural zero according to the atlas of Paxinos and Watson (1986). In a second group of animals, FG was injected into the right GP at the coordinates (in mm): AP -0.9, ML -2.8 and DV -5.0 from bregma according to the atlas of Paxinos and Watson (1986). In all cases, a 0.4 µl volume of FG was injected. Five days after the FG injections, all of the animals were deeply anesthetized with pentobarbital (100 mg/kg, i.p.) and perfused with saline (200 ml) followed by 200 ml of phosphate buffered saline (0.1 M) containing 4% paraformaldehyde. Each brain was removed immediately after perfusion and allowed to post fix in fresh fixative for at least 12 hr.

B. Immunohistochemistry

After postfixation, 20 μm coronal sections were cut from the striatum, GP and SNR using a vibratome. Sections cut at the level of the GP and SNR were used to verify the placement of FG injections. Antisera from two different suppliers was used to detect Fos-like immunoreactivity. One was a sheep polyclonal antibody (Cambridge Research Biochemicals, CRB OA-11-823) directed against residues 2-16 of the N-terminal region of Fos. The other antibody was raised in rabbits injected with a synthetic peptide corresponding to amino acids 128-152 of rat Fos (Young et al., 1991). Immunoblot analysis (CRB technical data sheet from batch 2447) indicates that the CRB antibody recognizes Fos_{2-16} as well as several other proteins (at 48/49 and 70 kDa). In addition to Fos, western blot analysis indicates that the antibody directed against amino acids 128-152 recognizes several other proteins (at 25, 35, 41 and 44 kDa) (Young et al., 1991). Hence, the two antibodies appear to recognize different Fos immunoreactive proteins.

In order to control for the performance of the N-terminally directed CRB antibody, sections from an animal which had been injected with haloperidol (2 mg/kg, s.c.) were processed in parallel with those from animals which had sustained unilateral 6-OHDA lesions of the nigrostriatal pathway. Using the CRB antibody, a large increase in haloperidol-induced Fos_{2-16}-like immunoreactivity was observed in the nuclei of striatal neurons (results not shown).

The striatal sections were washed three times (20 min per wash) with 0.01 M phosphate buffered saline (PBS) and incubated for 48 hr with primary antisera (CRB

diluted 1:500). Sections were then washed three times in PBS and incubated for 12 hr in PBS containing the appropriate biotin-labelled secondary antisera (donkey anti-sheep diluted 1:100) and 0.3% Triton X-100. Next, the sections were washed three times with PBS, and incubated for 3 hr in PBS containing Texas red-labelled streptavidin (Amersham; diluted 1:100) and 0.3% Triton X-100. Finally, the sections were washed three times in PBS, mounted and coverslipped with a mixture of glycerol and *d*-phenylenediamine. The slides were observed using a Zeiss fluorescence microscope either under ultraviolet light (340-380 nm) to examine FG, or green light (530-560 nm) to look at Texas red fluorescence.

C. Quantification of the Overlap between $Fos_{128\text{-}152}$-Like Immunoreactivity Nuclei and Retrogradely Labelled Striatal Neurons

For each animal, 2 sections through the head of the caudate-putamen, approximately 0.5 mm rostral to bregma, were selected for quantitative analysis. In each section, one representative area (covering 500 μm^2 of the section) was chosen for study in the medial aspect of the striatum which displayed dense FG and immunoreactive labelling (Fig.3). The number of retrogradely labelled, immunoreactive and double-labelled neurons in each of these areas were counted. Statistical comparisons for these three categories of neurons between GP and SNR FG-injected animals were made using multiple t-tests.

Fig 3. Camera lucid drawing of a representative section used for counting neurons in the medial aspect of 6-OHDA denevated striatum. The box corresponds to an area 500 μm^2 in size, within which the number of neurons retrogradely labelled with Fluoro-Gold (FG) and nuclei displaying $Fos_{128-152}$-like immunoreactivity were counted. In addition, the number of immunoreactive nuclei located in neurons retrogradely labelled with FG from globus pallidus (GP) and substantia nigra pars reticulata (SNR) were counted (number of overlaps).

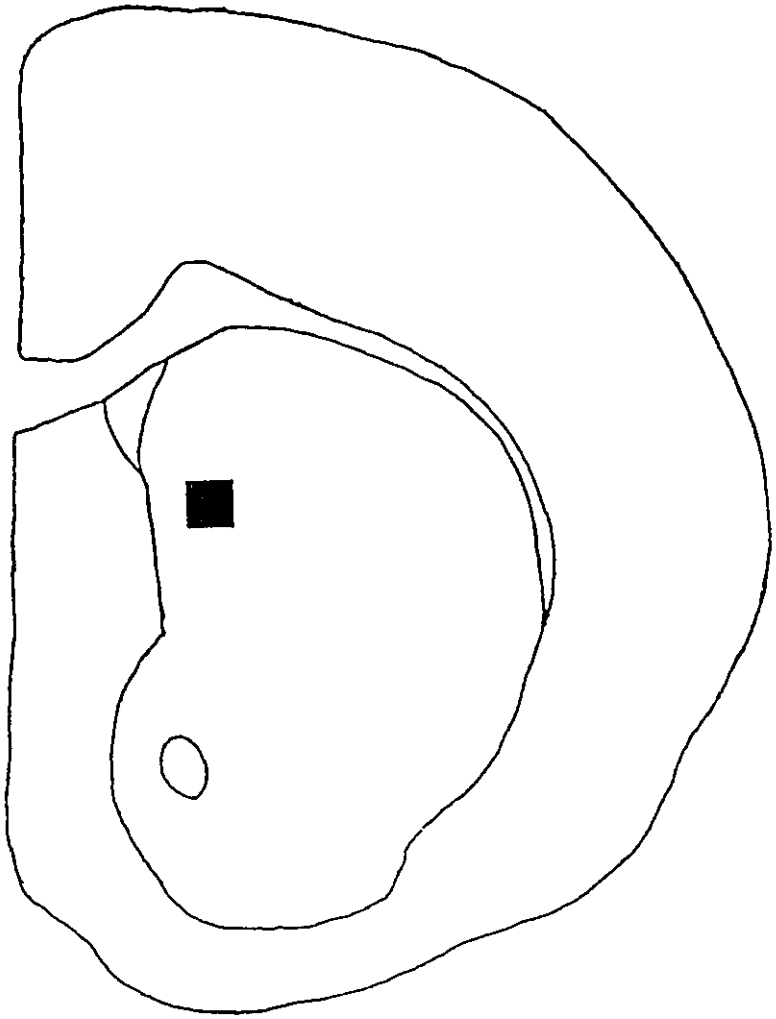

Figure 3.

8. Localization of D1-Like Agonist- and D2-Like Antagonist-Induced Fos_{2-16}-Like Immunoreactivity in Projections of the Nucleus Accumbens

A. Retrograde Labelling with Fluoro-Gold

The animals (275-375 g) used in this study were divided into three groups. The first group of animals which included 12 intact rats and four 6-OHDA-lesioned rats were given FG injections into the midbrain (ventral tegmental area and substantia nigra) in order to retrogradely label neurons of the nucleus accumbens projecting to these sites. The second group of animals which included 12 intact rats and four 6-OHDA-lesioned rats received FG injections into the VP in order to retrogradely label accumbal neurons projecting to this region. All the intact and 6-OHDA-lesioned rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame. In the first group of animals, FG dissolved in sterile saline (4 mg/100 µl) was injected unilaterally into the ventral tegmental area, and then the substantia nigra, using a 5 µl Hamilton syringe at the coordinates (in mm): AP 3.3, ML -1.0, DV 1.4 (ventral tegmental area) and AP 3.8, ML -2.3, DV 2.0 (substantia nigra) from interaural zero according to the atlas of Paxinos and Watson (1986). A 0.4 µl volume of FG was injected into both of these sites. In the second group of animals, FG (0.4 µl) was injected into the right VP at the coordinates (in mm): AP -0.2, ML -2.5, DV -8.5 from bregma according to the atlas of Paxinos and Watson (1986).

B. Retrograde Labelling with Dual Tracers

A third group which included 4 intact animals received injections of FG into the midbrain (ventral tegmental area and substantia nigra) as well as an injection of RLB into the VP. Tracer injections were made under pentobarbital anesthesia into both the VP and midbrain (ventral tegmental area and substantia nigra). RLB were suspended in sterile saline (1:2 dilution) and injected in a 0.4 µl volume with a Hamilton syringe into the right VP at the same coordinates as given above. FG was injected into the right ventral tegmental area and substantia nigra as described above.

C. Experimental Protocol for Drug Studies

Four intact animals which had received injections of FG into either the midbrain (ventral tegmental area and substantia nigra) or VP (first and second groups of animals) were injected subcutaneously (s.c.) with one of the following: haloperidol (2.0 mg/kg; dissolved in 1 ml of distilled water containing 40 µl of 20% acetic acid); *d*-amphetamine (2.5 mg/kg; dissolved in 1 ml of saline); clozapine (20 mg/kg, dissolved in 1 ml of distilled water containing 40 µl of 20% acetic acid). Animals with unilateral 6-OHDA lesions of the MFB (all lesioned rats in first and second groups) were injected with CY 208-243 (0.5 mg/kg, dissolved in 1 ml of saline containing 40 µl of 20% acetic acid). Animal behaviour was observed after drug treatments. Two hours later, all of the animals were deeply anesthetized with pentobarbital (100 mg/kg, i.p.) and perfused with saline

(200 ml) followed by 200 ml of 4% paraformaldehyde in phosphate-buffered saline (0.1 M). Each brain was removed and allowed to postfix in fresh fixative for at least 24 hr.

D. Immunohistochemistry

After postfixation, 20 µm coronal sections were cut from the nucleus accumbens, VP and midbrain (ventral tegmental area and substantia nigra) using a vibratome. Sections cut at the level of the VP and midbrain were used to verify the placement of FG injections. Fos_{2-16}-like immunoreactivity was detected using a sheep polyclonal antibody (Cambridge Research Biochemicals, CRB OA-11-823) directed against residues 2-16 of the N-terminal region of the Fos molecule. For verifying results obtained with CRB antibody, a second sheep polyclonal antibody (Serotec, PEPA 53) which also recognizes amino acids 2-16 of the N-terminal region of Fos was used at the same time. Both antibodies produced similar results.

Immunohistochemistry was performed on sections from the nucleus accumbens using the same procedure as outlined for detection of $Fos_{128-152}$-like immunoreactivity in the 6-OHDA denervated striatum. After immunohistochemical processing, the sections were mounted and coverslipped with a mixture of glycerol and *d*-phenylenediamine. The slides were observed using a Zeiss fluorescence microscope either under ultraviolet light (340-380 nm) to examine FG, or green light (530-560 nm) to look at Texas red fluorescence.

E. Quantification of the Overlap between Fos_{2-16}-Like Immunoreactive Nuclei and Retrogradely Labelled Accumbal Neurons

For each animal, 2 sections through the rostral-caudal middle of the nucleus accumbens, approximately 1.7 mm anterior to bregma according to the atlas of Paxinos and Watson (1986), were selected for quantitative analysis. In each section, two areas (each covering 500 μm^2 of the section) were chosen for study which displayed dense FG and immunoreactive labelling. One area was located in the nucleus accumbens core while the other corresponded to the dorsal aspect of the nucleus accumbens shell (Fig. 4). The number of retrogradely labelled, immunoreactive and double-labelled neurons in each of these areas were counted. Statistical comparisons for these three categories of neurons between VP and midbrain (ventral tegmental area and substantia nigra) FG-injected animals were made using multiple t-tests.

9. Distribution of D1-Like Agonist Induced Fos_{2-16}-Like Immunoreactivity in the Forebrain of 6-OHDA-Lesioned Animals

A. Experimental Protocol

One week after behavioral screening, 6-OHDA-lesioned rats were injected with the D1-agonist CY 208-243 (1 mg/kg, s.c.). In a second group of animals, SCH 23390 (0.1 mg/kg, s.c.) was given 20 minutes prior to CY 208-243 (1 mg/kg, s.c.). Rotational

Fig 4. Camera lucid drawing of a representative section used for counting neurons in the core (open box) and shell (dark box) regions of the nucleus accumbens. Each box corresponds to an area 500 μm^2 in size, within which the number of neurons retrogradely labelled with Fluoro-Gold and nuclei displaying $Fos_{2\text{-}16}$-like immunoreactivity were counted. In addition, the number of immunoreactive nuclei located in neurons retrogradely labelled with FG from the midbrain (substantia nigra and ventral tegmental area) and ventral pallium were counted (number of overlaps). In this figure, the area circled by the line of dashes is the nucleus accumbens core (AcbC) while the dorsal aspect is the nucleus accumbens shell (AcbSh).

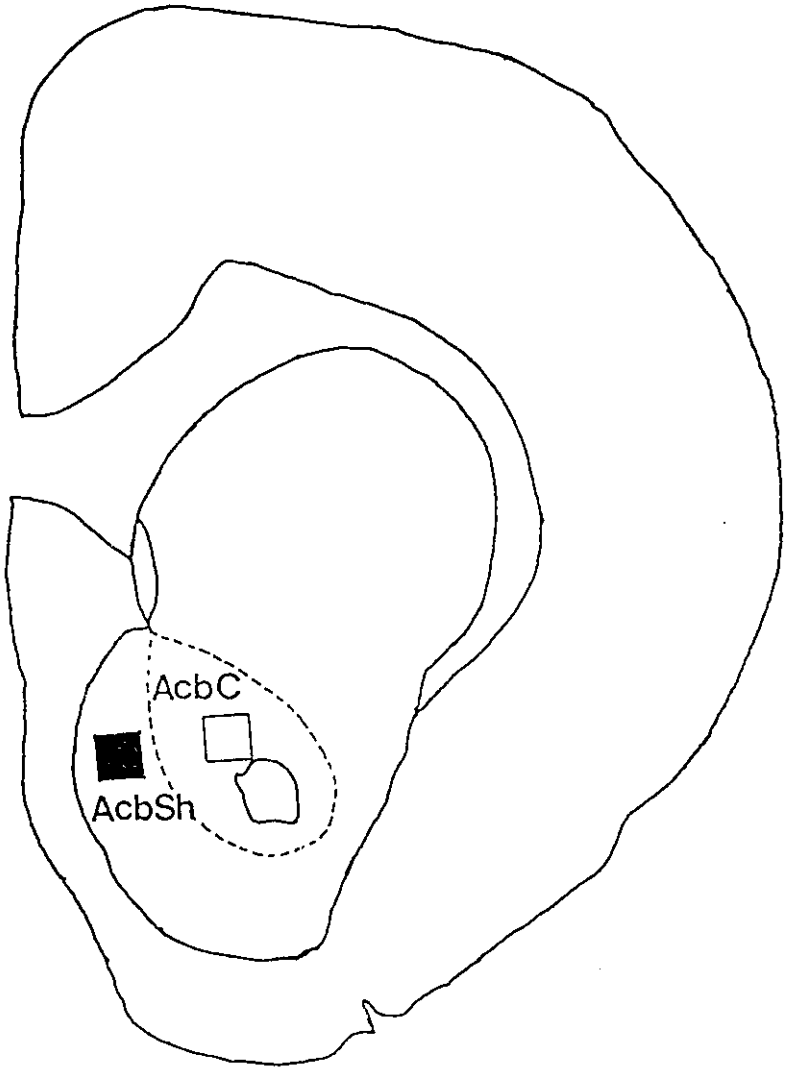

Figure 4.

behaviour was observed after injection of CY 208-243 for 30 minutes. Ninety minutes after CY 208-243, the animals were anesthetized with pentobarbital and perfused with 0.1 M phosphate buffer containing 4% paraformaldehyde. Brains were removed and immersed in the above fixative overnight. Immunohistochemistry was performed on 35 μm sections cut from various regions of the forebrain with a vibratome.

B. Fos_{2-16}-Like Immunohistochemistry

Fos_{2-16}-like immunoreactivity was detected by a glucose oxidase-3,3'-diaminobenzidine (DAB)-nickel method previously described by Shu et al.. Briefly, sections were washed one time with PBS for 10 min, then incubated in PBS containing 0.3% H_2O_2 (in 0.01 M PBS) for 10 min. Sections were then washed three times (10 min per wash) in PBS and incubated with primary antibody (a sheep polyclonal antibody; Cambridge Research Biochemicals, CRB OA-11-823) at a dilution of 1:2500 for 48 hr. Sections were again washed 3 times with PBS (10 min per wash) and incubated with biotinylated donkey anti-sheep (diluted 1:200) for 3 hr. After washing the sections three times in PBS, they were incubated with PBS containing 0.3% Triton X-100 and 1% horseradish peroxidase-avidin for 2 hr. Then the sections were rinsed three times in PBS, one time in 0.1 M acetate buffer and immersed in the chromogen diaminobenzidine for 5 min. Addition of glucose oxidase to the mixture permitted visualization of the hydrogen peroxidase reaction. After 2 washes in 0.1 M acetate buffer, sections were mounted on slides, defatted in alcohol and xylene. Lastly they were coverslipped with permount and examinated under the microscope.

RESULTS

1. Distribution of D1-Like Agonist Induced Fos_{2-16}-Like Immunoreactivity in the Forebrain of 6-OHDA-Lesioned Animals

Administration of the selective D1-like receptor agonist CY 208-243 (1.0 mg/kg, s.c.) to animals with unilateral 6-OHDA lesions resulted in rotational behaviour directed away from the side of the lesion. The D1-like agonist produced a dramatic increase in Fos_{2-16}-like immunoreactivity in the lesioned side of the forebrain particularly in the ipsilateral parietal cortex, striatum, nucleus accumbens, amygdala, lateral septal nucleus and olfactory tubercle (Fig. 5,6,7,8,9). By comparison, there was little D1-like agonist-induced Fos_{2-16}-like immunoreactivity in these structures in the intact side (Fig. 5,6,7,8,9). Pretreatment with the D1-like antagonist SCH 23390 (0.2 mg/kg, s.c.) abolished circling and all increases in Fos_{2-16}-like immunoreactivity produced by CY 208-243 (1.0 mg/kg, s.c., Fig. 5,6,7,8,9).

2. Localization of $Fos_{128-152}$-Like Immunoreactivity in the 6-OHDA Denervated Striatum

A. FG Injection Sites in the SNR and GP

Striatonigral and striatopallidal neurons were retrogradely labelled in separate groups of animals by injection of FG into the SNR and GP, respectively. Examination of the

Figure 5. Photomicrographs of CY 208-243-induced Fos_{2-16}-like immunoreactivity in the parietal cortex of a 6-OHDA lesioned rat. a. CY 208-243 had minor effects on Fos_{2-16}-like immunoreactivity in the parietal cortex on the intact side. b. CY 208-243 produced a dramatic increase in Fos_{2-16}-like immunoreactivity in the parietal cortex ipsilateral to the 6-OHDA lesion. c. Pretreatment with SCH 23390 abolished the induction of Fos_{2-16}-like immunoreactivity in the parietal cortex produced by CY 208-243. Scale bar = 250μm

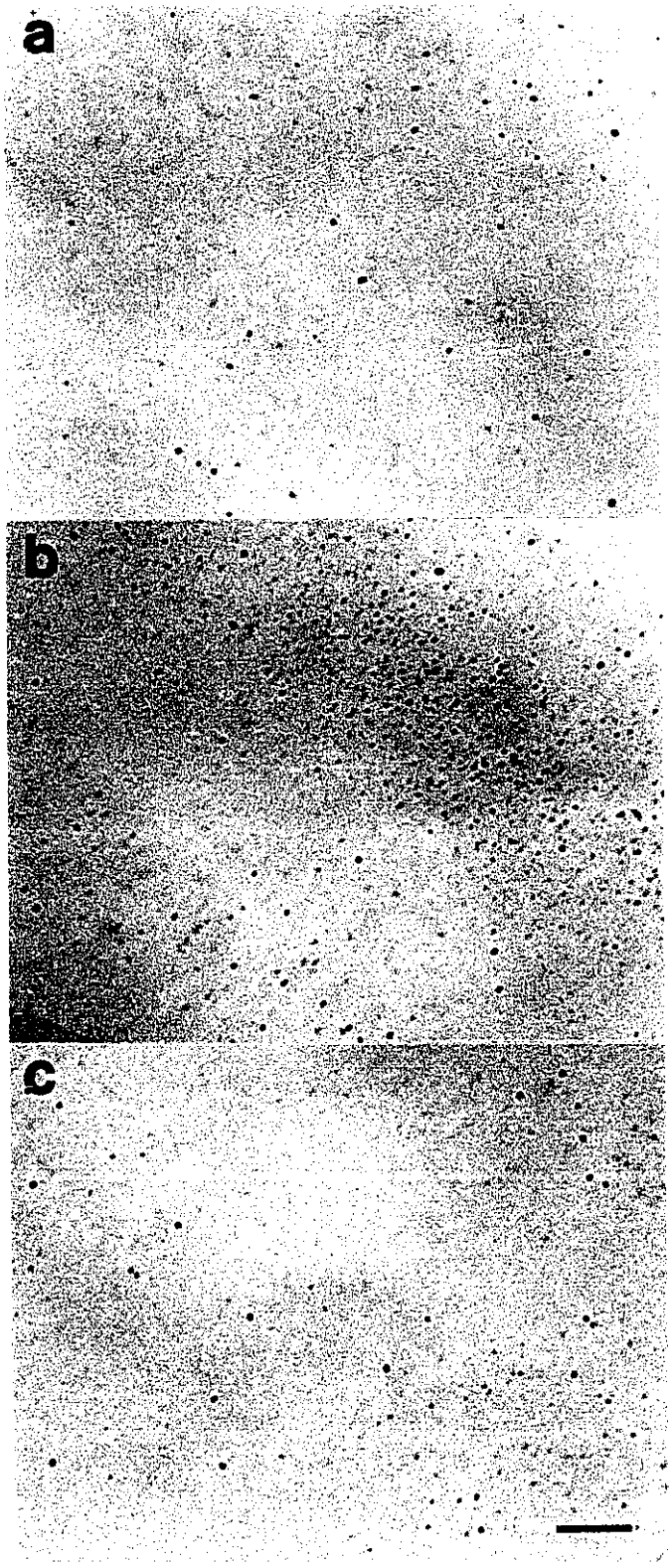

Figure 5.

Figure 6. Photomicrographs demonstrating the effect of CY 208-243 administration on Fos_{2-16}-like immunoreactivity in the intact and 6-OHDA-denervated striatum. a. CY 208-243 had minor effects on Fos_{2-16}-like immunoreactivity in the striatum on the intact side. b. CY 208-243 induced a dramatic increase in Fos_{2-16}-like immunoreactivity in the 6-OHDA-denervated striatum. c. Pretreatment with the selective D1-like receptor antagonist SCH 23390 abolished the induction of Fos_{2-16}-like immunoreactivity produced by CY 208-243 in the lesioned striatum. Scale bar = 500μm.

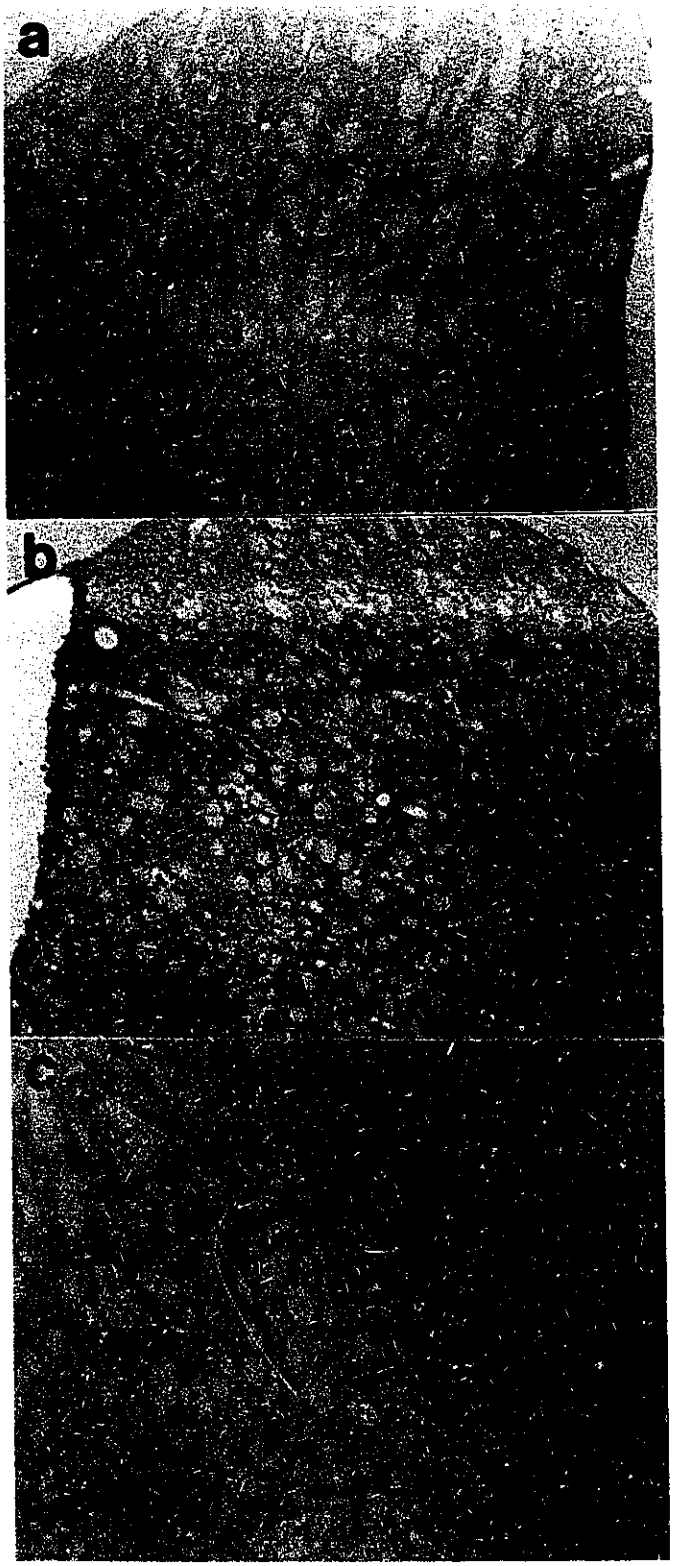

Figure 6.

Figure 7. Photomicrographs illustrating the effect of CY 208-243 on Fos_{2-16}-like immunoreactivity in the intact and 6-OHDA-denervated amygdala. a. CY 208-243 had minor effects on Fos_{2-16}-like immunoreactivity in the amygdala on the intact side. b. CY 208-243 induced a dramatic increase in Fos_{2-16}-like immunoreactivity in the 6-OHDA denervated amygdala. c. Pretreatment with the selective D1-like receptor antagonist SCH 23390 abolished the induction of Fos_{2-16}-like immunoreactivity by CY 208-243 in the lesioned amygdala. Scale bar = 500μm.

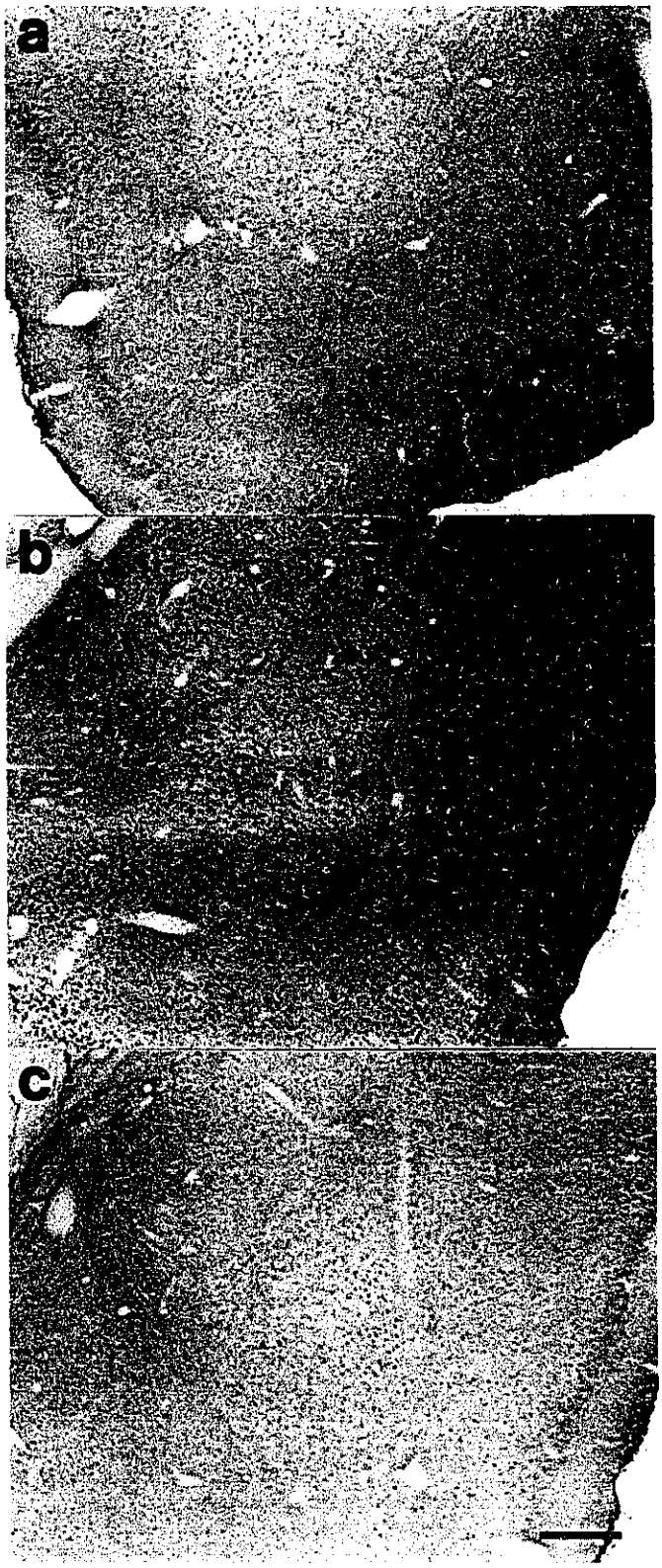

Figure 7.

Figure 8. Photomicrographs illustrating the effect of CY 208-243 on $Fos_{2\text{-}16}$-like immunoreactivity in the intact and 6-OHDA denervated lateral septal nucleus. a. CY 208-243 had minor effects on $Fos_{2\text{-}16}$-like immunoreactivity in the lateral septal nucleus on the intact side. b. CY 208-243 induced a dramatic increase in $Fos_{2\text{-}16}$-like immunoreactivity in the lesioned lateral septal nucleus. c. Pretreatment with the selective D1-like receptor antagonist SCH 23390 abolished the induction of $Fos_{2\text{-}16}$-like immunoreactivity produced by CY 208-243 in the denervated lateral septal nucleus. Scale bar = 500μm.

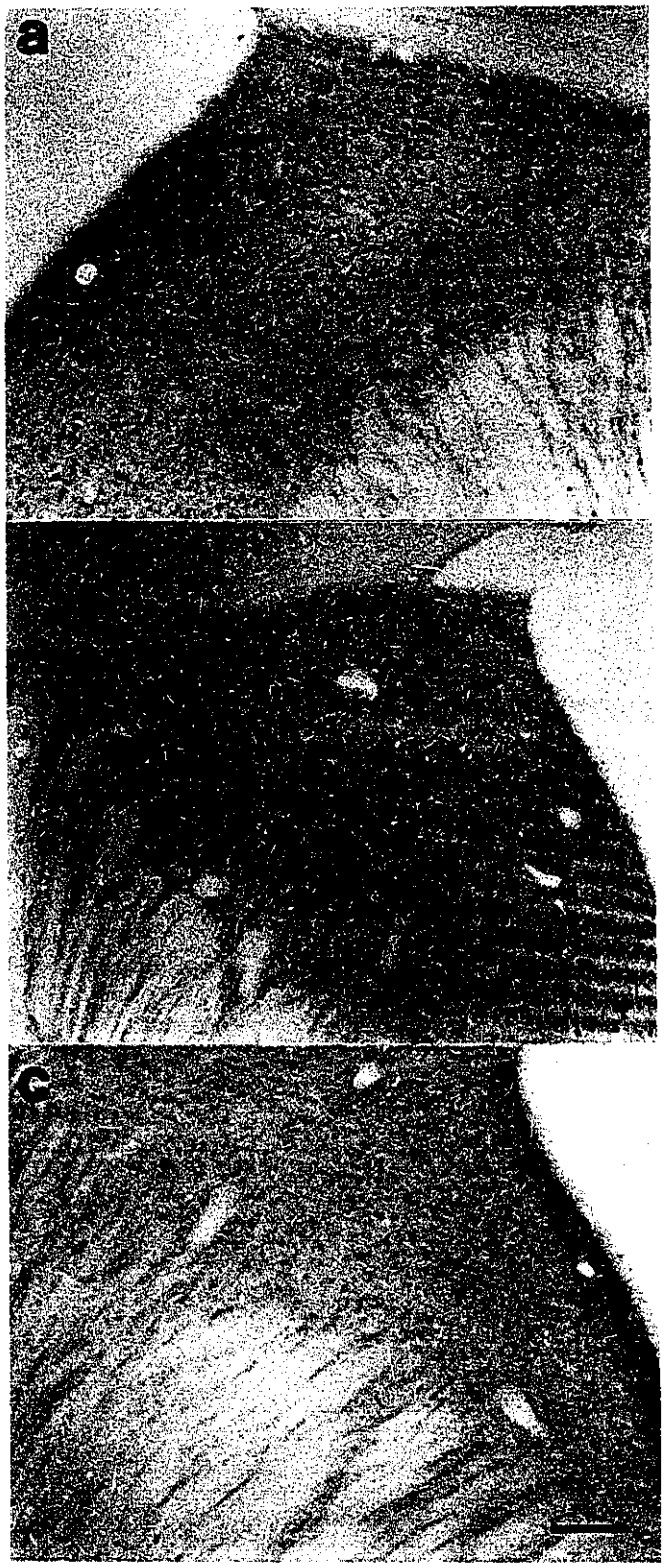

Figure 8.

Figure 9. Photomicrographs displaying the effect of CY 208-243 on $Fos_{2\text{-}16}$-like immunoreactivity in the intact and 6-OHDA denervated olfactory tubercle. a. CY 208-243 had minor effects on $Fos_{2\text{-}16}$-like immunoreactivity in the olfactory tubercle on the intact side. b. CY 208-243 induced a dramatic increase in $Fos_{2\text{-}16}$-like immunoreactivity in the 6-OHDA-denervated olfactory tubercle. c. Pretreatment with the selective D1-like receptor antagonist SCH 23390 abolished the induction of $Fos_{2\text{-}16}$-like immunoreactivity produced by CY 208-243 in the denervated olfactory tubercle. Scale bar = 250μm.

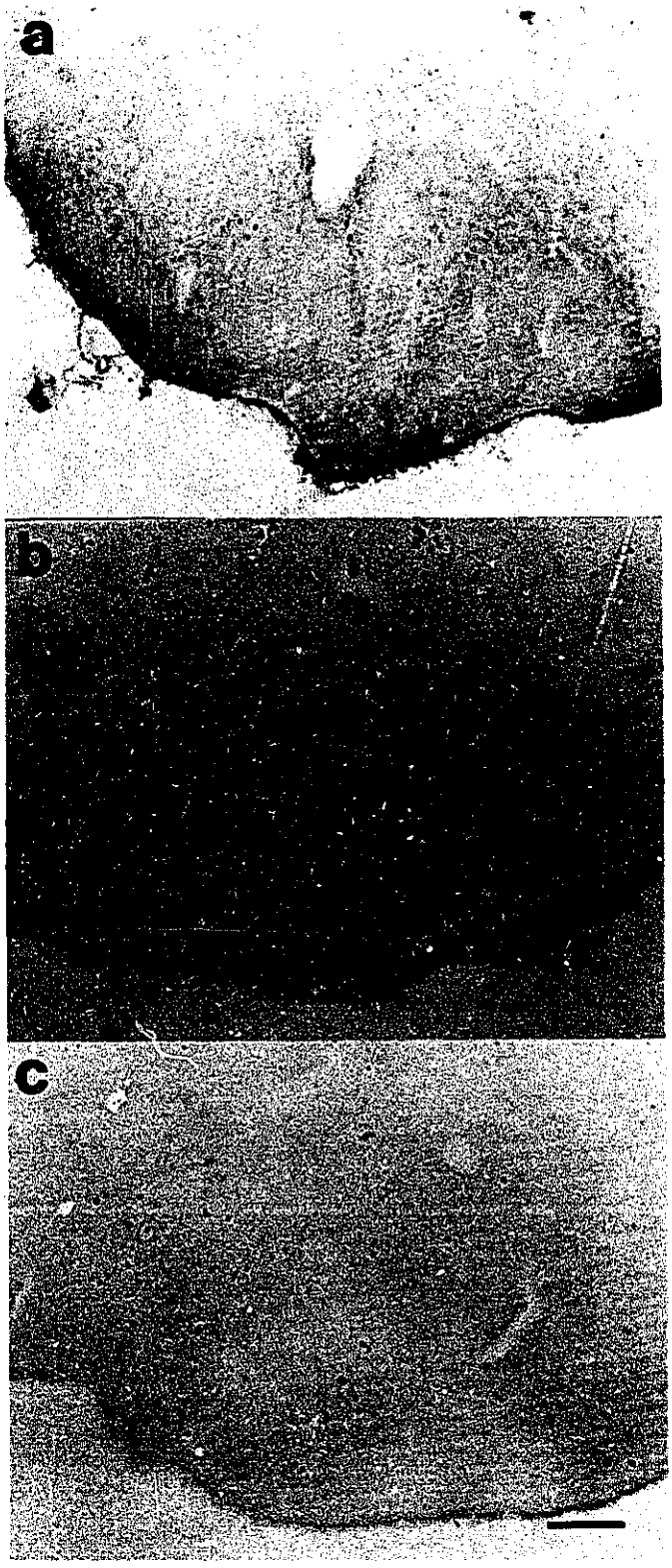

Figure 9.

injection sites for each of the 4 animals in these two groups revealed that the FG injections were largely confined to the target structure (Fig. 10). For instance, the GP was completely covered with limited diffusion into the adjacent caudate-putamen, internal capsule, substantia innominata and ventral pallidum (Fig. 10) while the SNR injection encompassed most of this structure with some encroachment into the SNC, deep mesencephalic nucleus, medial lemniscus and cerebral peduncle (Fig. 10).

B. Localization of $Fos_{128-152}$-Like Immunoreactivity in the Striatum after a 6-OHDA Lesion of the MFB

Using the N-terminally directed CRB antibody, it was not possible to detect an increase in immunoreactivity in the ipsilateral striatum 6 weeks after destruction of the nigrostriatal pathway, (results not shown). In contrast, an increase in $Fos_{128-152}$-like immunoreactivity was detected with the M-peptide antibody in medium-sized neurons (12-18 μm) of the 6-OHDA-denervated striatum (Fig. 11B, D). Injection of FG into the SNR and GP ipsilateral to the 6-OHDA lesion resulted in the retrograde labelling of medium-sized striatal cell bodies of the homolateral striatum (Fig. 11A, C). On average, 182 and 235 neurons were retrogradely labelled in the sampled areas by injection of FG into the SNR and GP, respectively (Table 1). Statistical comparison revealed that there was not a significant difference between the number of retrogradely labelled striatonigral and striatopallidal neurons (Table 1). Destruction of the nigrostriatal pathway by injection of 6-OHDA into the MFB increased the number of striatal neurons that expressed Fos_{128-}

Fig 10. Camera lucid drawings of Fluoro-Gold (FG) injection sites. The injection site is indicated in jet black while the shaded zone surrounding this area represents the extent of tracer diffusion. Top section: FG injections into the globus pallidus (GP) almost completely covered this structure with limited diffusion into the adjacent caudate-putamen (CP), internal capsule (ic), substantia inominate (SI) and ventral pallidum (VP). Bottom section: the FG injection into the substantia nigra pars reticulata (SNR) encompassed the majority of this structure with some encroachment into the overlying substantia nigra pars compacta (SNC), medial lemniscus (ml), deep mesencephalic nucleus (DpMe) and cerebral peduncle (CPE).

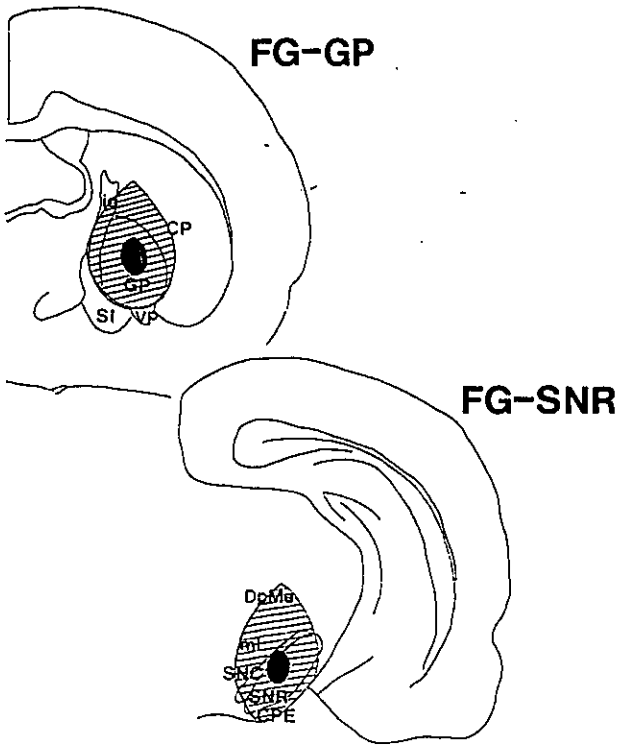

Figure 10.

Fig 11. Photomicrographs of striatal neurons retrogradely labelled with Fluoro-Gold (A and C) that contain (arrow heads) or do not contain (no arrow heads) nuclei displaying $Fos_{128\text{-}152}$-like immunoreactivity six weeks after a 6-OHDA lesion of the MFB (B and D). (A) Neurons in the medial aspect of the striatum retrogradely labelled by injection of Fluoro-Gold (FG) into the substantia nigra pars reticulata (SNR) ipsilateral to the 6-OHDA lesion. (B) Nuclei displaying $Fos_{128\text{-}152}$-like immunoreactivity in the same region of the striatum as shown in A. Note the lack of overlap between retrogradely labelled striatonigral neurons and immunoreactive nuclei. (C) Neurons in the medial aspect of the striatum retrogradely labelled by injection of FG into the globus pallidus ipsilateral to a 6-OHDA lesion. (D) Nuclei displaying $Fos_{128\text{-}152}$-like immunoreactivity in the same region of the striatum as shown in C. Note that immunoreactive nuclei can frequently be found in retrogradely labelled striatopallidal neurons. Scale bar = 40μm.

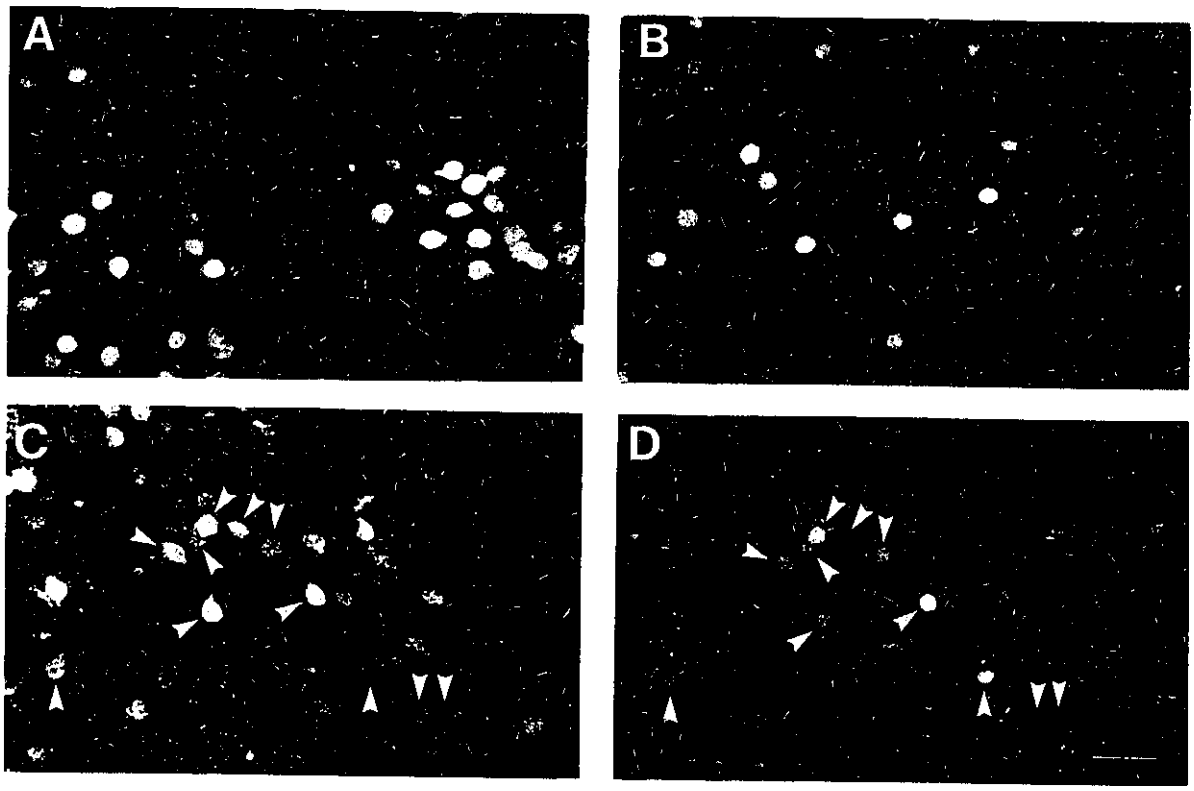

Figure 11.

Table 1. Summary of the results from rats which had received an injection of Fluoro-Gold (FG) into either the substantia nigra pars reticulata (SNR) or globus pallidus (GP) ipsilateral to a unilateral 6-OHDA lesion of the nigrostriatal pathway. The number of neurons retrogradely labelled with FG and nuclei displaying Fos-like immunoreactivity (FLI) were counted in an area 500 μm^2 in size located in the medial aspect of the striatum. In addition, the number of immunoreactive nuclei located in neurons retrogradely labelled with FG from the SNR and GP were counted in the sampled areas (number of overlaps). Lastly, the percentages of the total number of FLI nuclei which were located in retrogradely labelled striatopallidal and striatonigral neurons are given in the last row. Values represent the mean $\pm$ SEM of duplicate determinations for 4 animals.

Data derived from sampled areas of 6-OHDA-denervated striata	FG injected into SNR	FG injected into GP
Number of neurons labelled with FG	182.1±9.4	235.2±25.2
Number of FLI nuclei	94.3±11.1	71.0±13.4
Number of overlaps	10.7±3.4	51.3±8.2*
Percentage of overlaps	11.4%	72.3%

* P<0.01 relative to animals injected with FG in SNR, t test.

$_{152}$-like immunoreactivity. There was not a significant difference between the number of striatal neurons which expressed $Fos_{128\text{-}152}$-like immunoreactivity in animals which received FG injections in the SNR and GP. Comparison of the overlap between $Fos_{128\text{-}152}$-like immunoreactivity and retrogradely labelled striatonigral and striatopallidal neurons revealed that most of the $Fos_{128\text{-}152}$-like immunoreactivity was located in striatopallidal neurons. For example, only 11 of the 94 $Fos_{128\text{-}152}$-like immunoreactivity nuclei (11%), detected in the sampled areas, overlapped with retrogradely labelled striatonigral cells (Fig. 11A, B; Table 1). In contrast, 51 of the 71 $Fos_{128\text{-}152}$-like immunoreactivity nuclei (72%), observed on average in the sampled areas, were situated in retrogradely labelled striatopallidal neurons (Fig. 11C, D; Table 1). Hence, $Fos_{128\text{-}152}$-like immunoreactivity nuclei were frequently found in striatopallidal neurons but rarely in striatonigral neurons.

3. Localization of D1-Like Agonist- and D2-Like Antagonist-Induced $Fos_{2\text{-}16}$-Like Immunoreactivity in Projections of the Nucleus Accumbens

A. Effect of Drug Treatments on Behaviour

Alterations in behaviour were apparent in all of the animals 10 to 30 minutes after drug treatments. Haloperidol (2.0 mg/kg, s.c.) had a calming effect and produced catalepsy. Clozapine (20 mg/kg, s.c.) also produced sedation but not catalepsy. The dopaminergic stimulant *d*-amphetamine (2.5 mg/kg, s.c.) produced locomotion and mild stereotypy. CY 208-243 (1.0 mg/kg, s.c.) produced contralateral rotational behaviour as

well as sniffing, licking and grooming in 6-OHDA-lesioned rats that was directed away from the lesioned side.

B. Tracer Injection Sites in the VP, Substantia Nigra and Ventral Tegmental Area

The goal of tracer injections into the VP, substantia nigra and ventral tegmental area was to label as much of these areas as possible without involving neighbouring structures to a significant degree. Results from animals where a tracer injection was not located predominantly in the target region were excluded from the study. Representative examples of the areas covered by FG injections into the VP, substantia nigra and ventral tegmental area as well as RLB injections into the VP are shown in Fig. 12. The FG and RLB injections into the subcomissural VP encompassed most of this structure but encroached into a small portion of the overlying striatum and bed nucleus of stria terminalis. Injection of FG into the substantia nigra covered the bulk of this region with some spread dorsally. The ventral tegmental area injection completely covered this area with limited diffusion to the adjacent substantia nigra.

Fig 12. Camera lucid drawings of rhodamine-labelled beads (RLB) and Fluoro-Gold (FG) injection sites. Top section: RLB or FG injections into the ventral pallidum (VP) encompassed the majority of this structure with some encroachment into the overlying caudate-putamen (CP) and bed nucleus of stria terminalis (BST). Middle section: the FG injection into the substantia nigra (SN) covered most of the substantia nigra pars compacta (SNC) and substantia nigra pars reticulata (SNR). Bottom section: the FG injection into the ventral tegmental area (VTA) completely encompassed the VTA with limited diffusion into the adjacent SN.

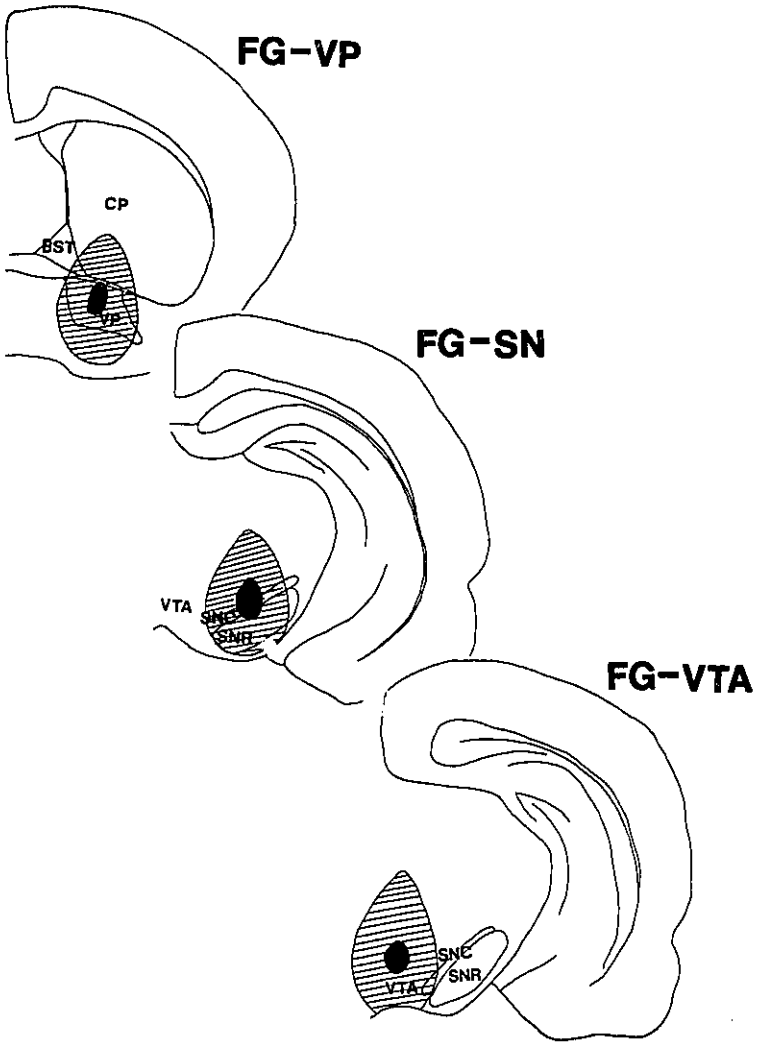

Figure 12.

C. Overlap between Neurons Retrogradely Labelled from the Midbrain and Ventral Pallidum

Injections of RLB into the VP and FG into the substantia nigra and ventral tegmental area resulted in the retrograde labelling of medium sized cells (12-18 μm) in the ipsilateral nucleus accumbens shell and core (Fig. 13). Approximately the same number of neurons were retrogradely labelled in the nucleus accumbens core and shell by the RLB injections (Table 2). Similarly, an equal number of neurons were retrogradely labelled in the core and shell by the FG injections in the midbrain (Table 2). Lastly, a comparable number of neurons were labelled by the FG and RLB injections in the midbrain and VP, respectively. Comparison of the number of neurons which displayed both tracers with the number of neurons that contained only one tracer revealed that approximately 19-23% of neurons in the core and shell were double labelled (Fig. 13; Table 2).

Fig 13. Photomicrographs of neurons in the nucleus accumbens shell retrogradely labelled by injection of Fluoro-Gold (FG) into the midbrain (MB) or rhodamine-labelled beads (RLB) into the ipsilateral ventral pallidum (VP). A: neurons retrogradely labelled with FG from midbrain (MB-FG). B: neurons retrogradely labelled with RLB from ventral pallidum (VP-RLB). Arrows indicate cells which contained both tracers. Comparison of the number of neurons which displayed both tracers with the total number of neurons which contained either RLB or FG revealed that approximately 20% of retrogradely labelled neurons in the core and shell were double labelled. Bar = 40μm.

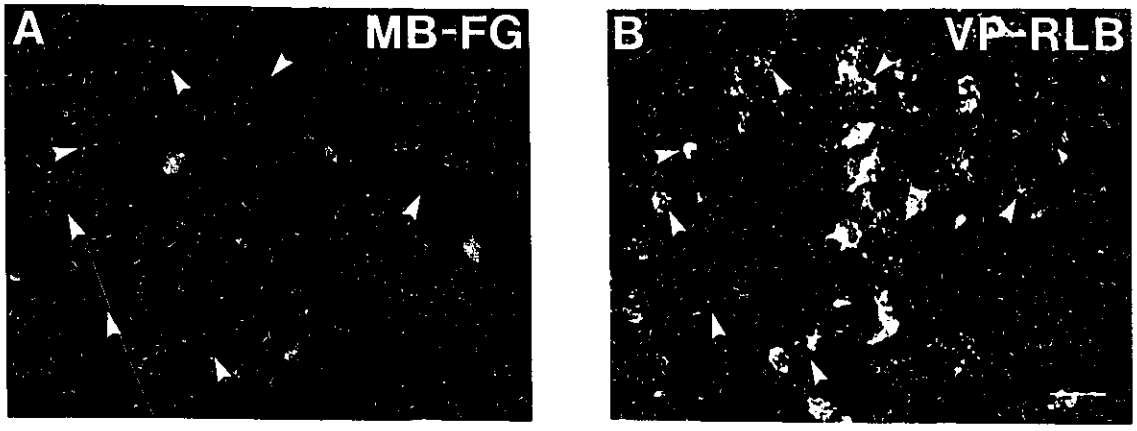

Figure 13.

Table 2. Summary of the results from rats which received injections of rhodamine-labelled beads (RLB) into the ventral pallidum (VP) and Fluoro-Gold (FG) into the ipsilateral midbrain (MB). Approximately the same number of neurons were retrogradely labelled in the nucleus accumbens core and shell by the RLB injections. Similarly, an equal number of neurons were retrogradely labelled in the core and shell regions by FG injections into the midbrain. Comparison of the number of neurons which displayed both tracers (OVERLAPS) revealed that approximately the same number of neurons were double labelled in the nucleus accumbens core and shell. The percentage of overlap (% OF OVERLAP) was obtained by dividing the average number of double labelled neurons (OVERLAPS) by the average number of neurons retrogradely labelled from either the ventral pallidum (RLB-VP) or midbrain (FG-MB) and multiplying the quotient by 100. This calculation revealed that approximately the same percentage of core and shell neurons retrogradely labelled from either the ventral pallidum or midbrain were double labelled (18-23%). For instance, 19% of core neurons retrogradely labelled from the ventral pallidum (RLB-VP) were double labelled by injection of FG into the midbrain (FG-MB). Similarly, about 18% neurons in the nucleus accumbens core retrogradely labelled from the midbrain with FG were also labelled by injection of RLB into the ventral pallidum. In the case of the nucleus accumbens shell, approximately 23% of neurons retrogradely from the ventral pallidum (RLB-VP) were double labelled by injection of FG into the midbrain (FG-MB) while about 19% of neurons retrogradely labelled from the midbrain were double labelled.

	RLB-VP	FG-MB	OVERLAPS	% OF OVERLAP $\frac{\text{OVERLAPS}}{\text{RLB-VP}} \times 100$	% OF OVERLAP $\frac{\text{OVERLAPS}}{\text{FG-MB}} \times 100$
CORE	182 ± 9.0	194 ± 9.0	34 ± 3.4	19%	18%
SHELL	164 ± 19.0	195 ± 19.0	37 ± 4.0	23%	19%

D. Overlaps between Haloperidol-Induced Fos_{2-16}-Like Immunoreactivity and Neurons Retrogradely Labelled from the Midbrain and Ventral Pallidum

Approximately the same number of neurons were retrogradely labelled in the core and shell regions of the nucleus accumbens by FG injections into the midbrain or by FG injections into the VP (Tables 3 and 4). Fos_{2-16}-like immunoreactivity was increased to a similar degree in the core and shell regions of the nucleus accumbens by haloperidol (2 mg/kg, s.c.) (Fig. 14; Tables 3 and 4). In both the core and shell, nuclei which displayed Fos_{2-16}-like immunoreactivity were very rarely located in neurons retrogradely labelled from the midbrain (ventral tegmental area and substantia nigra) (Fig. 14; Tables 3 and 4). By comparison, haloperidol-induced Fos_{2-16}-like immunoreactivity was found much more frequently in neurons retrogradely labelled from the VP (Fig. 14; Tables 3 and 4). Results presented in Fig. 14 are typical of each of the four cases which were examined.

E. Overlaps between Clozapine-Induced Fos_{2-16}-Like Immunoreactivity and Neurons Retrogradely Labelled from the Midbrain and Ventral Pallidum

In the nucleus accumbens shell, the number of neurons retrogradely labelled by FG injection into the VP was not significantly different from that produced by delivery of FG into the midbrain (Table 4). However, in the case of the nucleus accumbens core, FG injection into the VP resulted in the retrograde labelling of slightly more neurons than that

Fig 14. Photomicrographs of neurons in the nucleus accumbens shell retrogradely labelled with Fluoro-Gold (FG) from either the midbrain (MB) or ventral pallidum (VP) that contain (arrowheads) or do not contain (no arrowheads) haloperidol-induced $Fos_{2\text{-}16}$-like immunoreactivity (HAL-Fos). A: HAL-Fos in the shell region of the nucleus accumbens. B: neurons in the same region of the nucleus accumbens as shown in A retrogradely labelled by injection of FG into the MB (MB-FG). Note that HAL-Fos is seldom found in neurons retrogradely labelled from the MB (5% overlap). C: HAL-Fos in the nucleus accumbens shell. D: neurons in the same region of the nucleus accumbens as shown in C retrogradely labelled by injection of FG into the VP (VP-FG). HAL-Fos can frequently be found in neurons retrogradely labelled from the VP (53% overlap). Bar = 40μm.

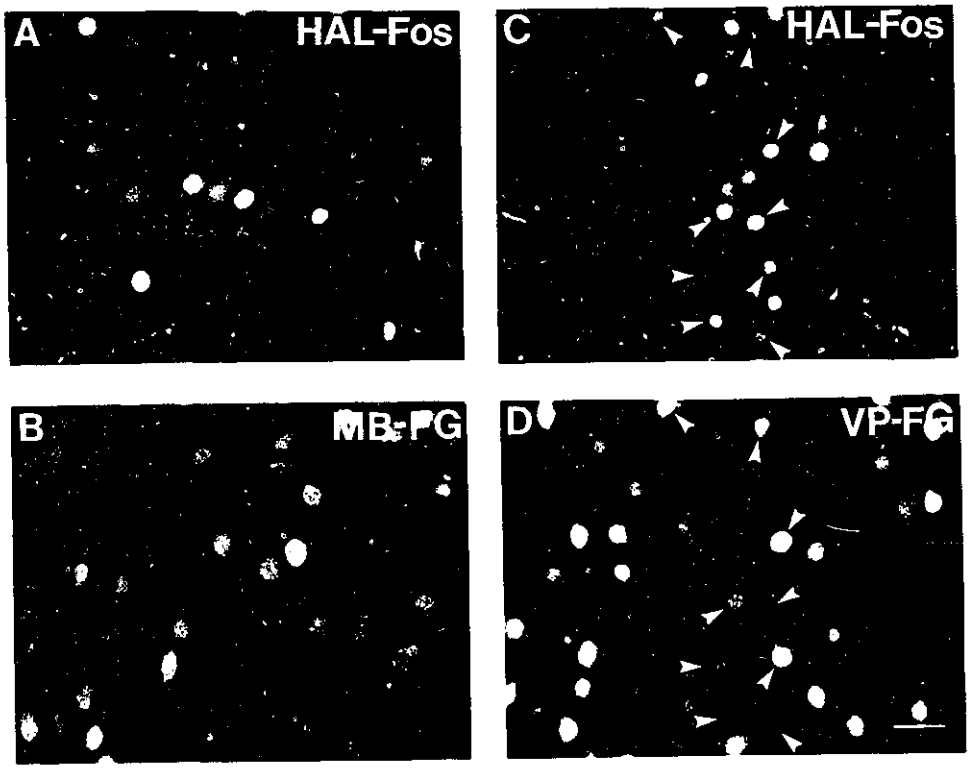

Figure 14.

Table 3. Summary of the localization of nuclei which expressed Fos-like immunoreactivity (FLI) in the nucleus accumbens core after the administration of either haloperidol (2 mg/kg, s.c.), clozapine (20 mg/kg, s.c.), *d*-amphetamine (2.5 mg/kg, s.c.) or CY 208-243 (0.5 mg/kg, s.c.) in neurons retrogradely labelled by injection of Fluoro-Gold (FG) into either the midbrain (FG-MB) or ventral pallidum (FG-VP). The number of neurons retrogradely labelled with FG and nuclei displaying FLI were counted in an area 500 μm^2 located in the nucleus accumbens core (see Fig. 1). In addition, the number of immunoreactive nuclei located in neurons retrogradely labelled with FG from the MB and VP were counted in the sampled areas (number of overlaps). Lastly, percentages of the total number of neurons which displayed FLI that were located in retrogradely labelled neurons are shown (percentage of overlaps). Values represent the mean ± S.E.M. of duplicate determinations for 4 animals. Haloperidol and clozapine increased FLI predominantly in neurons retrogradely labelled from the VP, whereas *d*-amphetamine and CY 208-243 increased FLI in a similar number of neurons labelled from the MB and VP.

DRUG TREATMENT	NUMBER OF NEURONS LABELLED WITH FG		NUMBER OF FLI NUCLEI		NUMBER OF OVERLAPS		PERCENTAGE OF OVERLAPS	
	FG-MB	FG-VP	FG-MB	FG-VP	FG-MB	FG-VP	FG-MB	FG-VP
HALOPERIDOL	206±24.6	312±45.5	26±4.3	30±3.1	2±0.3*	16±2.4	8%	53%
CLOZAPINE	236±20.9*	348±20.4	11±2.5	13±1.4	2±0.9*	9±1.0	18%	70%
d-AMPHETAMINE	241±16.3	329±24.6	43±5.3	42±4.0	26±3.6	18±2.1	59%	41%
CY 208-243	239±17.1	307±26.9	220±18.6	242±18.3	132±13.0	117±10.5	60%	48%

* $P < 0.01$ relative to FG-VP, t-test.

Table 4. Summary of the localization of nuclei which express Fos-like immunoreactivity (FLI) in the nucleus accumbens shell after the administration of either haloperidol (2 mg/kg, s.c.), clozapine (20 mg/kg, s.c.), *d*-amphetamine (2.5 mg/kg, s.c.) or CY 208-243 (0.5 mg/kg, s.c.) in neurons retrogradely labelled by injection of Fluoro-Gold (FG) into either the midbrain (FG-MB) or ventral pallidum (FG-VP). The number of neurons retrogradely labelled with FG and nuclei displaying FLI were counted in an area 500 μm^2 in size located in the shell region of nucleus accumbens (see Figure 1). In addition, the number of immunoreactive nuclei located in neurons retrogradely labelled with FG from the MB and VP were counted in the sampled areas (number of overlaps). Lastly, percentages of the total number of neurons which contained FLI that were located in retrogradely labelled neurons are shown (percentage of overlaps). Values represent the mean ± S.E.M. of duplicate determinations for 4 animals. Haloperidol and clozapine increased FLI predominantly in neurons retrogradely labelled from the VP, whereas *d*-amphetamine and CY 208-243 increased Fos-like immunoreactivity in a similar neurons retrogradely labelled from the MB and VP.

DRUG TREATMENT	NUMBER OF NEURONS LABELLED WITH FG		NUMBER OF FLI NUCLEI		NUMBER OF OVERLAPS		PERCENTAGE OF OVERLAPS	
	FG-MB	FG-VP	FG-MB	FG-VP	FG-MB	FG-VP	FG-MB	FG-VP
HALOPERIDOL	192±16.7	292±48.3	26±3.8	31±5.4	1±0.4*	17±3.4	5%	53%
CLOZAPINE	251±24.8	343±15.7	15±2.7	17±2.0	2.3±1.0*	11±1.1	14%	63%
d-AMPHETAMINE	255±10.0	295±23.9	41±5.2	46±4.6	23±2.9	17±2.3	57%	36%
CY 208-243	234±23.0	320±28.5	204±28.6	230±26.8	121±20.0	90±8.7	59%	42%

* $P < 0.01$ relative to FG-VP, t-test.Table

produced by injection of FG into the midbrain (Table 3). Clozapine (20 mg/kg, s.c.) increased Fos_{2-16}-like immunoreactivity to a greater extent in the shell than the core of the nucleus accumbens (Tables 3 and 4). In both the core and shell, immunoreactive nuclei were rarely located in neurons retrogradely labelled from the midbrain (ventral tegmental area and substantia nigra) (Fig. 15; Tables 3 and 4). In contrast, clozapine-induced Fos_{2-16}-like immunoreactivity was found more frequently in neurons retrogradely labelled from the VP (Fig. 15; Tables 3 and 4). The results shown in Fig. 15 are representative of each of the four cases.

F. Overlaps between d-Amphetamine-Induced Fos_{2-16}-Like Immunoreactivity and Neurons Retrogradely Labelled from the Midbrain and VP

FG injections into the VP and midbrain resulted in the retrograde labelling of an equal number of accumbal neurons in the core and shell regions (Tables 3 and 4). Fos_{2-16}-like immunoreactivity was increased to a comparable degree in the core and shell regions of the nucleus accumbens by *d*-amphetamine (2.5 mg/kg, s.c.). A similar number of neurons in the nucleus accumbens core which displayed Fos_{2-16}-like immunoreactivity were retrogradely labelled from the midbrain and VP (Fig. 16; Table 3). Likewise, in the nucleus accumbens shell, an equal number of neurons which contained Fos_{2-16}-like immunoreactivity were retrogradely labelled from the VP and midbrain (Table 4). Fig. 16 is representative of the results from four animals.

Fig 15. Photomicrographs of neurons in the nucleus accumbens shell retrogradely labelled with Fluoro-Gold (FG) from either the midbrain (MB) or ventral pallidum (VP) that contain (arrowheads) or do not contain clozapine-induced $Fos_{2\text{-}16}$-like immunoreactivity (CLZ-Fos). A: CLZ-Fos in the shell region of the nucleus accumbens. B: neurons in the same region of the nucleus accumbens as shown in A retrogradely labelled by injection of FG into the MB (MB-FG). Note that nuclei displaying CLZ-Fos are infrequently found in neurons retrogradely labelled from the MB (14% overlap). C: CLZ-Fos in the shell region. D: neurons in the same region of the nucleus accumbens as shown in C retrogradely labelled by injection of FG into the VP (VP-FG). Neurons displaying CLZ-Fos can often be found in neurons retrogradely labelled from the VP (63% overlap). Bar = 40μm.

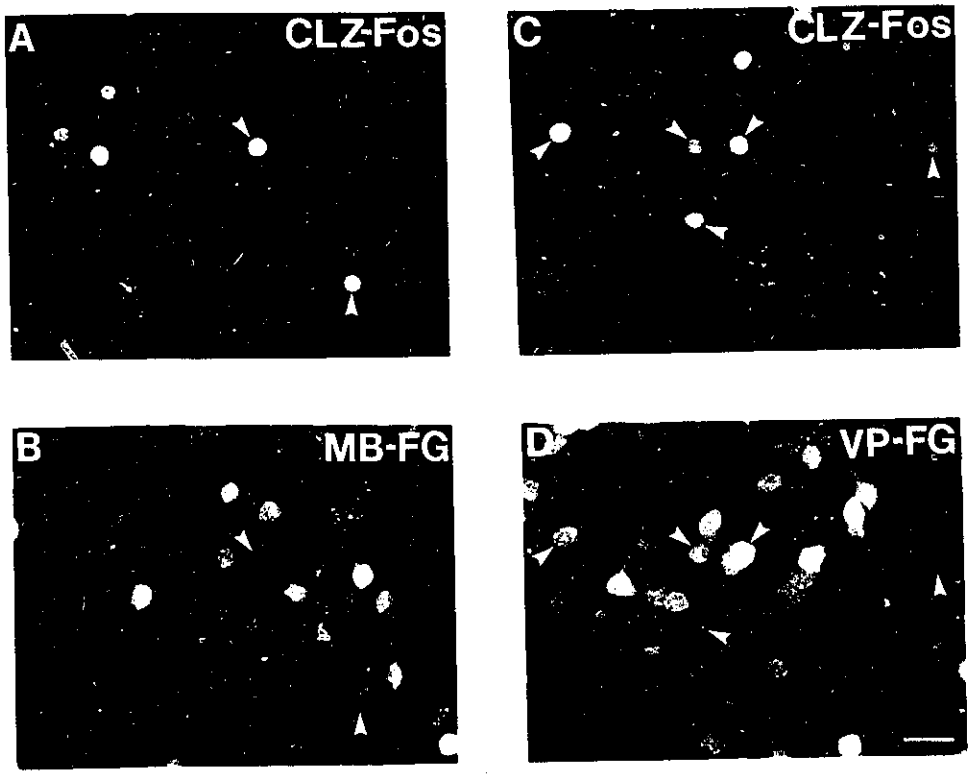

Figure 15.

Fig 16. Photomicrographs of neurons in the nucleus accumbens retrogradely labelled by injection of Fluoro-Gold (FG) into either the midbrain (MB) or ventral pallidum (VP) that contain (arrowheads) or do not contain (no arrow heads) d-amphetamine-induced Fos_{2-16}-like immunoreactivity (d-AMP-Fos). A: d-AMP-Fos in the shell region of the nucleus accumbens. B: neurons in the same region of the nucleus accumbens as shown in A retrogradely labelled by injection of FG into the MB (MB-FG). Note that approximately half of the nuclei which display d-AMP-Fos are located in neurons retrogradely labelled from the MB (57% overlap). C: d-AMP-Fos in the nucleus accumbens shell. D: neurons in the same region of the nucleus accumbens as shown in C retrogradely labelled by injection of FG into the VP (VP-FG). A significant number of accumbal neurons that display d-AMP-Fos were retrogradely labelled from the VP (36% overlap). Bar = 40μm.

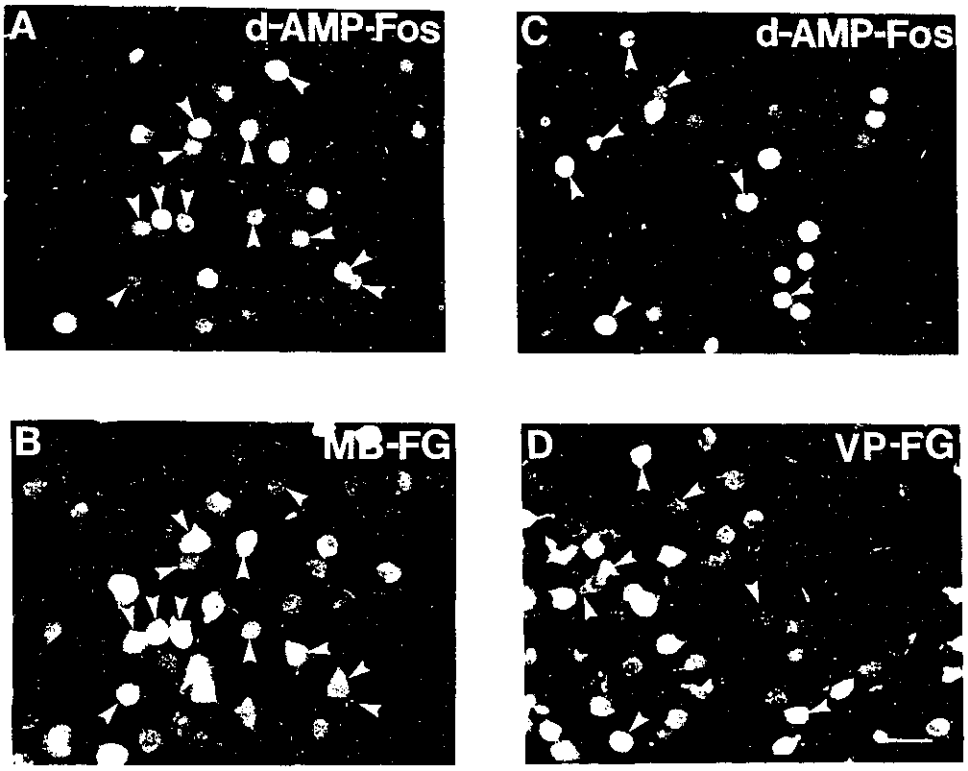

Figure 16.

G. Overlaps between CY 208-243-Induced Fos_{2-16}-Like Immunoreactivity and Neurons Retrogradely Labelled from the Midbrain and VP

An equivalent number of neurons in the core and shell regions of the nucleus accumbens were retrogradely labelled by FG injections into the midbrain and VP (Tables 3 and 4). Administration of the D1-like receptor agonist CY 208-243 (0.5 mg/kg, s.c.) dramatically increased Fos_{2-16}-like immunoreactivity in the nucleus accumbens ipsilateral to a 6-OHDA lesion of the MFB (Fig. 17; Tables 3 and 4). In both the nucleus accumbens core and shell, a similar number of neurons which displayed Fos_{2-16}-like immunoreactivity were retrogradely labelled from the midbrain and VP. Results presented in Fig. 17 are typical of the four animals which were examined.

H. Quantitative Comparisons between Retrogradely Labelled and Cresyl Violet Stained Cells

In order to determine the total number of medium-sized cells in the sampled areas of the nucleus accumbens, sections adjacent to those processed for Fos_{2-16}-like immunoreactivity were stained with cresyl violet. Counts performed on sections chosen at random from 5 animals indicated that there were 600 ± 45 and 575 ± 50 medium-sized cells in the sampled area of the nucleus accumbens core and shell, respectively. Averaging data derived from each of the four treatment groups (haloperidol, clozapine, *d*-amphetamine and CY 208-243), 233 and 312 neurons were retrogradely labelled in the

Fig 17. Photomicrographs of neurons in the nucleus accumbens shell retrogradely labelled with Fluoro-Gold (FG) from either the midbrain (MB) or ventral pallidum (VP) that contain (arrowheads) or do not contain CY 208-243-induced $Fos_{2\text{-}16}$-like immunoreactivity (CY-Fos). A: CY-Fos in the shell region of the nucleus accumbens. B: neurons in the same region of nucleus accumbens as shown in A retrogradely labelled by injection of FG into the MB (MB-FG). C. CY-Fos in the nucleus accumbens shell. D: neurons in the same region of the nucleus accumbens as shown in C retrogradely labelled by injection of FG into the VP (VP-FG). Note that nuclei displaying CY-Fos can frequently be found in neurons retrogradely labelled from both the MB and VP, 59% and 42% overlap, respectively. Bar = 40μm.

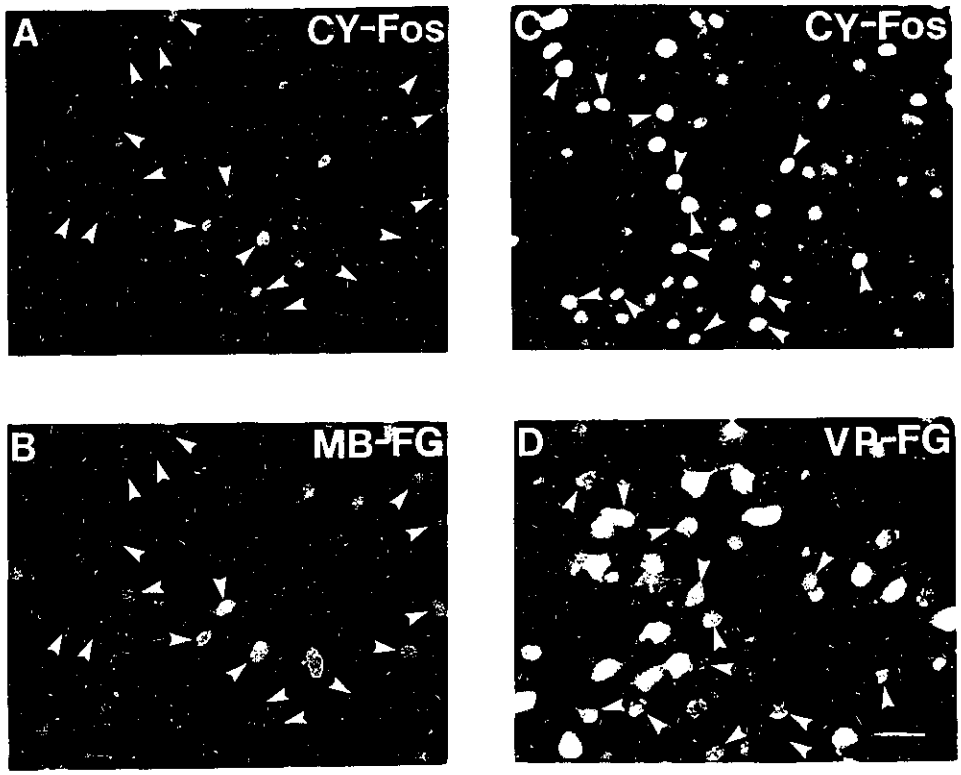

Figure 17.

shell region by injection of FG into the midbrain and VP, respectively. Similarly, 231 and 324 neurons were retrogradely labelled in the core region by injection of FG into the midbrain and VP, respectively. Thus, injections of FG into the midbrain and VP resulted in a total retrograde labelling of about 90-95% of the medium-sized neurons in the core and shell regions of nucleus accumbens.

DISCUSSION

1. Distribution of D1-Like Agonist Induced Fos_{2-16}-Like Immunoreactivity in the Forebrain of 6-OHDA-Lesioned Animals

The present studies demonstrated that administration of the selective D1-like agonist CY 208-243 (1.0 mg/kg, s.c.) to animals with unilateral 6-OHDA lesions resulted in rotational behaviour directed away from the side of the lesion and a dramatic increase in Fos_{2-16}-like immunoreactivity in the lesioned side of the forebrain. These increases occurred largely in the ipsilateral parietal cortex, striatum, nucleus accumbens, amygdala, lateral septal nucleus and olfactory tubercle. By comparison, CY 208-243 had minor effects on Fos_{2-16}-like immunoreactivity in these structures on the intact side. Pretreatment with the selective D1-like antagonist SCH 23390 abolished circling and the induction of Fos_{2-16}-like immunoreactivity produced by CY 208-243.

Autoradiographic localization of [^{3}H] SKF 38393 binding sites has shown that high densities of D1 receptors are located in the caudate-putamen, nucleus accumbens and olfactory tubercle (Scatton and DuBois, 1985). Intermediate levels of binding were detected in the lateral septal nucleus as well as prefrontal, perirhinal, piriform and cingulate cortices. *In situ* hybridization histochemistry has confirmed these findings by demonstrating that cells expressing D1 receptor mRNA are located in the caudate-putamen, nucleus accumbens, olfactory tubercle, limbic system, cortex, hypothalamus and thalamus (Fremeau et al., 1991; Gerfen et al., 1991). Similarly, D1 dopamine receptor localization by immunohistochemistry using a polyclonal anti-peptide antibody has also

shown that the receptor is concentrated in prefrontal, cingulate, parietal and piriform cortices as well as subcortically in the basal ganglia, amygdala, lateral septal area, substantia inominata and thalamus (Huang et al., 1992).

Thus, the distribution of D1-like agonist-induced Fos_{2-16}-like immunoreactivity very closely matches the distribution of D1 receptors in the forebrain. The close topographical relationship between D1-like agonist-induced Fos_{2-16}-like immunoreactivity and D1 receptors suggests that D1-like agonist-induced Fos_{2-16}-like immunoreactivity is located in neurons that express the D1 dopamine receptor.

The present results also demonstrate that there was little D1-like agonist induced Fos_{2-16}-like immunoreactivity in the intact side. This is consistent with previous studies which suggest that the activation of *c-fos* expression by selective D1-like agonists is dependent on the development of D1 receptor supersensitivity (Robertson GS et al., 1989; Robertson GS et al., 1992).

Pretreatment with the D1-like antagonist SCH 23390 abolished circling and the induction of Fos_{2-16}-like immunoreactivity in the forebrain produced by CY 208-243 demonstrating that CY 208-243-induced turning and Fos_{2-16}-like immunoreactivity was mediated by the activation of D1-like dopamine receptors. Lastly, since Fos expression is often associated with increased metabolic activity, it is tempting to speculate that those increases in D1-like agonist-induced Fos_{2-16}-like immunoreactivity that are here occurred in the parietal cortex, amygdala, lateral septum and olfactory tubercle are associated with elevated neuronal activity.

2. Localization of $Fos_{128-152}$-Like Immunoreactivity in the 6-OHDA Denervated Striatum

Using an antibody directed against a segment of the DNA binding region of Fos (amino acids 128-152) that is highly conserved among all members of the *fos* family, a large increase in $Fos_{128-152}$-like immunoreactivity has been reported to occur in the striatum after dopaminergic denervation (Dragunow et al., 1990). Antibodies raised against the N-terminus of Fos (amino acids 2-16), a region that is poorly conserved among each of the members of the *fos* family, fail to detect an increase in Fos_{2-16}-like immunoreactivity after dopaminergic denervation. This suggests that destruction of the nigrostriatal pathway produces a persistent elevation of a Fos-related protein(s), rather than Fos, in the denervated striatum. In order to determine the localization of $Fos_{128-152}$-like immunoreactivity, striatonigral and striatopallidal neurons were retrogradely labelled by injection of the fluorescent tracer FG into the SNR and GP, respectively. Results from these experiments demonstrated that $Fos_{128-152}$-like immunoreactivity was frequently found in striatopallidal neurons but rarely in striatonigral neurons.

Striatopallidal neurons, which constitute a major inhibitory input to the GP, are thought to be tonically inhibited by dopamine derived from nigrostriatal neurons (Dragunow et al., 1991; Nishina et al., 1990; Nguyen et al., 1992). Accordingly, dopamine depletion would be expected to increase striatopallidal activity resulting in a decrease in the spontaneous activity of pallidal neurons. Consistent with this proposal, electrophysiological studies have reported a decrease in the firing rate of spontaneously

active neurons in the GP 1-9 weeks after a 6-OHDA lesion of the ipsilateral nigrostriatal pathway (Pan and Walters, 1988). In the present study, $Fos_{128-152}$-like immunoreactivity detected 6 weeks post lesion was located predominantly in striatopallidal neurons. The long-lasting expression of $Fos_{128-152}$-like immunoreactivity by striatopallidal neurons may, therefore, be related to a sustained increase in excitatory transmembrane signalling which occurs in these neurons after dopamine deafferentation.

In addition to $Fos_{128-152}$-like immunoreactivity, dopamine depletion produces persistent increases in the expression of D2 dopamine receptors and several neuropeptides (Burgunder and Young, 1989; Gerfen et al., 1990; Le Moine et al., 1990; Young et al., 1986). *In situ* hybridization studies have revealed that the expression of mRNAs encoding D2 dopamine receptors and enkephalin are increased several weeks after the loss of midbrain dopamine neurons (Young et al., 1986). Furthermore, these increases occur preferentially in striatopallidal neurons (Gerfen et al., 1990). Since Fos is a member of the AP-1 transcriptional regulating complex (Nishina et al., 1990), it is tempting to speculate that AP-1 may participate in the intracellular events which result in the long-lasting elevation of mRNAs encoding striatopallidal D2 receptors and enkephalin after dopaminergic deafferentation. Indeed, transient co-transfection studies suggest that expression of the proenkephalin gene is increased by over expression of Fos and Jun (Sonnenberg et al., 1989).

It is not possible on the basis of the immunohistochemical results of the present study to confirm the identity of the protein(s) responsible for immunoreactivity detected in the striatum 6 weeks after the 6-OHDA-lesion. However, the fact that basal $Fos_{128-152}$-like

immunoreactivity is not observed in the 6-OHDA striatum using a sheep antibody (CRB OA-11-823) directed against the N-terminus of Fos (Dragunow and Faull, 1989; Paul et al., 1992; Robertson GS et al., 1992; Robertson HA et al., 1989) suggests that Fos does not contribute to immunoreactivity detected with the rabbit M-peptide antibody. In order to determine the nature of $Fos_{128\text{-}152}$ immunoreactive protein(s) responsible for this increase, Western blots were performed on striatal extracts. Approximately six weeks after the 6-OHDA lesion, expression of a 43kD Fos immunoreactive protein was dramatically enhanced. Since the molecular weight of the truncated form of FosB (ΔFosB) is close to this value, FosB and ΔFosB expression was examined using two antibodies. One antibody recognizes both FosB and ΔFosB while the other recognizes just FosB. Western blots revealed that dopaminergic denervation selectively increased expression of ΔFosB (Robertson GS et al., 1994). Thus, ΔFosB is responsible for the long-lasting increase in $Fos_{128\text{-}152}$-like immunoreactivity induced by dopaminergic denervation. These results also suggest that ΔFosB may participate in those intracellular events which maintain altered neuronal gene expression in medium-sized neurons of the striatum after dopaminergic denervation. Thus, like Fos, ΔFosB may dimerize with Jun to form AP-1 complexes capable of regulating the expression of genes which contain AP-1 binding sites (Nishina et al., 1990).

3. Localization of D1-Like Agonist- and D2-Like Antagonist-Induced Fos_{2-16}-Like Immunoreactivity in Projections of the Nucleus Accumbens

In accordance with previous findings (Bahat and Baraban, 1993; Berretta et al., 1992; Cole et al., 1992; Deutch et al., 1992; Graybiel et al., 1990; Hope et al., 1992; Robertson GS and Fibiger, 1992; Robertson HA et al., 1989), alterations in dopaminergic neurotransmission were found to produce significant increases in Fos_{2-16}-like immunoreactivity in the ventral striatum. *d*-Amphetamine (2.5 mg/kg, s.c.) enhanced Fos_{2-16}-like immunoreactivity to a similar degree in both the core and shell regions of the nucleus accumbens. The D1-like agonist CY-208-243 has weak effects on Fos_{2-16}-like immunoreactivity in intact animals. However, after dopaminergic denervation of the striatum, D1-like agonists profoundly increase Fos_{2-16}-like immunoreactivity in the dorsal striatum (Morelli et al., 1992; Paul et al., 1992). Our results indicate that CY 208-243 also dramatically enhances Fos_{2-16}-like immunoreactivity in the nucleus accumbens ipsilateral to a 6-OHDA-lesion of the MFB and that these increases occur to an equivalent degree in the core and shell regions. In contrast, the ability of D2-like antagonists such as haloperidol and clozapine to increase Fos_{2-16}-like immunoreactivity in the striatum is dopamine dependent. Removal of the dopaminergic innervation of the striatum eliminates the elevation in Fos_{2-16}-like immunoreactivity produced by these antipsychotics (Robertson GS and Fibiger, 1992). Since Fos is considered to be an activity marker, we have proposed that antipsychotics increase Fos_{2-16}-like immunoreactivity by a disinhibitory mechanism mediated by the blockade of inhibitory D2 receptors located in the striatum

(Dragunow and Faull, 1989; Robertson GS and Fibiger, 1992; Robertson GS et al., 1992).

Comparison of the number of neurons that displayed Fos_{2-16}-like immunoreactive after the administration of CY 208-243 (0.5 mg/kg, s.c.) (204-242; Tables 2 and 3) with the total population of medium sized cells (575-600) indicated that this D1-like agonist increased Fos_{2-16}-like expression in approximately 40% of the neurons in the denervated nucleus accumbens. By contrast, haloperidol (2 mg/kg, s.c.) and clozapine (20 mg/kg, s.c.) enhanced Fos_{2-16}-like immunoreactivity in considerably fewer neurons. Approximately 26-31 neurons (Tables 3 and 4) contained haloperidol-induced Fos_{2-16}-like immunoreactivity in the nucleus accumbens core and shell or about 5% of the total population of medium sized neurons. This value is far below the number of striatal neurons which are thought to express D2 receptors (Gerfen, 1993; Gerfen et al., 1990) suggesting that D2 receptor blockade is not the only factor governing immediate-early gene induction after haloperidol administration. On average, 11-17 neurons in the core and shell subdivisions of the nucleus accumbens displayed Fos_{2-16}-like immunoreactivity after clozapine (20 mg/kg, s.c.) or about 2% of the total population of medium sized neurons in the accumbens. Clozapine therefore induced Fos_{2-16}-like immunoreactivity is less than half as many accumbal neurons as haloperidol. In addition to this difference, it is now well established that clozapine and haloperidol also produce distinct patterns of Fos_{2-16}-like immunoreactivity in the nucleus accumbens. These differences may be reconciled by the finding that haloperidol and clozapine interact with distinct D2-like receptors. Molecular cloning studies have recently revealed the existence of three D2-like receptors termed D2, D3 and D4 (Bunzow et al., 1988; Sokoloff et al., 1990; Van Tol et

al., 1991). The most abundant is the D2 receptor which is located in all regions of the brain that receive dopaminergic innervation (Bouthenet et al., 1991; Giros et al., 1989). Next is the D3 receptor which is located preferentially in limbic regions such as the nucleus accumbens (Sokoloff et al., 1990). The concentration of D4 receptor mRNA in rat brain is very low, it is only detectable by PCR amplification in the frontal cortex and olfactory bulb and is present in the striatum in only a very few scattered cells (O'Malley et al., 1992). Hence, although clozapine and haloperidol have high affinity for this receptor (Lahti et al., 1993; Van Tol et al., 1991), its very low abundance in rat brain strongly suggests that D4 receptor blockade contributes little to antipsychotic-induced $Fos_{2\text{-}16}$-like immunoreactivity. Haloperidol has a considerably higher affinity than clozapine for the D2 receptor while the reverse is true for the D3 receptor. Thus, it is tempting to speculate that clozapine-induced $Fos_{2\text{-}16}$-like immunoreactivity is located predominantly in accumbal neurons that express the D3 receptor, whereas haloperidol-induced $Fos_{2\text{-}16}$-like immunoreactivity is situated principally in neurons that express the D2 receptor.

Retrograde tracing studies have established that D1-like agonists and D2-like antagonists increase $Fos_{2\text{-}16}$-like immunoreactivity in different populations of neurons in the dorsal striatum. D1-like agonists elevate $Fos_{2\text{-}16}$-like immunoreactivity primarily in striatonigral neurons, whereas D2-like antagonists increase $Fos_{2\text{-}16}$-like immunoreactivity predominantly in striatopallidal neurons (Cenci et al., 1992; Robertson GS et al., 1992). We have confirmed these findings by performing numerical comparisons in the dorsolateral striatum of the overlap between enkephalin neurons identified by *in situ*

hybridization and D1-like agonist- and D2-like antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity. Only 15% of the neurons in the dorsolateral striatum that displayed D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity were autoradiographically labelled with a proenkephalin oligonucleotide probe. In contrast, nearly 85% of those neurons which contained D2-like antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity were labelled with the proenkephalin probe. Given that striatopallidal, but not striatonigral, neurons are labelled with the enkephalin probe (Gerfen and Young, 1988), these results are consistent with the proposal that the majority of nuclei that display D1-like agonist- and D2-like antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity in the dorsolateral striatum are located in striatonigral and striatopallidal neurons, respectively.

The primary purpose of the present study was to determine if D1-like agonist- and D2 -like antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity in the nucleus accumbens is also located chiefly in efferents to the midbrain and pallidum, respectively. Before examining these relationships it was first necessary to determine whether accumbal projections to the midbrain and VP arise mainly from separate populations of neurons. This was done by counting the number of accumbal neurons which were double labelled after injection of RLB into the VP and FG into the midbrain. Approximately 18-23% of retrogradely labelled neurons in the nucleus accumbens core and shell contained both tracers indicating that the majority of these neurons project to either the midbrain or VP. Thus, the nucleus accumbens is similar to the dorsal striatum in that its projections to the midbrain and VP originate largely from distinct populations of neurons. Nevertheless, it remains to be determined if dorsal striatal and accumbal projections to the midbrain and pallidum share

a comparable degree of collateralization. Also like the dorsal striatum, haloperidol preferentially enhanced Fos_{2-16}-like immunoreactivity in accumbal neurons projecting to the pallidum (ventral pallidum). The atypical antipsychotic clozapine also enhanced Fos_{2-16}-like immunoreactivity principally in accumbal neurons projecting to the VP. In both the nucleus accumbens shell and core, the majority of neurons which displayed clozapine-induced Fos_{2-16}-like immunoreactivity were retrogradely labelled from the VP. These results suggest that accumbal neurons projecting to the VP may be an important target for the antipsychotic effects of these neuroleptics. On the basis of the present results, it is not possible to establish whether clozapine and haloperidol increased Fos_{2-16}-like immunoreactivity in the same population of accumbal neurons. Nevertheless, the different patterns of Fos_{2-16}-like immunoreactivity produced by clozapine and haloperidol in the nucleus accumbens suggest that these antipsychotics may enhance *c-fos* expression in distinct populations of neurons (Deutch et al., 1992; Nguyen et al., 1992; Robertson and Fibiger, 1992).

Both *d*-amphetamine and CY 208-243 elevated Fos_{2-16}-like immunoreactivity in accumbal neurons retrogradely labelled from the midbrain. Since few accumbal neurons projecting to the midbrain contained neuroleptic-induced Fos_{2-16}-like immunoreactivity, accumbal neurons projecting to the midbrain selectively expressed D1-like agonist-induced Fos_{2-16}-like immunoreactivity. This situation is reminiscent of the dorsal striatum where D1-like agonists preferentially increase Fos_{2-16}-like immunoreactivity in striatonigral neurons (Cenci et al., 1992; Robertson GS et al., 1992). Unlike the dorsal striatum where D1-like agonists seldom increase Fos_{2-16}-like immunoreactivity in neurons projecting to

the pallidum (Cenci et al., 1992; Robertson GS et al., 1992), approximately half of those accumbal neurons which expressed Fos_{2-16}-like immunoreactivity after either *d*-amphetamine or CY 208-243 projected to the pallidum. Indeed, an equal number of accumbal neurons which displayed D1-like agonist-induced Fos_{2-16}-like immunoreactivity were retrogradely labelled from the midbrain and VP. These results suggest that a significant number of accumbal neurons that contained D1-like agonist-induced Fos_{2-16}-like immunoreactivity may project to the pallidum but not the midbrain. However, this proposal is confounded by the fact that FG can be taken up and retrogradely transported by damaged fibers of passage (Dado et al., 1990). FG injections into the VP would likely injure the axons of accumbal neurons that pass through this structure on their way to the midbrain. Since D1-like agonist-induced Fos_{2-16}-like immunoreactivity is also located in accumbal neurons that project to the midbrain, tracer injections into the VP may also have retrogradely labelled these neurons. As a result, D1-like agonist-induced Fos_{2-16}-like immunoreactivity may have been located in neurons retrogradely labelled from the VP because of damage to fibers of passage. A further complication is that dorsal striatal neurons which project to the midbrain are known to give off small collaterals to the pallidum (Kawaguchi et al., 1990). Assuming that these collaterals are capable of taking up sufficient tracer to permit the retrograde labelling of cell bodies, collateralization may have been responsible for the retrograde labelling of accumbal neurons that project to the midbrain after FG injections into the VP. In this way, FG injections into the VP may have retrogradely labelled D1-like agonist-induced Fos_{2-16}-like immunoreactivity located in accumbal neurons that project to the midbrain.

It is unlikely, however, that damage to fibers of passage or collateralization can account for all of those cases in which D1-like agonist-induced Fos_{2-16}-like immunoreactivity was found in accumbal neurons retrogradely labelled form the VP. For example, in the case of the nucleus accumbens core, CY 208-243 increased Fos_{2-16}-like immunoreactivity in about 117 neurons that were retrogradely labelled from the VP. For this experiment, an average of 307 neurons were retrogradely labelled by injection of FG into the VP. On the basis of our dual tracer results (Table 2), 58 of these neurons (19%) would also be expected to project to the midbrain. Thus, there are considerably more accumbal neurons that display CY 208-243-induced Fos_{2-16}-like immunoreactivity than those which also project to the midbrain. To a lesser extent, the same holds true for the nucleus accumbens shell (Table 4). In this region, CY 208-243 increased Fos_{2-16}-like immunoreactivity in about 90 neurons that were retrogradely labelled from the VP. In this experiment, an average of 320 neurons were retrogradely labelled by injection of FG into the VP. On the basis of our dual tracer results, 74 of these neurons (23%) would be expected to be labelled by FG injections into the midbrain. Even if it is assumed that every one of these dual-projection neurons contained CY 208-243-induced Fos_{2-16}-like immunoreactivity, it is still not possible to account for the localization of D1-like agonist-induced Fos_{2-16}-like immunoreactivity in accumbal neurons projecting to the VP on the basis of collateralization or damage to fibers of passage. Thus, there appears to be a distinct population of neurons that project to the VP, and not to the midbrain, which contain D1-like agonist-induced Fos_{2-16}-like immunoreactivity.

Quantitative autoradiographic studies have revealed that there are a significant number

of D1 receptor binding sites in the VP (Beckstead, 1988 and Dawson et al., 1988). By comparison to the nucleus accumbens, the VP contains approximately 40% as many D1 receptor binding sites (Dawson et al., 1988). Since D1 receptor mRNA is found in the VP (Fremeau et al., 1991), it would appear that at least some of these D1 receptor binding sites reside on intrinsic neurons. However, the modest amount of D1 receptor mRNA found in the VP as well as the results of the present study suggest that the majority of D1 receptors in the VP may be located on the terminals of accumbal efferents. Our finding that both D1-like agonists and D2-like antagonists increase $Fos_{2\text{-}16}$-like immunoreactivity in accumbal neurons projecting to the VP raises the question as to whether these increases may be occurring in the same neuron. Although we have not addressed this question directly in the present study, two lines of evidence suggest that D1-like agonists and D2-like antagonists increase $Fos_{2\text{-}16}$-like immunoreactivity in distinct populations of accumbal neurons. First, recent evidence indicates that D1 and D2 receptors are located on separate populations of neurons in the nucleus accumbens. *In situ* hybridization studies which have simultaneously measured D1 and D2 receptor mRNAs in the same tissue section have shown that fewer than 5% of neurons in the striatum express both of these species of mRNA (Gerfen, 1993). Second, D1-like agonists and D2-like antagonists increase $Fos_{2\text{-}16}$-like immunoreactivity in distinct populations of neurons in the caudate-putamen. We have previously shown that D2-like antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity is located predominantly in striatopallidal neurons, whereas D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity is located principally in striatonigral neurons (Robertson GS et al., 1992). Thus, D1-like agonists and D2-like antagonists may increase $Fos_{2\text{-}16}$-like

immunoreactivity in different populations of accumbal neurons that project to the VP.

4. Technical Considerations

D1-like agonist- and D2-like antagonist-induced Fos_{2-16}-like immunoreactivity was localized in the nucleus accumbens using tract tracing techniques to retrogradely label accumbal neurons from either the midbrain or VP. D1-like agonists elevated Fos_{2-16}-like immunoreactivity not only in accumbal neurons retrogradely labelled from the midbrain but also in those labelled from the VP, whereas D2-like antagonists increased Fos_{2-16}-like immunoreactivity predominantly in accumbal neurons retrogradely labelled from the VP. These findings suggest that in the nucleus accumbens D1-like agonists increase Fos_{2-16}-like immunoreactivity in projections to the midbrain and VP, whereas D2-like antagonists elevate Fos_{2-16}-like immunoreactivity preferentially in projections to the VP. However, these conclusions should be viewed with caution in light of the technical limitations of retrograde tract tracing techniques. For instance, one problem with tracer injections is the inability to know the precise area from which the tracer is taken up and transported. The visible injection area changes in size during the survival period and the area seen at the time of death may not be an accurate representation of the actual uptake site. The area of tracer diffusion (Fig. 6) may therefore represent an overestimate of the region from which the tracer was actually taken up. As a result, FG injections into the midbrain and VP may not have labelled the majority of accumbal neurons projecting to these sites. This is particularly problematic when considering the possible collateralization of neurons

that project to both the VP and midbrain. For instance, the incomplete retrograde labelling of accumbal neurons projecting to the VP and midbrain may have resulted in an underestimate of the degree of collateralization to these sites. Although our tracer injections appeared to cover most of the VP and midbrain and resulted in the retrograde labelling of the vast majority of medium-sized neurons in the sampled areas (90-95%), it is impossible to rule out the problem of incomplete tracer uptake without preforming extensive control injections.

As mentioned earlier, another potential difficulty with retrograde tracing is the uptake of tracer by damaged fibers of passage. In the present study, accumbal neurons projecting to the midbrain may have been inadvertently labelled by Fluoro-Gold injections into the VP. Accumbal neurons that project to the midbrain course through the VP. Damage to these fibers of passage would likely have been produced by Fluoro-Gold injections into the VP. Since D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity is located in accumbal neurons that project to the midbrain, damage to their axons may have been responsible for the appearance of D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity in a significant number of accumbal neurons retrogradely labelled from the VP. One way to determine the degree to which damage to fibers of passage influenced our data would be to perform triple labelling studies. Accumbal neurons would be retrogradely labelled from both the midbrain and VP using different tracers, then D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity would be detected using a third fluorophore. A small number of triple labelled neurons would indicate that damage to fibers of passage is not responsible for the localization of D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity

in accumbal neurons retrogradely labelled form the VP. Another way to address the issue of damage to fibers of passage would be to perform anterograde tract tracing studies on accumbal neurons that have been labelled by intracellular injection of biocytin. This approach has been used elegantly in a recent study by Kawaguchi et al. (1990) to demonstrate the projectional nature of individual neurons in the dorsal striatum. The primary disadvantage of this technique is the small number of neurons that can be sampled.

CONCLUSIONS

1. Distribution of D1-Like Agonist Induced Fos_{2-16}-Like Immunoreactivity in the Forebrain of 6-OHDA-Lesioned Animals

Administration of the selective D1-like agonist CY 208-243 to animals with a unilateral 6-OHDA lesion resulted in rotational behaviour and a dramatic elevation of Fos_{2-16}-like immunoreactivity in the denervated forebrain. Fos_{2-16}-like immunoreactivity was located in the ipsilateral parietal cortex, striatum, nucleus accumbens, amygdala, lateral septal nucleus and olfactory tubercle, which very closely matches the distribution of D1 receptors in the forebrain revealed by autoradiography, *in-situ* hybridization and immunohistochemistry. Hence, these results suggest that the location of Fos_{2-16}-like immunoreactivity induced by CY 208-243 overlaps with the areas enriched with D1 receptors. The fact that pretreatment with the selective D1-like antagonist SCH 23390 abolished circling and the induction of Fos_{2-16}-like immunoreactivity in the forebrain produced by CY 208-243 indicates that D1-like agonist-induced circling and Fos_{2-16}-like immunoreactivity was mediated by the activation of D1-like dopamine receptors.

2. Localization of $Fos_{128-152}$-Like Immunoreactivity in the 6-OHDA Denervated Striatum

Destruction of the nigrostriatal pathway produced a long-lasting and widespread increase in the expression of striatal $Fos_{128-152}$-like immunoreactivity, which could be detected with a rabbit antibody raised against amino acids 128-152 (M-peptide) of the Fos molecule. The fluorescent tracer FG was used to retrogradely label striatonigral and striatopallidal neurons in order to establish the nature of striatal neurons which express $Fos_{128-152}$-like immunoreactivity in the 6-OHDA-denervated striatum. We found that nuclei expressing $Fos_{128-152}$-like immunoreactivity were frequently located in retrogradely labelled striatopallidal neurons but seldom in retrogradely labelled striatonigral neurons (Fig. 11 and 18). Western blot experiments indicated that the protein responsible for the persistent elevation of $Fos_{128-152}$-like immunoreactivity in striatopallidal neurons of the 6-OHDA-denervated striatum was the truncated form of FosB (Robertson et al., 1994).

3. Localization of D1-Like Agonist- and D2-Like Antagonist-Induced Fos_{2-16}-Like Immunoreactivity in Projections of the Nucleus Accumbens

In summary, D1-like agonists were found to elevate Fos_{2-16}-like immunoreactivity in accumbal projections to the VP and midbrain, whereas D2-like antagonists elevated Fos_{2-16}-like immunoreactivity preferentially in projections to the VP (Fig. 19). In contrast, striatal neurons projecting to the GP express few D1 receptors and as a consequence

Fig. 18. Proposed model for the localization of dopamine receptor-mediated Fos-like immunoreactivity increases in the basal ganglia. The previous studies indicate that, in the 6-hydroxydopamine-denevated striatum, selective D1-like receptor agonists increase Fos-like immunoreactivity primarily in striatonigral neurons. In contrast, selective D2-like receptor agonists fail to elevate Fos-like immunoreactivity in the 6-hydroxydopamine-denevated striatum. Instead, selective D2-like receptor agonists enhance Fos-like immunoreactivity expression in the ipsilateral globus pallidus. Unlike dopamine receptor agonists, D2-like receptor antagonists increase Fos-like immunoreactivity in the intact striatum, predominantly in striatopallidal neurons. In agreement with this later finding, the present studies demonstrate that $Fos_{128\text{-}152}$-like immunoreactivity in the 6-OHDA-denevated striatum is located primarily in the striatopallidal neurons.

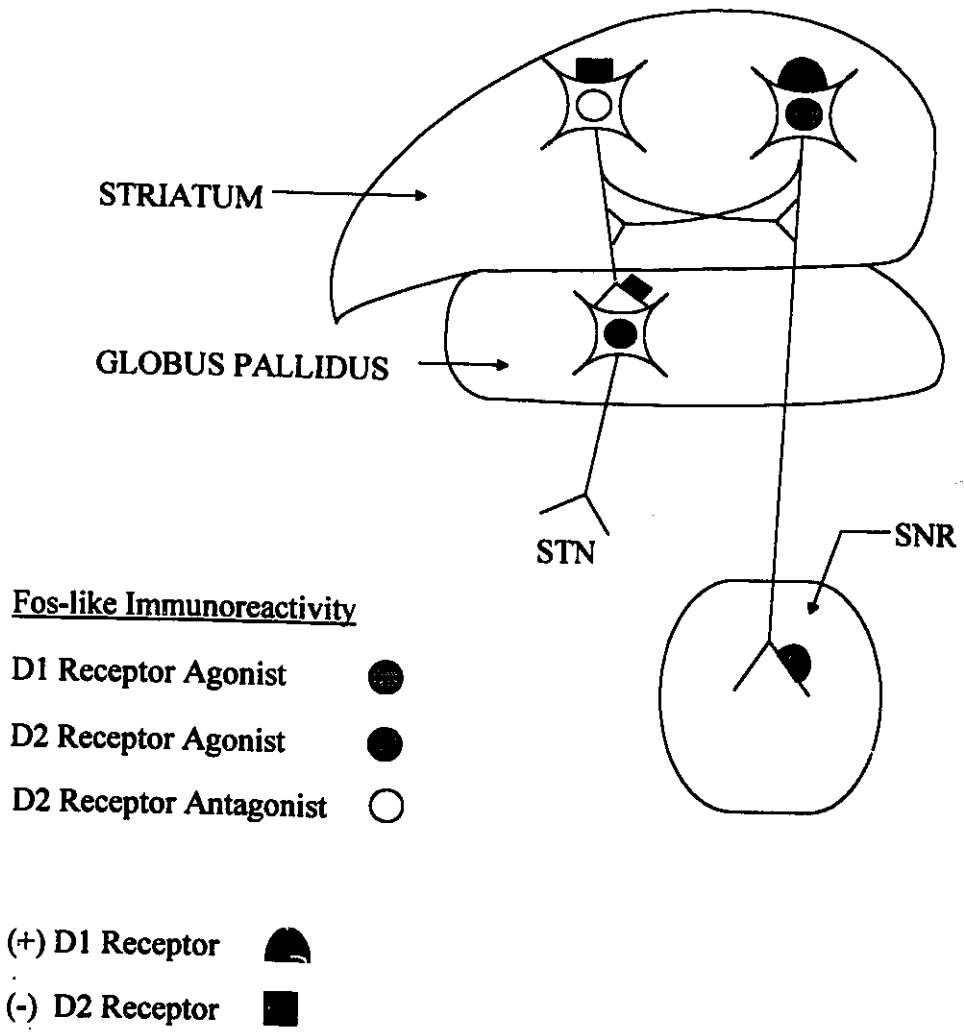
STRIATUM
GLOBUS PALLIDUS
STN
SNR
Fos-like Immunoreactivity
D1 Receptor Agonist
D2 Receptor Agonist
D2 Receptor Antagonist
(+) D1 Receptor
(-) D2 Receptor

Figure 18.

Fig 19. Proposed model for the localization of D1-like receptor agonist- and D2-like receptor antagonist-induced Fos_{2-16}-like immunoreactivity in accumbal afferents to the ventral pallidum and midbrain. D1-like receptor agonists (*d*-amphetamine and CY 208-243) increase Fos_{2-16}-like immunoreactivity in neurons of the nucleus accumbens core and shell that project to such midbrain structures as the substantia nigra pars reticulata (SNR), substantia nigra pars compacta (SNC) and ventral tegmental area (VTA). In addition, D1-like agonists increase Fos_{2-16}-like immunoreactivity in a separate population of accumbal neurons that project to the ventral pallidum. Thus, the majority of D1-like dopamine receptors in the core and shell regions of the nucleus accumbens are considered to reside on these two efferent populations. The facilitatory action of D1-like receptor agonists on Fos expression suggests that D1-like receptors may have an excitatory action over these neurons. D2-like receptor antagonists (haloperidol and clozapine) elevate Fos_{2-16}-like immunoreactivity preferentially in neurons of the nucleus accumbens core and shell which project to the ventral pallidum. Therefore, D2-like receptors in this structure are thought to be located predominantly on accumbopallidal efferents. The enhancement of Fos_{2-16}-like immunoreactivity after D2-like receptor blockade is consistent with an inhibitory effect of D2-like receptor stimulation on the activity of at least some accumbopallidal neurons.

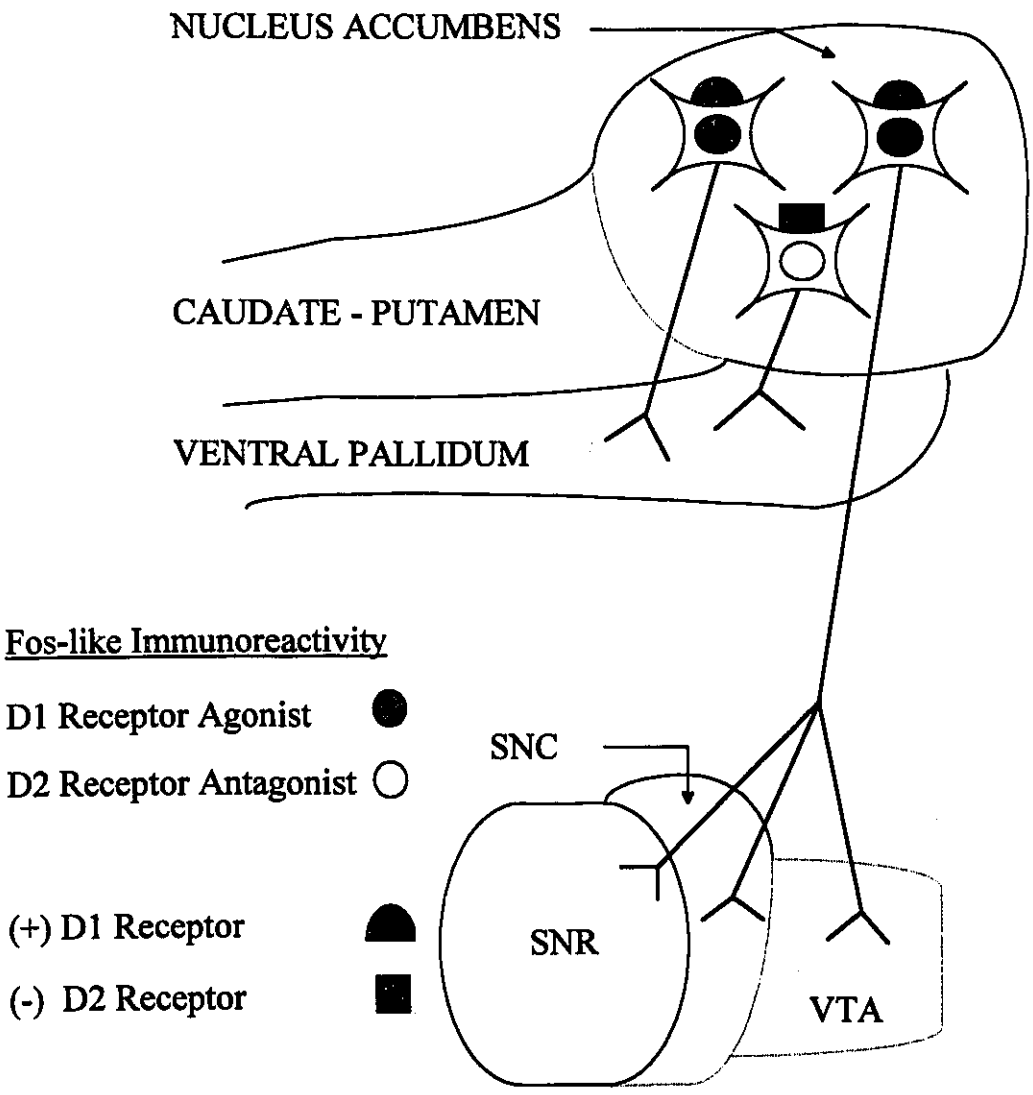
NUCLEUS ACCUMBENS
CAUDATE - PUTAMEN
VENTRAL PALLIDUM
Fos-like Immunoreactivity
D1 Receptor Agonist
D2 Receptor Antagonist
(+) D1 Receptor
(-) D2 Receptor
SNC
SNR
VTA

Figure 19.

seldom contain D1-like agonist-induced $Fos_{2\text{-}16}$-like immunoreactivity. Instead, striatopallidal neurons predominantly express D2 receptors and often contain D2-like antagonist-induced $Fos_{2\text{-}16}$-like immunoreactivity (Gerfen et al., 1990; Harrison et al., 1992; Robertson GS et al., 1992). Hence, the VP differs from the GP in that its striatal afferents are directly regulated by both D1 and D2 receptors. This proposal is supported by the results of electrophysiological studies in which the effects of selective D1-like and D2-like agonists on single unit activity in the GP and VP were compared. Maskowski and Napier (1991) have shown that the D1-like agonist SKF 38393 altered the activity of 52 of 61 neurons examined in the VP. In contrast, neurons in the GP are insensitive to these doses of SKF 38393, and at 20 mg/kg of SKF 38393, only 54% of recorded neurons were reported to be affected (Carlson et al., 1988). However, activity in both the VP and GP are altered by comparable doses of the selective D2-like agonist quinpirole. The high sensitivity of VP neurons to systemic administration of selective agonists for either D1- or D2-like receptors is therefore consistent with the results of the present study which suggest that the activity of accumbal efferents to the VP are directly regulated by both D1- and D2-like receptors. Dopamine receptors in the nucleus accumbens are considered to play an important role in mediating not only the reinforcing properties of stimulants such as cocaine and *d*-amphetamine but also the antipsychotic actions of neuroleptics. Consequently, the results of the present study suggest that accumbal projections to both the VP and midbrain may be important sites for the central actions of dopaminergic stimulants, whereas accumbal neurons that project to the VP may be vital for the antipsychotic effects of neuroleptics.

FUTURE WORK

1. Phenotypical Characterization of Accumbal Neurons that Display D1-Like Agonist- and D2-Like Antagonist-Induced Fos_{2-16}-Like Immunoreactivity

From my studies on the localization of D1-like agonist- and D2-like antagonist-induced Fos_{2-16}-like immunoreactivity in the nucleus accumbens, I have found that D1-like agonist-induced Fos_{2-16}-like immunoreactivity is present in both accumbal neurons projecting to the midbrain and VP. In contrast, D2-like antagonist-induced Fos_{2-16}-like immunoreactivity was found mainly in accumbal neurons projecting to the VP. As mentioned previously, these findings are compromised by several technical limitations of retrograde tract tracing, i.e. incomplete tracer uptake and damage to fibers of passage. One way to circumvent these problems would be to use neurochemical markers rather than retrograde tract tracing to distinguish between major classes of neurons in the nucleus accumbens. For example, accumbal neurons could be labelled using oligonucleotide probes complimentary to either D1 or D2 dopamine receptor mRNAs. Combining the detection of D1 and D2 receptor mRNAs by *in situ* hybridization could then be combined with the detection of D1-like agonist- and D2-like antagonist-induced Fos_{2-16}-like immunoreactivity by immunohistochemistry. In this way it would be possible to determine if D1-like agonists and D2-like antagonists elevate Fos_{2-16}-like immunoreactivity in accumbal neurons that express either D1 or D2 receptor mRNA. Since the nucleus accumbens contains a variety of neuropeptides, it should also be possible to double label accumbal neurons for neuropeptide mRNA and Fos_{2-16}-like

immunoreactivity. Enkephalin and dynorphin, two neuropeptides which are very abundant in the basal ganglia, are thought to be contained within distinct neuronal populations in the striatum. Prior investigation has shown that D2-like antagonist-induced Fos_{2-16}-like immunoreactivity is localized primarily within dorsal striatal neurons that express proenkephalin mRNA. If the same is true for the ventral striatum, D2-like antagonists should selectively elevate Fos_{2-16}-like immunoreactivity in neurons that express proenkephalin mRNA. In contrast, the D1 receptor is thought to reside primarily on striatal neurons that express prodynorphin mRNA. Consequently, D1-like agonist-induced Fos_{2-16}-like immunoreactivity should be located predominantly in accumbal neurons that express prodynorphin mRNA.

A second approach would simply be to use antibodies selective for either the D1 or D2 dopamine receptor to double label neurons which display D1-like agonist- and D2-like antagonist-induced Fos_{2-16}-like immunoreactivity. D1-like agonist-induced Fos_{2-16}-like immunoreactivity would be expected to be located primarily in accumbal neurons stained with the D1 receptor antibody, whereas D2-like antagonist-induced Fos_{2-16}-like immunoreactivity would be predicted to be located primarily in accumbal neurons labelled with D2 receptor antibody.

2. Identification of the D2-Like Receptor(s) Responsible for Clozapine-Induced Fos_{2-16}-Like Immunoreactivity

It has been well established that clozapine and haloperidol produce distinct patterns

of Fos_{2-16}-like immunoreactivity in the nucleus accumbens (Deutch et al., 1992; Nguyen et al., 1992; Robertson GS and Fibiger, 1992). Clozapine and haloperidol are thought to increase Fos_{2-16}-like immunoreactivity in the nucleus accumbens by blocking D2-like dopamine receptors (Robertson and Fibiger, 1992, Nguyen et al., 1992). Consequently, the distinct patterns of Fos_{2-16}-like immunoreactivity produced by these compounds in the nucleus accumbens may result from the blockade of different D2-like receptors. Molecular cloning studies have established that there are at least 3 types of D2-like receptors termed D2, D3 and D4. The D4 receptor is present only in very low amounts in rodent brain suggesting that it contributes little to clozapine- and haloperidol-induced *c-fos* expression. Haloperidol has a much higher affinity for the D2 than the D3 receptor, whereas clozapine has similar affinity for both the D2 and D3 receptor. Moreover, the pattern of clozapine-induced Fos_{2-16}-like immunoreactivity closely matches the distribution of the D3 receptor which is concentrated in the ventral striatum. This suggests that D3 receptor blockade may contribute significantly to clozapine-induced Fos_{2-16}-like immunoreactivity. Similarly, the pattern of haloperidol-induced Fos_{2-16}-like immunoreactivity matches well the distribution of the D2 receptor suggesting that D2 receptor blockade may mediate haloperidol-induced *c-fos* expression.

If these proposals are correct, it should be possible to selectively label neurons that contain haloperidol- and clozapine-induced Fos_{2-16}-like immunoreactivity by *in situ* hybridization using oligonucleotide probes complementary to D2 and D3 receptor mRNAs, respectively. Alternatively, antibodies against the D2 and D3 receptor may be used to double label neurons which express haloperidol- and clozapine-induced Fos_{2-16}-like immunoreactivity.

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