ANTIPSYCHOTIC-INDUCED IMMEDIATE-EARLY GENE EXPRESSION IN THE LIMBIC SYSTEM

by

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IN THE NAME OF GOD

THE COMPASSIONATE, THE MERCIFUL

DEDICATION

To my devoted parents

ABSTRACT

The mechanism by which the atypical neuroleptic clozapine is able to relieve the symptoms of schizophrenia without causing extrapyramidal side effects that are characteristic of most other antipsychotic drugs is unclear. *c-fos* is a proto-oncogene that encodes a 55 kDa phosphoprotein, Fos, which is thought to be an activity marker in the central nervous system. Recently, it has been shown that clozapine and haloperidol produce distinc patterns of increased *c-fos* expression in the rat forebrain. Haloperidol induces Fos-like immunoreactivity (FLI) in the nucleus accumbens, dorsolateral striatum and lateral septal nucleus, whereas clozapine preferentially increases in FLI in limbic structures such as the prefrontal cortex, nucleus accumbens, mediolateral striatum and lateral septal nucleus. These findings suggest that clozapine's unique therapeutic profile may be related to its failure to induce FLI in the striatum as well as its idiosyncratic actions in the medial prefrontal cortex. Given the significant clinical implications of these results, the present study attempted to identify these receptors which mediate the different effects of clozapine and haloperidol on *c-fos* expression.

The dramatic effects of haloperidol on FLI in the dorsolateral striatum, a region implicated in the control of movement, suggest that these increases may occur in neurons which mediate haloperidol-induced catalepsy. Thus, the first major goal of this study was to determine whether the dose-response relationships for haloperidol-induced *c-fos* expression in the dorsolateral striatum and catalepsy are similar. The dose-response curves for haloperidol-induced increases in FLI, *c-fos* mRNA and catalepsy yielded comparable EC_{50} and E_{max} values suggesting that haloperidol-induced *c-fos* expression in

the dorsolateral striatum occurs in those neurons which mediate the cataleptic effects of this antipsychotic.

Both clozapine- and haloperidol-induced increases in FLI are mediated by the blockade of D_2 receptors. However, the different patterns of FLI induced by haloperidol and clozapine suggest that these neuroleptics may block distinct dopamine receptors. Consequently, a second major goal of this study was to determine whether clozapine- and haloperidol-induced FLI are mediated by different dopamine receptors. Indeed, the distribution of haloperidol-induced FLI closely matches that for the D_2 receptor while clozapine-induced FLI has excellent correspondence with those areas which express the D_3 receptor. Hence, the blockade of distinct dopamine receptors may account for the ability of these compounds to elevate FLI in different regions of the forebrain. To determine the relative roles of D_2 and D_3 receptors blockade in haloperidol- and clozapine-induced FLI, the ability of prior administration of relatively selective D_3 receptor agonists (7-OH-DPAT and quinpirole) to competitively reduce these increases were compared. Both quinpirole (0.5 mg/kg, 1 mg/kg, s.c.) and 7-OH-DPAT (0.05 mg/kg, 0.5 mg/kg, s.c.) produced significant decreases in clozapine (20 mg/kg, s.c.)-induced FLI in the prefrontal cortex, nucleus accumbens, lateral septal nucleus and islands of Calleja. In contrast, haloperidol-induced FLI in the dorsolateral striatum was only attenuated when a high dose of quinpirole (1 mg/kg, s.c.) was administrated before a low dose of haloperidol (0.1 mg/kg, s.c.). These results suggest that D_3 receptor blockade may contribute to clozapine-induced increases in FLI in limbic structures while

D_2 receptor blockade may be responsible for haloperidol-induced FLI in the dorsolateral striatum.

The fact that neuroleptics must be administered chronically in order to generate both extrapyramidal side effects and an optimal therapeutic response calls into question the relevance of acute changes in FLI for these slowly developing events. FLI cannot be used to identify neurons activated by chronic neuroleptic administration because the increase in FLI produced by an acute antipsychotic injection is dramatically reduced following repeated neuroleptic administration. In contrast, expression of the immediate-early gene product ΔFosB is persistently elevated in the striatum by chronic haloperidol administration. This suggests that ΔFosB-like immunoreactivity may be used to identify neurons activated by chronic antipsychotic administration. Since typical and atypical neuroleptics elevate FLI in different regions of the forebrain acutely, a final series of studies were conducted to determine whether typical (haloperidol) and atypical (clozapine) antipsychotics produce distinct patterns of elevated ΔFosB-like immunoreactivity in the forebrain after chronic administration.

Administration of haloperidol (2 mg/kg/day) for 16 days produced a homogeneous elevation of neurons which displayed ΔFosB-like immunoreactivity in the ventral, medial and dorsolateral aspects of the striatum. Chronic haloperidol administration did not enhance ΔFosB-like immunoreactivity in the prefrontal cortex and lateral septal nucleus. Repeated administration of clozapine (20 mg/kg/day) for 16 days elevated ΔFosB-like immunoreactivity not only in the ventral striatum but also in the prefrontal cortex and lateral septal nucleus. However, this compound had weak effects on ΔFosB-like

immunoreactivity in the dorsolateral striatum. These results suggest that a preferential action on limbic structures such as the prefrontal cortex, ventral striatum and lateral septal nucleus may account for the ability of chronic clozapine administration to reduce the symptoms of schizophrenia without generating extrapyramidal side effects.

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LIST OF ABBREVIATION

CNS	central nervous system
CRB	Cambridge Research Biochemicals
CT	computerized tomography
DAB	diaminobenzidine
D_1-like receptors	dopamine receptor positively coupled to adenylate cyclase
D_2-like receptors	dopamine receptor negatively coupled to adenylate cyclase
DL-Str	dorsolateral striatum
EPS	extrapyramidal side effects
FLI	Fos-like immunoreactivity
GABA	δ-aminobutyric acid
gr	gram
hr	hour
IEG	immediate-early gene
i.p.	intraperitoneal
ISHH	*in situ* hybridization histochemistry
kg	kilogram
LSN	lateral septal nucleus
mg	miligram
min	minute
ML-Str	mediolateral striatum
MRI	magnetic resonance imaging
NAc	nucleus accumbens
PBS	phosphate-buffered saline
PET	positron emission tomography
PFA	paraformaldehyde
PFC	prefrontal cortex
6-OHDA	6-hydroxydopamine
s.c.	subcutaneous

INTRODUCTION

I. BIOCHEMICAL ASPECTS OF SCHIZOPHRENIA

A. Clinical perspective

Schizophrenia is a psychotic disorder that affects about 1% of the population and usually first arises during adolescence disrupting perception, thought, language, and behaviour. The symptoms of schizophrenia have recently been divided into two categories termed type I and type II symptoms (Crow, 1989). Type I or positive symptoms include delusions, hallucinations, and thought disorder; type II or negative symptoms include affective flattening, apathy and poverty of speech. One way to distinguish between these two types of symptoms is by the response to antipsychotic drugs: Type I symptoms are usually more responsive than type II symptoms.

In terms of etiology of schizophrenia, there is some evidence for a genetic contribution. Holzman et al. (1990) have determined that the incidence of schizophrenia in the parents of schizophrenics is about 4 percent, that in their siblings it is about 8 percent and that in their children it is about 12 percent. If both parents are schizophrenic, the chance of an offspring having the disorder is about 40 percent (Holzman et al., 1990). Brain morphology may be altered in some patients with schizophrenia, particularly those unresponsive to antipsychotic drugs. Computerized tomography (CT) and magnetic resonance imaging (MRI) have demonstrated that these schizophrenics display ventricular enlargement suggestive of neuronal loss (Pfefferbaum et al., 1991).

Perhaps the most popular theory regarding the cause of schizophrenia is neurochemical. According to this theory, schizophrenics suffer from imbalances in the activity of neurotransmitter system(s) that regulate cognitive, perceptual and emotional processes.

B. Dopamine hypothesis of schizophrenia

The dopamine hypothesis of schizophrenia is the most fully developed of several neurochemical hypotheses and is the basis for much of the rationale for drug therapy. According to this hypothesis, schizophrenics suffer from excessive activity of mesocortical and/or mesolimbic dopamine systems. This hypothesis is supported by several lines of circumstantial evidence. Firstly, compounds which increase dopaminergic neurotransmission such as amphetamine and cocaine produce schizophrenic-like symptoms (paranoia, hallucinations). Similarly, the dopamine precursor L-Dopa has also been reported to produce psychotic side effects in patients with Parkinson's disease. Secondly, antipsychotics are potent dopamine receptor antagonists. Indeed, there is a strong correlation between the affinity of an antipsychotic for the D_2 dopamine receptor and the amount of the drug which is required to control the symptoms of schizophrenia (Seeman, 1981; Farde et al., 1989). Thirdly, D_2-like dopamine receptor binding sites have been found postmortem to be increased in the brains of schizophrenics that have not been treated with antipsychotic drugs (Seeman et al., 1990). In contrast, the density of D_1-like receptors is normal in schizophrenics (Joyce et al., 1988). Positron emission tomography (PET) approaches have also shown

increased dopamine D_2-like receptor densities in the brains of untreated schizophrenics (Seeman et al., 1990).

II. ANATOMY OF THE BASAL GANGLIA AND ASSOCIATED DOPAMINE SYSTEMS

A. Dopaminergic pathways

Using histofluorescence techniques for visualizing catecholamines and immunohistological localization of tyrosine hydroxylase, the rate-limiting enzyme (Shiman et al., 1971) in catecholamines biosynthesis, it has been shown that dopamine is the principal neurotransmitter in four major neural systems in the brain (Dahlström et al., 1964; Fallon et al., 1978; Graybiel et al., 1983; Lindvall and Björklund., 1983). The largest of these neuronal systems is the nigrostriatal pathway, which originates in the midbrain substantia nigra complex and projects to the dorsal striatum (caudoputamen). Degeneration of this pathway leads to Parkinson's disease (Hornykiewicz, 1966). The mesolimbic system, relatively spared in Parkinson's disease, arises in the midbrain ventral tegmental area and innervates the ventral striatum (nucleus accumbens and olfactory tubercle) and parts of the limbic system. This system is thought to influence motivated behaviours, including motor activity related to reward (Koob and Bloom, 1988; Koob, 1992a; 1992b). The ventral tegmental area also gives rise to the smaller mesocortical pathway, which projects to the frontal cortex, and may be involved

in certain aspects of learning and memory (Le Moal and Simon, 1991). The fourth dopamine-containing pathway is the hypothalamic tuberoinfindibular system, which innervates the pituitary stalk and regulates prolactin secretion (Fuxe et al., 1969).

B. Dorsal striatum vs ventral striatum: structure and function

The striatum is divided into dorsal and ventral regions based on a variety of anatomical and behavioral distinctions. The dorsal striatum (caudate-putamen) regulates sensorimotor processes while the ventral striatum (nucleus accumbens and olfactory tubercle) controls affective and motivational aspects of behaviour (Delong, 1990; Le Moel and Simon, 1991). One way to distinguish ventral from dorsal striatum is by the efferent and afferent connections of these regions. For instance, the nucleus accumbens receives telencephalic connections from the hippocampus, amygdala, medial prefrontal and entorhinal cortices while the dorsal striatum receives afferents from sensory/motor cortex (Kelley and Domesick, 1982; Groenwegan et al., 1987; McGeorge and Faull, 1989). Furthermore, the nucleus accumbens is innervated by dopaminergic neurons originating from the ventral tegmental area whereas dopaminergic projections to the dorsal striatum arise from the substantia nigra pars compacta (Dahlström and Fuxe, 1964; Dragunow and Faull, 1989).

In terms of their efferent connections, both the dorsal striatum and nucleus accumbens project to the pallidum and midbrain, however, the nucleus accumbens also projects to a variety of limbic nuclei, e.g. amygdala, bed nucleus of stria terminalis,

sublenticular innominate and lateral hypothalamus (Swanson and Cowan, 1975; Conrad and Pffaf, 1976; Nauta et al., 1978; Zahm and Heimer, 1993).

The nucleus accumbens has been further divided into shell and core parts, based on the distribution of neuropeptides and other neuroactive substances including cholecytokinin, angiotensin II and neurotensin (Voorn et al., 1989; Heimer et al., 1991). Like the dorsal striatum, core neurons project to the ventral pallidum and ventral mesencephalon while accumbal projections to the limbic system arise from the shell (Zahm and Brog, 1992).

III. NEUROLEPTIC DRUGS

A. Biochemical aspects of antipsychotic drugs

Antipsychotic drugs are currently the most effective treatment for schizophrenia. Unfortunately, most of these drugs known as typical antipsychotics produce extrapyramidal side effects (EPS) resembling Parkinson's disease (dystonia, bradykinesia, akathesia and dyskinesia) which limit their clinical utility. Clozapine is unique in that it reduces the symptoms of schizophrenia without producing EPS. Furthermore, some patients who are unresponsive to typical neuroleptics such as chlorpromazine and haloperidol benefit from clozapine (Kane et al., 1988; Meltzer et al., 1990). Another attractive feature of clozapine is that it reduces both the positive and negative symptoms of schizophrenia (Meltzer, 1989). This is in contrast to most other neuroleptics which

are only effective against the positive symptoms of schizophrenia (Borison et al., 1983; Claghorn et al., 1987). Indeed, clinical comparisons of clozapine and chlorpromazine suggest that clozapine's utility in the treatment of neuroleptic-resistant schizophrenics may be related to its unique ability to reduce negative psychotic symptoms (Meltzer, 1989). On the basis of *in vivo* imaging and neuropsychological studies, Weinberger (1988) has proposed that hypoactivity in the frontal cortex may be responsible for the negative symptoms of schizophrenia. Hence, clozapine may improve negative symptoms by enhancing activity in the frontal cortex.

B. Clozapine: Biochemical effects

CSF levels of dopamine, noradrenaline and serotonin metabolites are altered by 20 days treatment with clozapine (100 mg/kg, p.o.) suggesting that clozapine influences a variety of monoamine systems (Ackenheil, 1989). Radioligand binding studies support this finding by demonstrating that clozapine has moderate to high affinity for dopamine D_1, D_2, D_4, $5HT_{2A}$, $5HT_3$, muscarinic, α_1-adrenergic and histamine H_1 receptors (for review see Baldessarini and Frankenburg, 1991). Indeed, clozapine's high affinity for muscarinic and serotonin receptors has been proposed to underlie its low propensity to induce EPS (Stille et al., 1971; Synder et al., 1974). In the case of clozapine's antipsychotic actions, its ability to block D_2 dopamine receptors were initially thought to be crucial (Creese and Synder, 1976). However, the advent of molecular cloning techniques, resulting in the identification of several novel dopamine receptor subtypes, have forced a re-evaluation of this notion.

IV. Dopamine receptor subtypes

Historical background: 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 et al., 1979). The parathyroid gland was considered to contain just the D_1 dopamine receptor while the pituitary was proposed to contain only the D_2 receptor. Since dopamine activated adenylate cyclase activity in the parathyroid, the D_1 receptor was proposed to be linked positively to adenylate cyclase. In contrast, because dopamine inhibited adenylate cyclase activity in the pituitary, the D_2 receptor was hypothesized to be linked negatively to this enzyme. The two dopamine receptor theory was strengthened considerably by the development of selective compounds for the D_1 and D_2 receptor. The benzazepine derivative SCH 23390 is a selective D_1 receptor antagonist while SKF 38393 and CY 208-243 are preferential D_1 receptor agonists. Raclopride is a selective D_2 receptor antagonist while quinpirole is a selective D_2 agonist (Ögren et al., 1986). Using radiolabelled versions of these compounds, it has been possible to study the distribution of D_1 and D_2 receptors in the brain by autoradiography.

Molecular cloning studies have revealed the existence of 5 distinct dopamine receptor subtypes termed D_1, D_2, D_3, D_4 and D_5 (for review see Sibley and Monsma, 1992). On the basis of structure and pharmacological similarities, these 5 receptors have been categorized as either D_1- or D_2-like receptors. The D_1 and D_5 receptors are called D_1-like receptors because of their similar amino acid composition and high affinity for the benzazepine analog SCH 23390. The D_2, D_3 and D_4 receptors are termed D_2-like

receptors because they are structurally alike and all have high affinity for antipsychotic drugs.

A. Dopamine D_{1a} (D_1) and D_{1b} (D_5) receptors

The gene for the human D_1 receptor encodes a protein of 446 amino acids with a molecular mass of 49,296 when expressed in cell lines. The D_{1a} receptor is the rodent homolog of the human D_1 receptor. Both receptors display high affinity and saturable binding of the benzazepine ligand [^{3}H]SCH 23390 and couple positively to adenylyl cyclase-important hallmarks of the D_1 receptor (Dearry et al., 1990).

Recently, another clone has been isolated from both human and rat genetic libraries that is structurally and functionally homologous to the dopamine D_1 receptor termed the D_5 receptor (Tiberi et al., 1991). The D_{1b} receptor is the rodent homolog of the human D_5 receptor. Dopamine binds to this receptor with an affinity that is 3- or 10-fold higher than the affinity with which dopamine binds to the D_1 receptor (Tiberi et al., 1991). The human D_5 receptor gene codes for a protein of 477 amino acids with a molecular mass of 52,950. Amino acid homology between members of the dopamine D_1-like receptors is presented in Fig. 1. Several features of D_1-like receptors (D_1 and D_5) are common to the super-family of G protein-linked receptors. These include seven putative transmembrane domains connected by 3 intra- and extracellular loops. These receptors also have extracellularly directed consensus sites for asparagine-linked (N-linked) glycosylation. The binding sites of catecholamine agonist and antagonist ligands consist of an aspartate in the third transmembrane domain and two serine residues in the

fifth transmembrane domain. Another common feature of G protein-linked receptors is the presence of consensus sites for phosphorylation by regulatory kinases. Thus, these receptors have multiple putative sites at which protein kinases (PKA, PKC) can phosphorylate the receptor protein.

At present, the greatest difference observed in the characteristics of D_1/D_{1a} and D_5/D_{1b} receptors relates to their distribution within the central nervous system. Northern blot and *in situ* hybridization histochemistry analyses have demonstrated that the mRNA for the D_{1a} receptor is present in relatively high amounts in the rat caudate-putamen, nucleus accumbens and olfactory tubercle (Dearry et al., 1990). These regions also contain relatively high levels of D_1-like receptor binding (Asio et al., 1987). In contrast, D_5/D_{1b} receptor mRNA is expressed most highly within the hippocampus, lateral mamilliary bodies, and the parafascicular nucleus of the thalamus (Meador-Woodruff et al., 1992). Hence, the disparate distributions of the D_{1a} and D_5/D_{1b} receptors are suggestive of differences in the functional consequences of activation of these receptors within the brain.

B. The dopamine D_2 receptor

The D_2 receptor was first among the dopamine receptors to be cloned. Exploiting the known homologies among the G protein-linked receptor family, Bunzow et al. (1988) used a β-adrenoreceptor probe to isolate clones encoding putatively novel receptors from a rat genomic library. All of these clones encoded a protein 415 amino acids that when expressed in cell lines exhibited pharmacological characteristics expected for a D_2

receptor. Hydropathy analysis of this protein indicated the presence of seven transmembrane domains that contain 3 intracellular and extracellular loops, a long extracellular N-terminus and a short C-terminus which projects to the cytosol (Fig. 1). The N-terminus contains consensus sequences for three potential N-linked glycosylation sites while the C-terminus possesses a Cys residue that may be a site for palmitoylation (Dolhamn et al., 1991). Another important feature of this receptor is the presence of a large third intracellular loop which contains one potential site for phosphorylation by the cAMP-dependent protein kinase A. Northern blots and *in situ* hybridization histochemistry studies indicate that this dopamine receptor is located in nearly all regions innervated by dopaminergic neurons (Bunzow, 1988). The areas of highest expression are the caudate putamen, nucleus accumbens, and olfactory tubercle. In addition, D_2 receptor mRNA is expressed by dopamine neurons in the substantia nigra pars compacta and ventral tegmental area. Within the striatum, D_2 receptor mRNA is expressed by 50% of medium-sized neurons and has been also observed in large-diameter neurons thought to be cholinergic interneurons (Le Moine et al., 1990).

Depending on the cell line, the D_2 receptor has been shown to couple to a variety of second messenger system (Sibley and Monsma, 1992). For instance, the D_2 receptor has been shown to reduce adenylyl cyclase activity, prolactin release, Ca^{2+} levels and K^+ conductance with the latter resulting in hyperpolarizing. Furthermore, when the dopamine D_2 receptor is expressed in Chinese hamster ovary cell lines, it potentiates ATP-mediated arachidonic acid release (Kenterman et al., 1991), a common feature of several G_i coupled receptors (Felder et al., 1991).

Radioligand binding studies performed on cells transfected with the D_2 receptor have confirmed that this receptor displays a pharmacological profile consistent with that of a D_2-like receptor. That is, the rank order of K_i values for displacement of [^{3}H]spiperone is spiperone > (+)-butaclamol > haloperidol > sulpiride >> (-)-butaclamol (Senogles et al., 1988). The dopamine D_1-selective antagonist SCH 23390 and the serotonin $5HT_2$ antagonist ketanserine were much less potent at displaying [^{3}H]spiperone binding. Furthermore, these binding data were fit best by assuming the presence of only one class of binding sites.

After the initial cloning of the D_2 dopamine receptor, it was discovered that two isoforms of the D_2 receptor which differ in length by 29 amino acids are generated by alternative splicing. The splice occurs within the third cytoplasmic loop of the D_2 receptor. Both isoforms termed D_{2L} and D_{2S} share a similar pharmacology and distribution within the brain (Gandelman et al., 1991).

C. The dopamine D_3 receptor

Sokoloff et al. (1990) used probes derived from the D_2 receptor sequence to clone a novel dopamine receptor cDNA from a rat library encoding a protein of 446 amino acids in length termed the D_3 receptor. Similar to the D_2 receptor, the D_3 receptor contains a relatively large third cytoplasmic loop and a short C-terminus as well as consensus sequences for N-linked glycosylation sites and a cAMP dependent phosphorylation site in the third cytoplasmic loop and a conserved Cys residue at the C-terminus. Radioligand binding studies on cells transfected with the D_3 receptor indicated

that AJ76 and UH 232 are somewhat selective D_3 receptor antagonists (Sokoloff et al., 1990) while quinpirole is a relatively selective D_3 receptor agonist (Sibley and Monsma, 1992). However, more recently, the ability to preferentially express a single dopamine subtype in cell lines has led to the identification of 7-OH-DPAT (7-Hydroxy-N-N'-di-n-propyl-2-aminotetralin) as a selective ligand for the D_3 receptor (Lévesque et al., 1992). Radioligand-binding experiments have shown that the concentration of 7-OH-DPAT ([^{3}H]7-OH-DPAT) required to competitively displace binding at the D_3 receptor is much lower than that needed to displace binding at D_1, D_2, and D_4 receptors (Fig. 2).

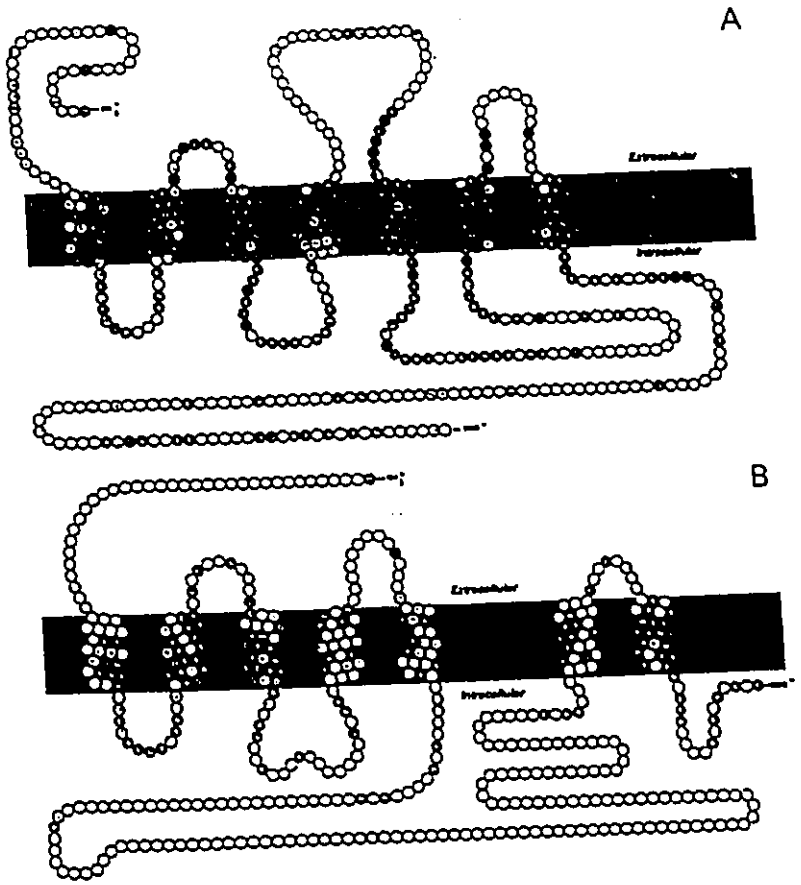


Fig. 1. Amino acid identity between dopamine receptor subtypes. Shown here are the hypothetical transmembrane (TM) organization of the D_1-like (panel A) and the D_2-like (panel B) dopamine receptors. Each circle represents an amino acid residue. Blackened circles indicate amino acid residues that are conserved between all three receptor subtypes, and blank circles indicate residues that are not conserved between any of the three receptor types. In many instances, gaps were necessarily inserted to preserve the alignment of these receptors. (A) The structure of the human D_{1A} dopamine receptor subtype is depicted (Dearry et al., 1990). Residues that are conserved in this receptor with the D_{1B} and D_5 (Tiberi et al., 1991) receptor are showed according to the description above. (B) The structure of the rat D_2 receptor is depicted and compared with the D_3 and the D_4 receptors (Bunzow et al., 1988) according to description above.

In addition, [^{3}H]7-OH-DPAT binding sites are located in the same regions which display high levels of D_3 receptor mRNA further suggesting that 7-OH-DPAT is a selective D_3 receptor ligand (Lévesque et al., 1992) (Figures 3, 4). Northern blot analysis indicates that D_3 receptor mRNA is about one order of magnitude less abundant than D_2 receptor mRNA (Sokoloff et al., 1990, Lévesque et al., 1992). D_3 receptor mRNA is expressed predominantly in limbic regions of the brain such as the olfactory tubercle, nucleus accumbens, island of Calleja and hypothalamus. This distribution pattern has led to the suggestion that the D_3 receptor mediates dopaminergic control of cognitive and emotional functions that may be related to antipsychotic therapy using dopamine antagonists.

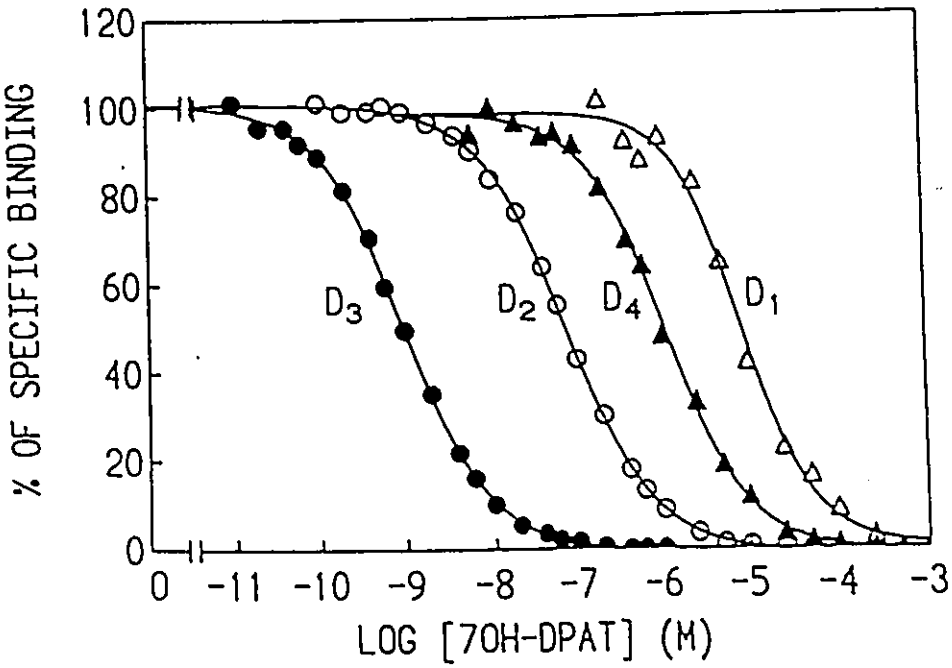


Fig. 2. [^{3}H]7-OH-DPAT as a selective radioligand for the D_3 receptor. The selectivity of 7-OH-DPAT is shown in competition experiments of ligand binding to D_1, D_2, D_4 receptors expressed in transfected CHO cells (Taken from Lévesque et al., 1992).

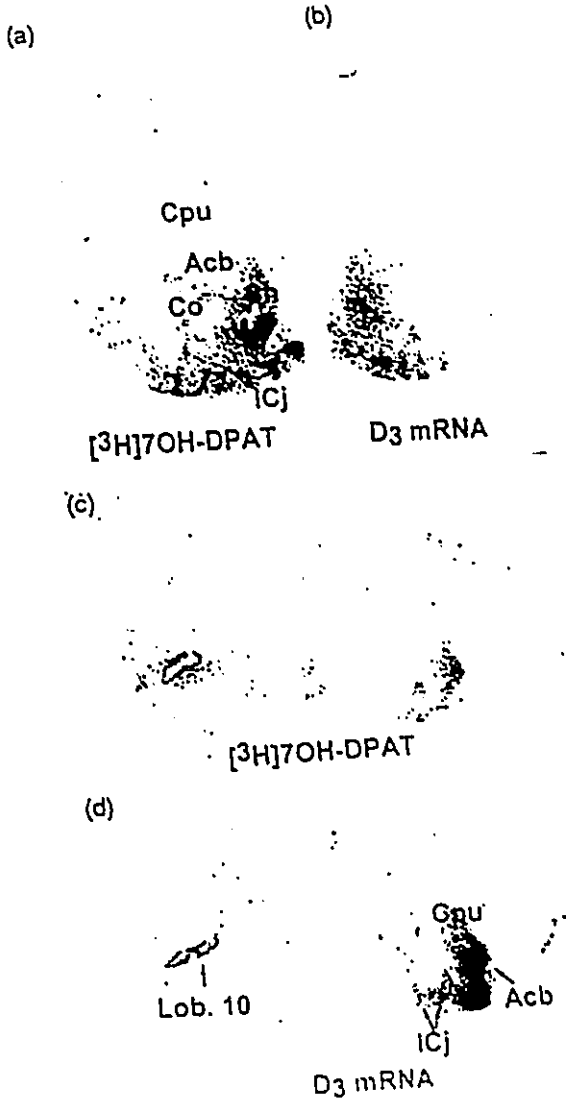


Fig. 3. Comparison of autoradiographic localization of [^{3}H]7-OH-DPA^T binding to rat brain sections (a, c) with the distribution of D_3 receptor mRNA measured by *in situ* hybridization (b, d). Sections at similar levels were either incubated with [^{3}H]7-OH-DPAT (0.5 nM) or hybridized with a specific ^{32}P-labeled complementary RNA probe. Note in both cases the labelling of the island of Calleja (ICj), of the shell part of nucleus accumbens (Acb), and of lobule 10 in cerebellum (Lob. 10). Other parts of the striatal complex, i.e., caudate putamen (CPu) and the core part of nucleus accumbens (Co) are only sparingly labeled (Adapted from Niznik, 1994).

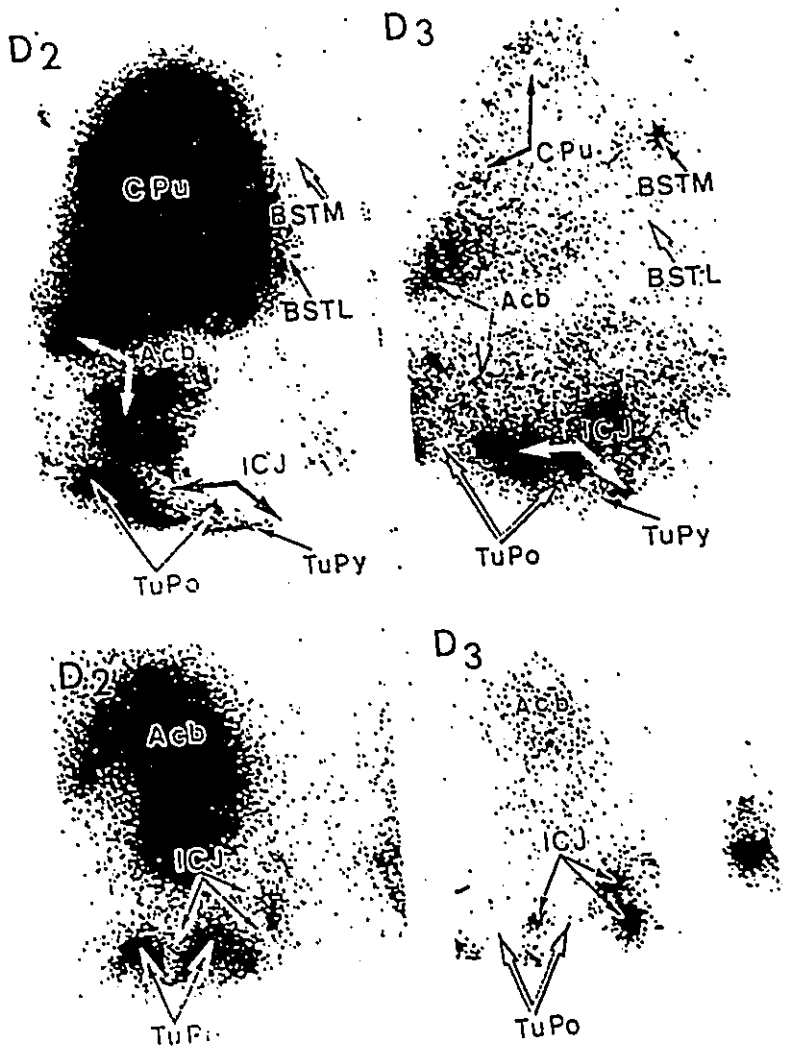


Fig. 4. Compared distribution of D_2 and D_3 receptor mRNAs established by *in situ* hybridization in sagittal (top) and frontal (bottom) sections of rat telencephalon. Note the complementary distributions of the two transcripts in the ventral striatum, particularly at the level of the olfactory tubercle, islands of Calleja and bed nucleus of stria terminals. Abbreviations: Acb: accumbens nucleus; BSTL and BSTM: bed nucleus of stria terminals, lateral or medial part; CPu: caudate putamen; ICj: islands of Calleja; TuPo and TuPy: polymor and pyramidal layers of the olfactory tubercle (Adapted from Niznik, 1994).

D. The dopamine D_4 receptor

The last dopamine receptor to be cloned in the D_2 subfamily is the D_4 receptor which was identified by screening of a human neuroblastoma cell cDNA library using a rat D_2 receptor cDNA probe (Van Tol et al., 1991). The D_4 receptor comprises a protein of 387 residues in length with seven putative membrane-spanning domains. The proposed D_4 receptor membrane topography is similar to that seen with the D_2 and D_3 receptors, but like the human D_3 receptor, the dopamine D_4 receptor has a slightly smaller third cytoplasmic loop. The D_4 receptor contains one potential site for N-linked glycosylation in the N-terminus and one consensus sequence cAMP-dependent phosphorylation site in the third cytoplasmic loop. Similar to D_2 and D_3 receptors, there is a conserved Cys residue in the C-terminus of the D_4 receptor. Radioligand binding studies have shown that the D_4 receptor displays very high affinity for the atypical antipsychotic clozapine. The concentration of D_4 receptor mRNA in rat brain is very low and it is only detectable by polymerase chain reaction (PCR) amplification (O'Malley et al., 1992). The areas of highest D_4 expression include the frontal cortex, midbrain, amygdala and medulla, with lower levels observed in the striatum and olfactory tubercle.

V. NEUROANATOMICAL SITES OF ANTIPSYCHOTIC ACTION

A. Fos as a metabolic marker in the brain

Neuronal expression of Fos, the protein product of the immediate-early gene *c-fos*, is increased by a wide variety of pharmacological and physiological stimuli in regions of the central nervous system (CNS) thought to be activated by these treatments (Sagar et al., 1988; Morgan and Curran, 1989). This has led to the proposal that it might be possible to use Fos immunohistochemistry to map functional pathways in the CNS (Sagar et al., 1988; Dragunow and Faull, 1989). Consistent with this proposal, pharmacological studies suggest that immunohistochemical detection of Fos may be used to identify potential neuroanatomical sites of drug action (Nakajima et al., 1989; Presely et al., 1990). Since most antibodies used to detect Fos also recognize other Fos-related antigens, the term Fos-like immunoreactivity (FLI) will be used rather than Fos immunoreactivity.

After translation in the cytoplasm, Fos is rapidly translocated to the nucleus where it dimerizes with Jun, the protein product of the immediate-early gene (IEG) *c-jun* (Curran et al., 1984). The heterodimerization of Fos and Jun is mediated by a conserved domain called the "leucine zipper". The Fos / Jun dimer is a transcriptional factor known as AP-1 that binds with high affinity and specificity to genes that contain a consensus sequence of DNA (-TGACTCA-) called the AP-1 binding site. AP-1 binding facilitates transcription of the target gene by an unknown mechanism (Sheng et al., 1990). In contrast, heterodimers of Fos/JunB (the protein product of the IEG *junB*)

represse transcription of genes with AP-1 sites in their promoter region (Chiu et al., 1989). There are three mammalian Jun proteins (Jun, JunB, JunD) (Ryder and Nathans, 1988; Ryder et al., 1988) and at least four Fos family members (Fos, FosB, Fra1, Fra2) (Cohen and Curran, 1988; Cohen et al., 1989; Zerial et al., 1989; Matsui et al., 1990; Nishina et al., 1990). Since each member of the Fos family can dimerize with each member of the Jun family, there are a large number of possible AP-1 combinations.

B. *c-fos* induction kinetics

The time-course for *c-fos* induction is the same regardless of the stimulus or tissue type. Transcriptional activation occurs within 5 minutes (Greenberg et al., 1985) and reaches peak values at 35-45 minutes post-stimulation (Muller et al., 1984). However, because *c-fos* mRNA is unstable it has a relatively short half-life of about 12 minutes.

C. Neuroleptics increase *c-fos* expression in the forebrain: contrasting effects of haloperidol and clozapine

Recently, it has been shown that alterations in dopaminergic neurotransmission dramatically increase neuronal expression of Fos in the nucleus accumbens and dorsolateral striatum (Robertson et al., 1992; Dragunow et al., 1989; Graybiel et al., 1990). Stimulants such as cocaine and d-amphetamine elevate FLI by increasing D_1 receptor activity, whereas antipsychotics like haloperidol and raclopride increase FLI and *c-fos* mRNA by blocking D_2 receptors (Graybiel et al., 1990; Miller, 1990; Deutch et al., 1992; Bahat et al., 1993). Retrograde tracing studies have shown that

psychostimulants increase FLI predominantly in striatonigral neurons, whereas haloperidol-induced FLI is located chiefly in striatopallidal neurons (Robertson et al., 1992; Cenci et al., 1992). These results are consistent with studies indicating that D_1 receptors are situated principally on striatonigral neurons while D_2 receptors are located primarily on striatopallidal neurons (Gerfen et al., 1990; Harrison et al., 1990). Furthermore, these findings are consistent with the proposal that the ability of dopamine to activate striatonigral neurons and inhibit striatopallidal neurons is mediated by the largely separate localization of D_1 and D_2 receptors on these outputs (Weick et al., 1987; Gerfen et al., 1990; Robertson et al., 1992). Furthermore, it has been reported that the typical neuroleptic haloperidol increases the number of neurons which display FLI in the striatum (Dragunow et al., 1990; Deutch et al., 1992; Robertson and Fibiger, 1992; Robertson et al., 1995). The selective D_2 receptor antagonist raclopride has similar effects, whereas the dopamine D_1 receptor antagonist SCH 23390 actually reduces the number of neurons that contained FLI in the striatum (Dragunow et al., 1990; Robertson and Fibiger, 1992). Unlike haloperidol, clozapine, an atypical neuroleptic does not increase FLI in the dorsolateral striatum (Deutch et al., 1991; Robertson and Fibiger, 1992). Instead, clozapine-induced increases in FLI are restricted to limbic structures such as the prefrontal cortex, nucleus accumbens and lateral septal nucleus (Robertson and Fibiger, 1992). Since the dorsolateral striatum is associated with the regulation of movement (Pisa, 1988; Carelli and West, 1991), these findings led to the proposal that clozapine may be free of EPS because it fails to increase FLI in the dorsolateral striatum (Deutch et al., 1992; Robertson and Fibiger, 1992). Conversely, by increasing *c-fos*

expression in the dorsolateral striatum, haloperidol may promote long lasting changes in this structure which contribute to the development of EPS (Robertson et al., 1995).

Catalepsy is a condition whereby a subject's limbs maitain any position in which they are placed. All typical neuroleptics share the ability to produce catalepsy in the rat. By contrast, atypical neuroleptics have weak cataleptic effects in the rodents. Thus, the ability to produce catalepsy in rats is considered to be predictive of EPS potential in humans. Given that antipsychotic-induced increases in FLI in the dorsolateral striatum may be related to the EPS produced by typical neuroleptics, it is possible that those neurons which express FLI in the dorsolateral striatum may mediate the cataleptic effects of typical neuroleptics such as haloperidol.

If this hypothesis is valid, then the dose-response relationships for haloperidol-induced FLI in the dorsolateral striatum and catalepsy should be similar.

D. Antipsychotic-induced *c-fos* expression: receptor mechanisms

Previous studies have shown that haloperidol and the selective D_2-like antagonist raclopride produce similar patterns of neurons that display increased FLI in both the ventral and dorsal striatum (Dragunow et al., 1990; Robertson et al., 1992). In related studies which introduced an agonist reversal strategy, Miller (1990) showed that the dopamine D_2-like receptor agonist quinpirole reduced haloperidol-induced *c-fos* expression in the striatum. These results suggest that haloperidol-induced increases in striatal *c-fos* expression may be mediated by blockade of D_2-like receptors. Furthermore, they suggest that it may be possible to determine the specific dopamine subtype(s) that

mediate antipsychotic-induced *c-fos* expression by agonist reversal studies with highly selective dopamine agonists.

Like haloperidol, clozapine elevates FLI in the nucleus accumbens, however, these compounds produce distinct patterns of increased FLI (Robertson and Fibiger, 1992). Haloperidol increases FLI in small patches throughout the rostral to caudal extent in the nucleus accumbens. In contrast, clozapine-induced FLI is homogenously distributed across the anterior nucleus accumbens but is limited to the medial aspect of this nucleus at more caudal levels (Robertson and Fibiger, 1992). The different distributions of FLI produced by these drugs suggests that clozapine-induced FLI may not be related to D_2 receptor blockade and may occur in different populations of nucleus accumbens neurons than those activated by haloperidol. The failure of the selective D_1 antagonist SCH 23390 to increase FLI in the nucleus accumbens also indicates that D_1 blockade is not responsible for clozapine-induced FLI in the nucleus accumbenes. Taken together, these observations raise the possibility that clozapine increases FLI in the nucleus accumbens by blocking a dopamine receptor(s) that is/are pharmacologically distinct from the D_1 and D_2 subtypes (Robertson and Fibiger, 1992).

Molecular cloning studies have recently revealed the existence of a novel dopamine receptor (D_3) which differs in its pharmacology and signalling system from D_1 and D_2 receptors (Sokoloff et al., 1990). The D_3 receptor is concentrated in limbic regions such as the nucleus accumbens and has been suggested to be a potential target for antipsychotic actions (Sokoloff et al., 1990). Unlike, the D_2 receptor which is highly abundant in both ventral and dorsal striatum, there are few D_3 receptors in the dorsal

striatum (Boyson et al., 1986; Joyce et al., 1985). Indeed, the distribution of D_2 and D_3 receptors correlates with the neuroanatomical localization of haloperidol- and clozapine-induced FLI, respectively (Robertson et al., 1992). Accordingly, haloperidol may elevate FLI predominantly in striatal neurons which express the D_2 receptor, whereas clozapine-induced FLI may be located principally in neurons which express the D_3 receptor. Thus, if D_3 receptor blockade contributes to the ability of clozapine to increase *c-fos* expression in the nucleus accumbens and the other areas, it should be possible to reduce this increase by prior administration of selective D_3 agonist, e.g. 7-OH-DPAT.

VI. CHRONIC MARKERS OF NEURONAL ACTIVITY: ΔFosB

Although acute increases in FLI are predictive of both antipsychotic activity and EPS liability, FLI is limited by the fact that it cannot be used to study the long term effects of neuroleptic administration. Chronic administration of haloperidol or clozapine results in a rapid desensitization of the acute increases in both *c-fos* mRNA and FLI produced by these drugs (Sebens et al., 1995; Merchant et al., 1995). Down regulation of the *c-fos* response is a general phenomenon that has been reported to occur with repeated exposure to a variety of treatments (Winston et al., 1990; Hope et al., 1994). Since antipsychotics must be administered for several weeks in order to produce both an optimal therapeutic response and EPS such as tardive dyskinesia (Baldessarini et al.,

1990), it is uncertain whether acute increases in FLI identifies neurons that are involved in these delayed events.

The IEG *fos*B encodes two proteins, FosB and ΔFosB, which are generated by alternative splicing (Mumberg et al., 1991; Nakabeppu and Nathans, 1991; Yen et al., 1991). The ΔFosB protein is identical in structure to FosB except that it lacks the last 101 amino acids found in the C-terminus of FosB (Fig. 5). Because of this truncation, ΔFosB is a weaker transcriptional activating factor than FosB. Compared to most other IEG products, ΔFosB has prolonged induction kinetics (Nakabeppu and Nathans, 1991; Mumberg et al., 1991). Indeed, it has been recently discovered that destruction of the dopaminergic nigrostriatal pathway produces a prolonged elevation (>3 months) of ΔFosB-like immunoreactivity in the striatum (Jian et al., 1993; Robertson et al., 1994; Doucet et al., 1995). Chronic haloperidol administration also increases striatal ΔFosB-like imunoreactivity suggesting that its elevation after dopaminergic denervation is mediated by reduced activation of D_2-like receptors (Doucet et al., 1995). Retrograde tract tracing studies indicate that these increases occur primarily in striatopallidal neurons which are thought to be activated by dopaminergic deafferentation and chronic haloperidol administration (Doucet et al., 1995). Furthermore, chronic administration of haloperidol increases striatal Jun- and JunB-like immunoreactivity. Taken together, these findings suggest that ΔFosB may be a marker for neurons activated by chronic neuroleptic administration.

Given that acute administration of typical and atypical neuroleptics produce different patterns of increased FLI in the forebrain, it is predicted that chronic

administration of haloperidol and clozapine will also produce different patterns of elevated ΔFosB-like immunoreactivity in the prefrontal cortex, nucleus accumbens, medial and dorsolateral striatum and the lateral septal nucleus.

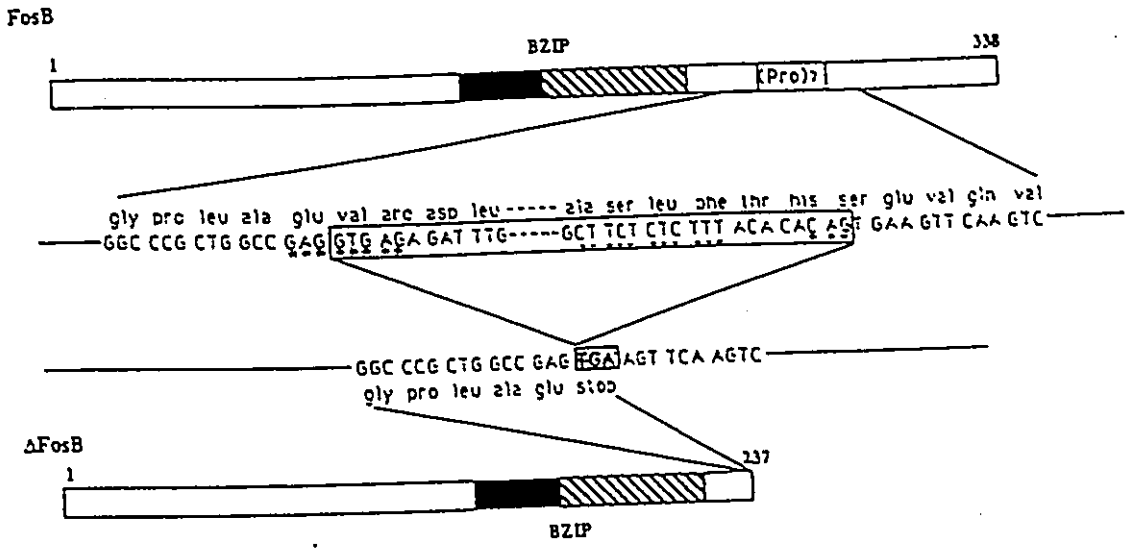


Fig. 5. Comparison of FosB and ΔFosB cDNAs. BZIP, DNA-binding domain (B, basic region, and ZIP, leucine zipper). The number of amino acids encoded by FosB and ΔFosB is indicated. The sequence of FosB that is missing in ΔFosB is shown in the box. Asterisks indicate the residues that are conserved in typical splice donor and acceptor sequences. The pyrimidine stretch adjacent to the acceptor site, which is conserved in a typical intron, is underlined. (Pro)7 indicates a heptaproline sequence (Nakabeppu and Nathans, 1992).

VII. RESEARCH OBJECTIVES

A. Dose-dependent effects of haloperidol on *c-fos* expression in the striatum and catalepsy

It has been hypothesized that haloperidol-induced increases in *c-fos* occur in neurons that mediate the cataleptic effects of this drug. If this the case, the dose-response relationships for haloperidol-induced *c-fos* expression and catalepsy should be similar. To test this hypothesis, the effects of several doses of haloperidol, ranging from 0.125 mg/kg to 2 mg/kg, on catalepsy, FLI and *c-fos* mRNA levels in the ventral, dorsal and medial aspects of striatum were compared.

B. The role of D_2 and D_3 dopamine receptor blockade in the mediation of haloperidol- and clozapine-induced FLI in the forebrain and antipsychotic activity

It has been demonstrated that clozapine and haloperidol produce distinctly different patterns of FLI in the nucleus accumbens. Both haloperidol- and clozapine-induced increases in FLI appear to be mediated by blockade of D_2-like dopamine receptors. However, the different distributions of FLI produced by clozapine and haloperidol suggest that these increases may be mediated by distinct D_2-like dopamine receptors. Indeed, the distribution of haloperidol-induced FLI matches that for the D_2 dopamine receptor, whereas there is excellent correspondence between the pattern of neurons which display clozapine-induced FLI and those which express mRNA encoding the D_3 dopamine receptor. Thus, one of the major objectives was to determine the

degree to which D_2 and D_3 receptor blockade contributes to clozapine- and haloperidol-induced FLI in the forebrain. Given that haloperidol-induced increases in *c-fos* mRNA levels can be competitively reduced by prior administration of quinpirole, a similar strategy was used in the present study to determine the relative roles of D_2 and D_3 receptor blockade in clozapine- and haloperidol-induced FLI. Consequently, dose response relationships for the inhibitory effects of quinpirole and 7-OH-DPAT on clozapine- and haloperidol-induced FLI in several forebrain areas as well as haloperidol-induced catalepsy were determined. The ability of prior administration of either 7-OH-DPAT or quinpirole to reduce haloperidol- and clozapine-induced FLI in the prefrontal cortex, nucleus accumbens, mediolateral striatum, dorsolateral striatum and lateral septal nucleus were compared.

C. Effects of the selective D_3 antagonists UH 232 and naphtamide on FLI in the forebrain

Given that D_3 receptor blockade may be responsible for clozapine's ability to selectively elevate FLI in the limbic system, the effects of the selective D_3 antagonists UH 232 and naphtamide on FLI in the prefrontal cortex, nucleus accumbens, mediolateral striatum, dorsolateral striatum and lateral septal nucleus were compared.

D. The role of D_1 dopamine receptor activation in the mediation of antipsychotic induced FLI

Pharmacological studies have shown that psychostimulants such as cocaine and d-amphetamine which dramatically enhance exteracellular dopamine levels elevate FLI in the striatum by increasing D_1 receptor activation (Assunta Imperato and Gaetano Di Chiara, 1988). Antipsychotics also elevate dopamine levels in the striatum because they block inhibitory autoreceptors (D_2-like receptors) on dopaminergic neurons (Assunta Imperato and Gaetano Di Chiara, 1985). Consequently, increases in D_1 receptor activation may also contribute to the mediation of antipsychotic-induced FLI in the striatum. In order to test this hypothesis, the effects of the D_1 receptor antagonist SCH 23390 on clozapine- and haloperidol- induced FLI in the prefrontal cortex, nucleus accumbens, mediolateral striatum, dorsolateral striatum and lateral septal nucleus were examined.

E. Effects of chronic clozapine and haloperidol administration on immediate-early gene expression in the forebrain

Chronic administration of haloperidol and clozapine result in a down regulation of the *c-fos* acute response. Given that typical and atypical neuroleptics produce different patterns of increased FLI in the forebrain, the present study addressed the effects of chronic haloperidol and clozapine administration on Jun-, JunB- and ΔFosB-like immunoreactivity in the prefrontal cortex, nucleus accumbens, medial and dorsolateral striatum and the lateral septal nucleus.

MATERIALS AND METHODS

I. ANIMALS

Adult male Wistar rats (275-375 g; Charles River, Montréal) were housed two or three per cage in a temperature-controlled environment with a 12-hr light/dark cycle and free access to standard laboratory chow and water. All animals were treated in strict accordance with procedures outlined in the Guide for the Care and Use of Experimental Animals endorsed by the Medical Research Council of Canada.

II. DRUGS AND IMMUNOREAGENTS

Reagents, antibodies and drugs used in this thesis are listed below with the respective sources and vehicles.

DRUG	SUPPLIER	VEHICLE
7-OH-DPAT (7-Hydroxy-N,N' -di-n-propyl- 2-aminotetralin)	Research Biochemicals International	distilled water
Clozapine	Sandoz, Dorval, P.Q.	distilled water containing 0.8 mg/ml acetic acid
Haloperidol	Research Biochemicals International	distilled water containing 0.8 mg/ml acetic acid

DRUG	SUPPLIER	VEHICLE
Pentobarbital	MTC pharmaceutical, Cambridge, Ontario	sodium salt in aqueous propylene glycol base
Quinpirole	Research Biochemicals International	0.9% saline
UH 232	UpJohn Co., USA	distilled water
Naphtamide	Bioproject, Paris, France	0.9% saline
SCH 23390	Research Biochemicals International	distilled water
ANTIBODY	**SUPPLIER**	**SOLVENT**
Sheep anti $Fos_{(2-16)}$ (polyclonal); 1:2500	Cambridge, England (OA-11-823)	0.01 M sodium phosphate, 0.1% azide
Rabbit anti JunB (polyclonal); 1:2000	Dr. R. Bravo, Bristol-Myers Squibb Princeton, NY	0.05 M sodium phosphate, 0.1% azide
Rabbit anti Jun (polyclonal); 1:4000	Dr. R. Bravo, Bristol-Myers Squibb Princeton, NY	0.05 M sodium phosphate, 0.1% azide
Rabbit anti FosB(N) (polyclonal); 1:1500	Dr. Yusaku Nakabeppu, Kuyshu Univ., Japan	0.05 M sodium phosphate, 0.1% azide
Rabbit anti FosB(C)	Dr. Yusaku Nakabeppu, Kuyshu Univ., Japan	0.05 M sodium phosphate, 0.1% azide
Donkey anti-sheep; 1:200	Jackson Immuno Research Laboratories, Inc.	0.01 M sodium phosphate, 0.25 M NaCl, pH 7.6
Donkey anti-rabbit; 1:200	Jackson Immuno Research Laboratories, Inc.	0.01 M sodium phosphate, 0.25 M NaCl, pH 7.6
Streptavidin; 1:100	Amersham Life Sciences	0.01 sodium phosphate

REAGENT	SUPPLIER	SOLVENT
Cobalt reaction buffer	Gibco/BRL	0.7 mM potassium cacodylate, pH 7.2, 150 mM Tris base, 5 mM $CoCl_2$, 0.5 mM DTT
c-fos oligonucleotide probe	DNA synthesis lab, Univ. of Calgary	distilled water
^{35}S ATP	Nen/Dupont	
Terminal Deoxynucleotidyl Transferase (TDT)	Gibco/BRL	0.1 M potassium phosphate, pH 7.2
dithiothreitol (DTT)	BIO RAD	
formamide	VWR scientific	deionised

III. INDUCTION OF CATALEPSY AND Fos-LIKE IMMUNOREACTIVITY BY HALOPERIDOL

A. Experimental protocol

Four groups, composed of 7 rats each, were injected subcutaneously (s.c.) with either vehicle (1 ml/kg; 40 μl of 20% acetic acid in 1 ml distilled water) or one of the following doses of haloperidol: 0.125 mg/kg, 0.025 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg or 2 mg/kg. Catalepsy was measured 30 min after administration of vehicle or haloperidol.

Animals were allowed to survive for 90 min, and then deeply anaesthetized by intraperitoneal (i.p.) injection of sodium pentobarbital (100 mg/kg). Subjects were then perfused transcardially with 200 ml of saline (0.9%) followed by 150 ml of 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde (PFA), at room temperature. Brains were then removed and post fixed in 4% PFA for 24-48 hours at 4°C. Coronal sections (30 μm thick) were cut from the anterior to caudal nucleus accumbens using a vibratome, (plates 9 to 15 of the atlas of Paxinos and Watson, 1986).

B. Measurement of catalepsy

The animals were tested for catalepsy by measuring the time required to remove both front paws from the top of a 10 cm tall box. Subjects were not permitted to stay in this position for longer than 2 min.

C. Polyclonal antisera and immunohistochemistry

Fos-like immunoreactivity was detected using an affinity-purified sheep antibody [OA-11-823; Cambridge Research Biochemical (CRB), Cambridge, U.K.] raised against a synthetic peptide corresponding to amino acids 2-16 in the NH_2 terminal region of Fos, a sequence that is conserved in mouse and human Fos. Immunoblot analysis indicates that this antibody recognizes Fos (62 kD) and Fos-related antigens (48/49 and 70 kD). Immunohistochemistry was performed using a standard procedure described previously by Robertson and Fibiger (1992). Free floating sections (30 μm thick) were washed in 0.01 M phosphate buffer saline (PBS) for 10 min followed by PBS containing 0.9% hydrogen peroxidase for 10 min to block endogenous peroxidase activity. Next, sections were washed 3 times in PBS and incubated in PBS containing 0.3% Triton X-100 (a detergent to disrupt the plasma and nuclear membranes permitting the primary antibody access to the Fos protein located in the nucleus); 0.025% sodium azide and primary antisera (CRB OA-11-823; 1:2500) for 48 hours at 4°C. The sections were then washed 3 times with PBS to remove non-specifically bound primary-antibody and incubated with biotin-labelled donkey anti-sheep secondary antibody (1:200) overnight at 4°C. Sections were then washed 3 times with PBS and incubated for 3 hours with PBS containing 0.3% Triton X-100 and streptavidin-horseradish peroxidase (1:100, Amersham). After 3 washes in PBS, the sections were rinsed in acetate buffer (0.1 M, pH 6.0). The reaction was visualized by a glucose oxidase-DAB (3',3'-Diaminobenzidine)-nickel method (Shu et al., 1988).

The reaction was terminated by washing the sections in acetate buffer (0.1 M, pH 6.0). Sections were then mounted on chrome-alum coated slides. After drying, the sections were dehydrated through a graded series of ethanol, and two changes of xylene, and coverslipped for microscopic observation.

D. Quantification of Fos-like immunoreactivity

Fos-like immunoreactivity (FLI) was quantified in anterior nucleus accumbens (NAc), mediolateral striatum (ML-Str) and dorsolateral striatum (DL-Str) using an image analysis system equipped with Image 1.47 software (Wayne Rasband, NIMH). Digitization of sampled areas (660 X 800; Fig. 6) was performed at 100X magnification using a CCD camera linked to a microscope. Thresholding was performed on the digitized image in order to eliminate small positive profiles such as fragments of nuclei and weakly stained cells from the final analysis. To ensure consistency between measurements, the appropriate threshold value was chosen on the basis of the nuclear size. Thresholding was performed on the digitized image until the average diameter of stained nuclei was 7-8 μm. A total of two measurements, each performed on a separate section, were done for every animal.

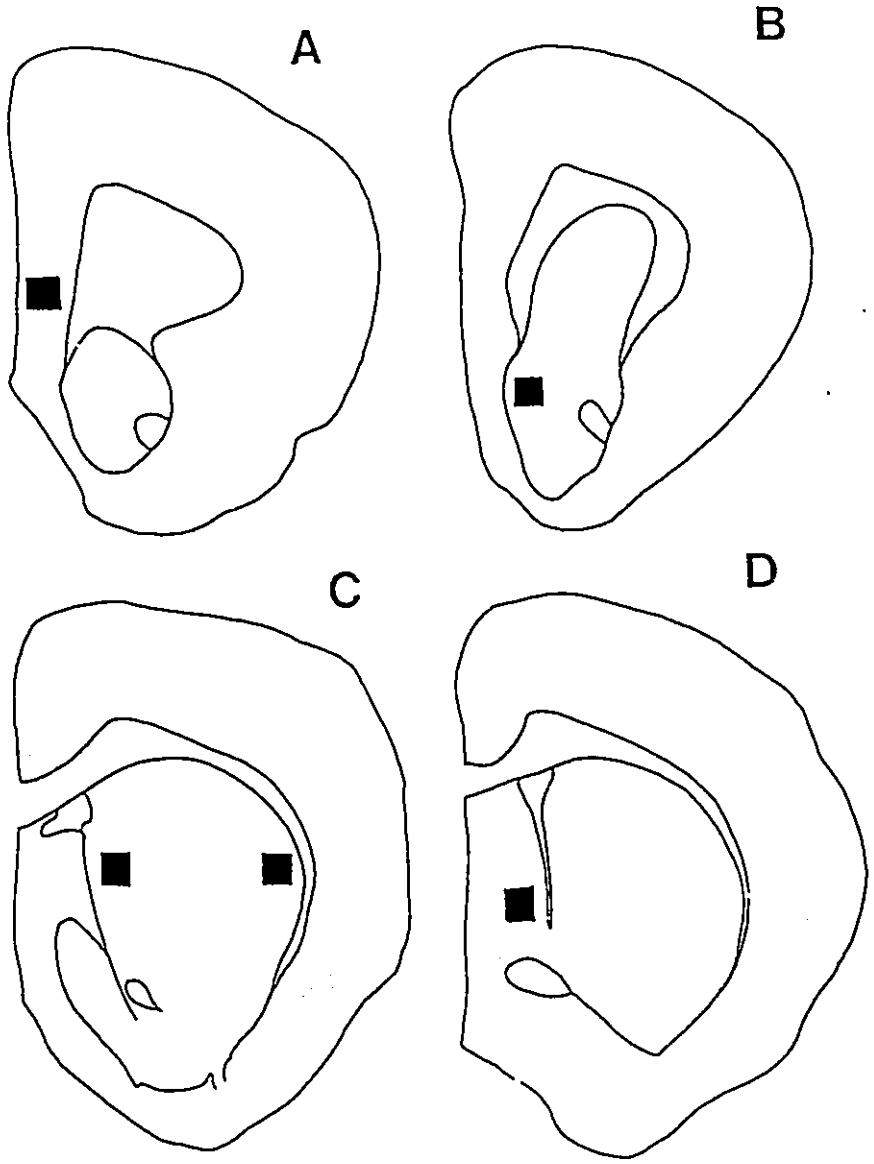


Fig. 6. Camera lucida drawings of representative sections used for counting immunoreactive nuclei in the medial prefrontal cortex (PFC) (A), nucleus accumbens (NAc) (B), mediolateral striatum (ML-Str) and dorsolateral striatum (DL-Str) (C) and lateral septal nucleus (LSN) (D). Dark boxes indicate the sampled areas (600 x 800 μm) in each structure (Robertson et al., 1992).

IV. AGONIST REVERSAL OF ANTIPSYCHOTIC-INDUCED Fos-LIKE IMMUNOREACTIVITY

A. Experimental protocol

i. Effects of 7-OH-DPAT on clozapine-induced Fos-like immunoreactivity

The purpose of this study was to determine whether a prior 7-OH-DPAT injection could reverse clozapine-induced FLI. In a first experiment, 4 groups, composed of 4 rats each, were given one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), 7-OH-DPAT (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.) or 7-OH-DPAT (0.5 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to the second one.

In a second experiment, 4 groups, composed of 4 rats each, were injected with one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), 7-OH-DPAT (0.05 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.) or 7-OH-DPAT (0.05 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to the second one.

All subjects from these two experiments were killed 90 min after the last injection with an overdose of pentobarbital (100 mg/kg, i.p.). Sections (30 μm thick) were cut from the prefrontal cortex to lateral septal nuclei and processed for Fos-like immunoreactivity as described previously. Alterations in Fos-like immunoreactivity were

quantified by counting the number of immunoreactive nuclei in the prefrontal cortex (PFC), nucleus accumbens (NAc), mediolateral striatum (ML-Str), dorsolateral striatum (DL-Str) and lateral setal nuclei (LSN) as described above.

ii. Effects of 7-OH-DPAT on haloperidol-induced Fos-like immunoreactivity

The purpose of this study was to determine whether a prior 7-OH-DPAT injection could reduce haloperidol-induced Fos-like immunoreactivity (FLI) and catalepsy. In a first experiment, 4 groups, composed of 4 rats each, were given one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), 7-OH-DPAT (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.) or 7-OH-DPAT (0.5 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.). The first injection occurred 30 min prior to the second one.

In a second experiment, 4 groups, composed of 4 rats each, were injected with one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), 7-OH-DPAT (0.05 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.) or 7-OH-DPAT (0.05 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. Catalepsy was measured 30 min after the last injection.

All animals from these two experiments were deeply anaesthetized with an injection of sodium pentobarbital (100 mg/kg, i.p.) and perfused 90 min after the last injection with PFA. Sections (30 μm thick) were cut from the prefrontal cortex to lateral

septal nuclei and processed for Fos-like immunoreactivity as described previously. Alterations in Fos-like immunoreactivity were quantified by counting the number of immunoreactive nuclei in the prefrontal cortex (PFC), nucleus accumbens (NAc), mediolateral striatum (ML-Str), dorsolateral striatum (DL-Str) and lateral setal nuclei (LSN) as described above.

iii. Reversal of clozapine-induced Fos-like immunoreactivity by quinpirole

The purpose of this study was to determine whether a prior injection of quinpirole could reduce clozapine-induced FLI. In a first experiment, 4 groups, composed of 4 rats each, were injected with one of the following: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), quinpirole (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.) or quinpirole (0.5 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to the second one.

In a second experiment, 4 groups, composed of 4 rats each, were injected with one of the following: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), quinpirole (1 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.) or quinpirole (1 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to the second one.

All animals from these two experiments were deeply anaesthetized with an injection of sodium pentobarbital (100 mg/kg, i.p.) and perfused 90 min after the last injection with paraformaldehyde. Sections (30 μm thick) were cut from the prefrontal

cortex to lateral septal nuclei and processed for Fos-like immunoreactivity as described previously. Alterations in FLI were quantified by counting the number of immunoreactive nuclei in the prefrontal cortex (PFC), nucleus accumbens (NAc), mediolateral striatum (ML-Str), dorsolateral striatum (DL-Str) and lateral septal nuclei (LSN) as described previously.

iv. Reversal of haloperidol-induced Fos-like immunoreactivity and catalepsy by quinpirole

The purpose of this study was to determine whether a prior injection of quinpirole could reduce FLI- and catalepsy- induced by haloperidol. The effects of several doses of quinpirole and haloperidol were examined. In a first experiment, 4 groups, composed of 4 rats each, were injected with one of the following : saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), quinpirole (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (2 mg/kg, s.c.) or quinpirole (0.5 mg/kg, s.c.) + haloperidol (2 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. Catalepsy was measured 30 min after the last injection.

In a second experiment, 4 groups, composed of 4 rats each, were injected with one of the following: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), quinpirole (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (1 mg/kg, s.c.) or quinpirole (0.5 mg/kg, s.c.) +

haloperidol (1 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. Catalepsy was measured 30 min after the last injection.

In a third experiment, 4 groups, composed of 4 rats each, were injected with one of the following: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), quinpirole (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (0.5 mg/kg, s.c.) or quinpirole (0.5 mg/kg, s.c.) + haloperidol (0.5 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. Catalepsy was measured 30 min after the last injection.

In a fourth experiment, 4 groups, composed of 4 rats each, were injected with one of the following: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, 40 μl of 20% acetic acid in 1 ml of distilled water, s.c.), quinpirole (1 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.) or quinpirole (1 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. Catalepsy was measured 30 min after the last injection.

All animals from these four experiments were deeply anaesthetized with an injection of sodium pentobarbital (100 mg/kg, i.p.) and perfused 90 min after the last injection with PFA. Sections (30 μm thick) were cut from the prefrontal cortex to lateral septal nuclei and processed for Fos-like immunoreactivity as described previously. Alterations in Fos-like immunoreactivity were quantified by counting the number of immunoreactive nuclei in the prefrontal cortex (PFC), nucleus accumbens (NAc), mediolateral striatum (ML-Str), dorsolateral striatum (DL-Str) and lateral septal nuclei (LSN) as described previously.

V. D_1-LIKE RECEPTOR ANTAGONIST REVERSAL OF ANTIPSYCHOTICS-INDUCED Fos-LIKE IMMUNOREACTIVITY

The purpose of this study was to determine whether a prior injection of the D_1-like receptor antagonist SCH 23390 will reduce the ability of several D_2-like receptor antagonists to elevate Fos-like immunoreactivity in the forebrain.

A. Experimental protocol

i. Effects of SCH 23390 on haloperidol-induced Fos-like immunoreactivity

Four groups, composed of 3 rats each, were injected with one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c) + haloperidol (0.1 mg/kg, s.c.), or SCH 23390 (0.1 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.).

ii. Effects of SCH 23390 on clozapine-induced Fos-like immunoreactivity

Four groups, composed of 3 rats each, were injected with one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c) + clozapine (20 mg/kg, s.c.), or SCH 23390 (0.1 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.).

iii. Effects of SCH 23390 on UH 232-induced Fos-like immunoreactivity

Dose Response study: A dose-response study was performed first to determine the optimal dose of UH 232 for future experimentation. Four groups, composed of 3 rats

each, were injected with one of the following: distilled water (1 ml/kg, s.c.) + distilled water (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + UH 232 (10 mg/kg, s.c.), or distilled water (1 ml/kg, s.c.) + UH 232 (30 mg/kg, s.c.). The optimal dose of UH 232 was considered to be 10 mg/kg based on the Fos-like immunoreactivity results (data not shown).

In order to determine whether a prior SCH 23390 injection could reverse FLI induced by UH 232, four groups, composed of 3 rats each, were given one of the following: distilled water (1 ml/kg, s.c.) + distilled water (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + UH 232 (10 mg/kg, s.c.), or SCH 23390 (0.1 mg/kg, s.c.) + UH 232 (10 mg/kg, s.c.).

iv. Effects of SCH 23390 on naphtamide-induced Fos-like immunoreactivity

Dose-Response study: A dose-response study was performed in order to determine the optimal dose of naphtamide for future experimentation. Four groups, composed of 5 rats each, were injected with one of the following: saline (1 ml/kg, s.c.) + distilled water (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + naphtamide (0.1 mg/kg, s.c.), distilled water (1 ml/kg, s.c.) + naphtamide (0.5 mg/kg, s.c.), distilled water (1 ml/kg, s.c.) + naphtamide (1 mg/kg, s.c.), or distilled water (1 ml/kg, s.c.) + naphtamide (5 mg/kg, s.c.). The optimal dose of naphtamide was considered to be 5 mg/kg based on FLI results (data not shown).

In order to examine the effects of a prior SCH 23390 injection on naphtamide-induced FLI, 4 groups, composed of 3 rats each, were given one of the following:

distilled water (1 ml/kg, s.c.) + saline (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + naphtamide (5 mg/kg, s.c.), or SCH 23390 (0.1 mg/kg, s.c.) + naphtamide (5 mg/kg, s.c.).

All subjects were transcardially perfused with PFA 90 min after the second injection. Several sections (30 μm thick) were cut from the prefrontal cortex to lateral septal nucleus and processed for FLI. Changes in FLI were quantified by counting the number of immunoreactive nuclei in the prefrontal cortex (PFC), nucleus accumbens (NAc), mediolateral striatum (ML-Str), dorsolateral striatum (DL-Str) and lateral septal nuclei (LSN) as described previously.

VI. EFFECTS OF CHRONIC ANTIPSYCHOTIC ADMINISTRATION ON IMMEDIATE-EARLY GENE EXPRESSION IN THE FOREBRAIN

A. Experimental protocol

i. Effects of chronic treatment of haloperidol and clozapine

Three groups, composed of 5 rats each, were injected once a day for 16 days with one of the following: vehicle (1 ml/kg, i.p.), haloperidol (2 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.). Twenty four hours after the last injection, all subjects were perfused transcardially with 200 ml saline followed by 150 ml of phosphate buffer (0.1 M) containing 4% paraformaldehyde. Brains were removed and after postfixation overnight, 30 μm thick sections were cut from the medial prefrontal cortex to the lateral septal

nucleus. The next day, immunohistochemistry was performed on sections from the medial prefrontal cortex (PFC), anterior nucleus accumbens (NAc), mediolateral striatum (ML-Str), dorsolateral striatum (DL-Str) and lateral septal nuclei (LSN) (representative sections shown in Fig. 6).

ii. Immunohistochemical detection of the immediate-early gene products Fos, FosB, ΔFosB, JunB and Jun

ΔFosB- and FosB(C)-like immunoreactivity were detected using two different affinity purified rabbit polyclonal antibodies (Nakabeppu and Nathans, 1991). One antibody, raised against amino acids 79-131 of the N-terminus of FosB, recognizes both FosB and a truncated form of FosB known as ΔFosB (FosB(N) antibody). The second antibody, raised against a portion of the C-terminus of FosB that is absent from ΔFosB (amino acids 245-315), recognizes just FosB (FosB(C) antibody) (Nakabeppu and Nathans,1991). JunB- and Jun-like immunoreactivity were detected using antisera generously provided by Dr. Bravo (Bristol-Myers Squibb Co.). These antibodies were generated in rabbits by immunization with bacterially expressed fusion proteins (Karla Kovary et. al, 1991). The following sequences were used for fusion: JunB, amino acids 46-344; Jun, amino acids 80-344 (Karla Kovary et. al, 1991). JunB and Jun were expressed in bacteriafused to β-galactosidase. The specificity of these antibodies have been established by immunopercipitation and Western blot experiments (Karla Kovary et. al, 1991).

The sections were incubated in primary antibodies (2 sections/area/animal), CRB (1:2500), FosB(N) (1:1500), FosB(C) (1:500), JunB (1:2000), Jun (1:4000) for 48 hours at 4°C. Sections were then washed in PBS (0.01 M) and incubated in the appropriate biotinylated secondary antibody at the dilution of 1:200 for 24 hr. Next, sections were washed in PBS, incubated with streptavidin for 3 hrs, washed in PBS and immunoreactive nuclei were visualized by a DAB reaction as described previously. Lastly, sections were mounted, dehydrated and coverslipped for microscopic observation.

B. Data analysis

Quantification of immunoreactive nuclei in the different regions of the forebrain was performed using an image analysis system equipped with Image 1.47 software (Wayne Rasband, NIMH). Digitization of the sampled areas (660 X 800; Fig. 6) was performed at 100X magnification using a CCD camera linked to a microscope as described previously.

VII. EFFECTS OF HALOPERIDOL AND CLOZAPINE ON *c-fos* mRNA LEVELS IN THE FOREBRAIN

A. Experimental protocol

i. Induction of *c-fos* mRNA expression by haloperidol

The purpose of this study was to determine the dose-response relationships for haloperidol-induced *c-fos* mRNA in the medial, dorsolateral and ventral striatum. Four groups, composed of 7 rats each, were injected with vehicle (1 ml/kg, s.c.) or one of the following doses of haloperidol: 0.025 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, or 2 mg/kg (dissolved in vehicle, 1 ml of distilled water containing 40 μl of 20% acetic acid , s.c.). All subjects were decapitated 45-50 min later, and their brains removed and frozen in isopentane (-65°C).

ii. Induction of *c-fos* mRNA expression by clozapine

The purpose of this study was to determine whether haloperidol and clozapine elevate Fos-like immunoreactivity (FLI) and *c-fos* mRNA in the same regions of forebrain. Four groups, composed of 3 rats each, were injected with one of the following: clozapine (20 mg/kg, s.c.), haloperidol (2 mg/kg, s.c.), or 0.9% saline (1 ml/kg, s.c.). All subjects were decapitated 45-50 min later and their brains removed and frozen in isopentane (-65°C).

B. Oligodeoxynucleotide probes and *in situ* hybridization histochemistry

Expression of *c-fos* mRNA was detected by *in situ* hybridisation histochemistry (ISHH). An oligonucleotide 39 bases in length and complimentary to bases 1238-1276 of *c-fos* (Curran et al., 1987) was used for the ISHH studies. The sequences used for the *c-fos* probe was not significantly homologous to other sequences catalogued in the GENEBANK and EMBL data banks. The probe was 3'end-tailed with ^{35}S-labelled dATP using terminal deoxynucleotidyl transferase to a specific activity of 6 x 10^9 d.p.m./μg. ISHH was performed on fresh frozen brain. Sections 12 μm thick were cut using cryostat from the prefrontal cortex to lateral septal nucleus. These sections were immersed into 4% paraformaldehyde for 5 min at 21°C, rinsed 2 x 5 min in water and then allowed to dry. Next, the sections were hybridized overnight at 42°C in 4 X standard saline citrate buffer (SSC); 0.6 M NaCl, 0.06 M sodium citrate (pH 7.0), 50% deionized formamide, 1 X Denhardt's (0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 10% dextran sulphate, 1% N-lauroylsarcosine, 1 mg/kg salmon sperm DNA, 0.2 M dithiothreitol and the ^{35}S-labelled probe (1 x 10^6 d.p.m./100 μl/slide). After hybridization, the sections were washed at 21°C (20 min) and 1 X SSC (4 x 20 min), then at 45°C with 1 X SSC (20 min) followed by 0.5 X SSC (20 min) at 55°C. Lastly, the sections were washed in double-distilled water at 30°C (1 min) and 21°C (2 x 5 min). After drying, the slides were dipped in Kodak NTB-2 emulsion (diluted 1:1 with distilled water) at 42°C, dried and exposed for 2-4 weeks at 4°C. The slides were developed with Kodak D-19 for 4 min at 18°C, rinsed in water, fixed in Kodak fixer and washed in water. The section were then counterstained with Cresyl Violet (0.01%,

Sigma), dehydrated through a graded series of alcohol and two changes of xylene, and coverslipped for microscopic observation.

C. Data analysis

Analysis of ISHH labelling was performed by digitized image analysis. This process entailed the digitization of sampled areas through a microscope at 100X magnification with a CCD camera to generate a digitized image of 256 X 256 pixel. Each sample area (660 X 800; Fig. 6) was first digitized under bright-field illumination and then under dark-field illumination. The camera settings and lighting conditions were held constant for all of the measurements. The matched set of bright-field and dark-field images was then analyzed using a redirected analysis routine. With this routine, neurons in the different regions of the forebrain stained with Cresyl Violet were selected from the bright-field image using a circular field of constant size (400 μm^2), and the average optical density (OD) of silver grains over neurons containing *c-fos* was measured in the corresponding area under dark-field illumination. The average optical density value obtained for each cell was subtracted from 255, which was the maximum optical density value, to provide an inverse optical density, because the silver grains were visualized with dark-field optics. The OD_i value was then used as a measure of label per cell, with higher values indicating greater labelling. A cell was considered to be labelled if its OD_i value was three times greater than background. A total of four measurements, each performed on a separate section, were done for every animal.

D. Statistical analysis

Single factor analysis of variance (ANOVA) was used to determine whether a significant difference existed between the means of 3 or more groups. If the ANOVA yielded a significant F, multiple comparisons between the individual groups were performed using the Student-Newman Keuls test (SNK) according to Zar (1974).

RESULTS

I. EFFECTS OF D_2-LIKE RECEPTOR ANTAGONIST ADMINISTRATION ON Fos-LIKE IMMUNOREACTIVITY IN THE FOREBRAIN

The purpose of this study was to determine the dose-response relationships for haloperidol-induced Fos-like immunoreactivity (FLI) in the limbic system and catalepsy.

A. Dose-dependent effects of haloperidol on FLI in the striatum

The dose-response relationships for haloperidol-induced FLI in the nucleus accumbens (NAc), mediolateral striatum (ML-Str), and dorsolateral striatum (DL-Str) are shown in Fig. 7. Haloperidol produced a dose-dependent elevation of the number of neurons which displayed FLI in all of these regions. Maximal induction was observed at a dose of 0.1 mg/kg (s.c.) in the medial and dorsolateral regions of the striatum. In contrast, maximal increases in FLI occurred in the NAc with a haloperidol dose of 0.5 mg/kg (s.c.). The EC_{50} dose for haloperidol-induced FLI in the ML-Str and DL-Str was about 0.03 mg/kg (s.c.) while the EC_{50} for haloperidol-induced FLI in the NAc was approximately 0.05 mg/kg (s.c.).

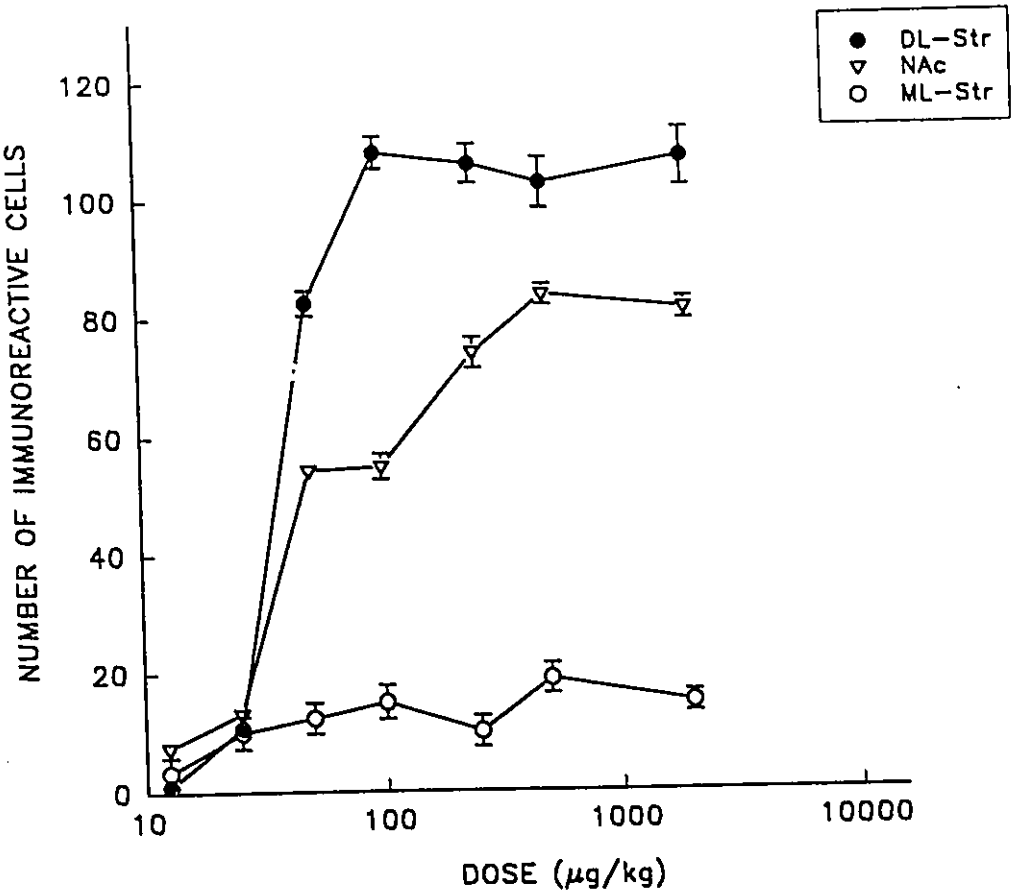


Fig. 7. Dose-dependent effects of haloperidol on Fos-like immunoreactivity in the striatum. Seven groups, composed of 4 rats each, received one of following doses of haloperidol: (0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, or 2 mg/kg, s.c.). Fos-like immunoreactivity was quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the NAc (nucleus accumbens), ML-Str (mediolateral striatum) and DL-Str (dorsolateral striatum). Each value represents the mean $\pm$ SEM of duplicate determinations performed on separate sections from 4 animals.

B. Dose-dependent effects of haloperidol on catalepsy

The dose-response curve for haloperidol-induced catalepsy is shown in Fig. 8. A dose of 0.05 mg/kg (s.c.) of haloperidol produced a maximal increase in catalepsy. The EC_{50} dose for haloperidol-induced catalepsy was approximately 0.03 mg/kg.

II. EFFECTS OF PRIOR ADMINISTRATION OF D_2-LIKE RECEPTOR AGONISTS ON ANTIPSYCHOTIC-INDUCED FLI IN THE FOREBRAIN

The purpose of this experiment was to determine whether clozapine- and haloperidol-induced FLI were reversible by prior administration of the D_2-like receptor agonists 7-OH-DPAT and quinpirole.

A. Effects of 7-OH-DPAT on clozapine-induced FLI

In agreement with previous work, clozapine (20 mg/kg, s.c.) selectively increased FLI in limbic structures of the forebrain such as the prefrontal cortex (PFC), nucleus accumbens (NAc) and lateral septal nuclei (LSN) (Fig. 9). The ability of clozapine (20 mg/kg, s.c.) to enhance FLI in the PFC, NAc and LSN was substantially reduced by prior administration of the selective D_3 receptor agonist 7-OH-DPAT (0.5 mg/kg, s.c.) (Fig. 9, 13). Clozapine-induced FLI was also markedly reduced in the NAc, and LSN by prior administration of a significantly lower dose of 7-OH-DPAT (0.05 mg/kg, s.c.) (Fig. 10). The lower dose of 7-OH-DPAT also significantly reduced clozapine-induced FLI in the PFC (30% reduction) .

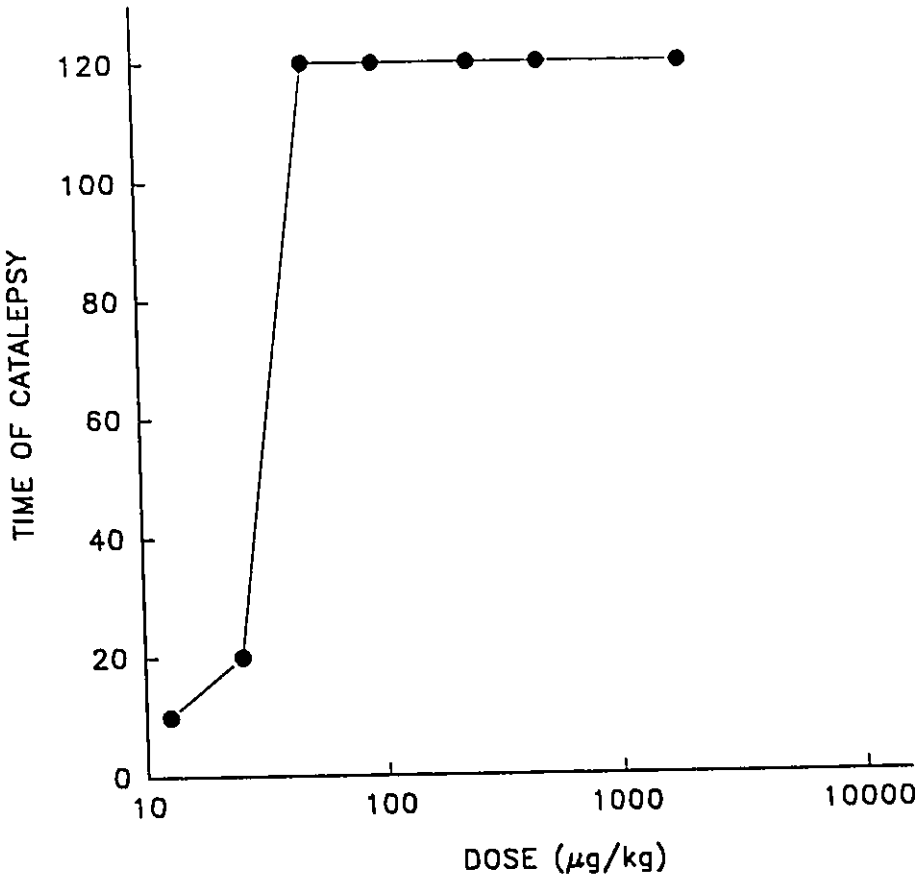


Fig. 8. Dose-dependent effects of haloperidol on catalepsy. Seven groups, composed of 4 rats each, received one of the following doses of haloperido: (0.0125, 0.025, 0.05 , 0.1, 0.25, 0.5, or 2 mg/kg, s.c.). Each value represents the time required for animals to remove both front paws from the top of a 10 cm tall box.

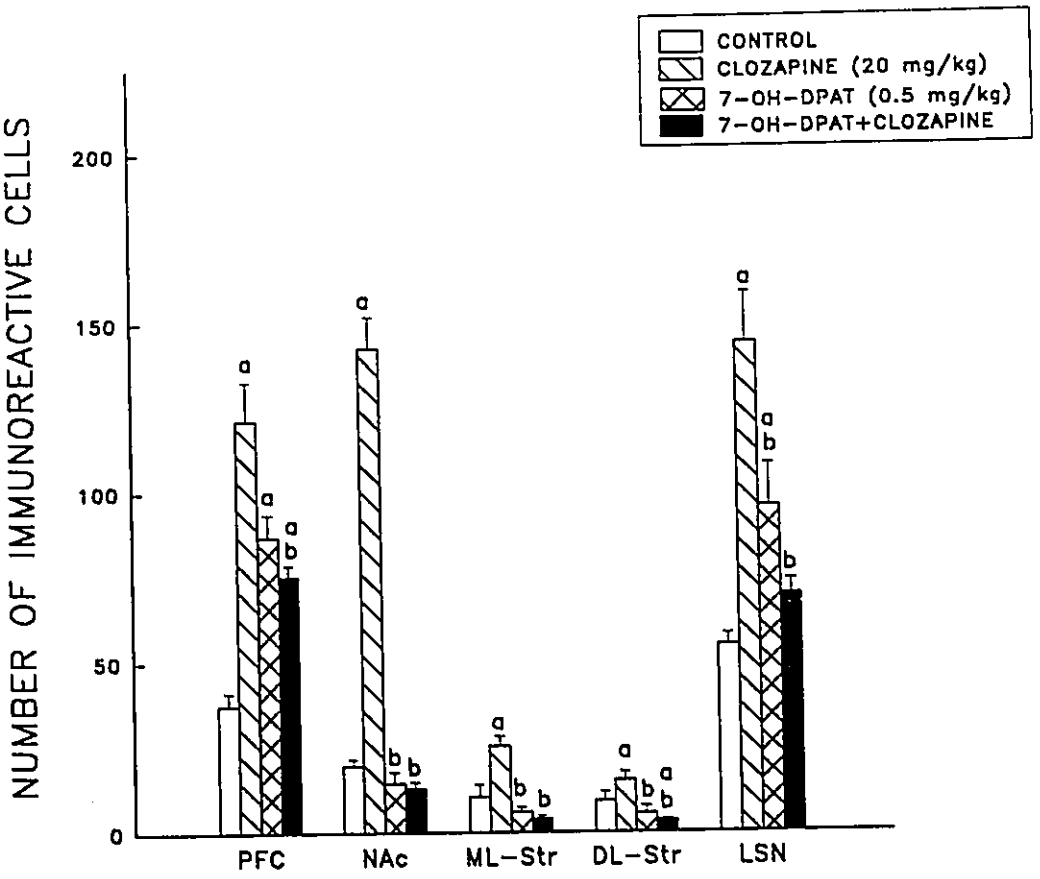


Fig. 9. Effects of 7-OH-DPAT pretreatment on clozapine induced-increases in Fos-like immunoreactivity in the forebrain. Four groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.), 7-OH-DPAT (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.) or 7-OH-DPAT (0.5 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from clozapine group ($p<0.01$).

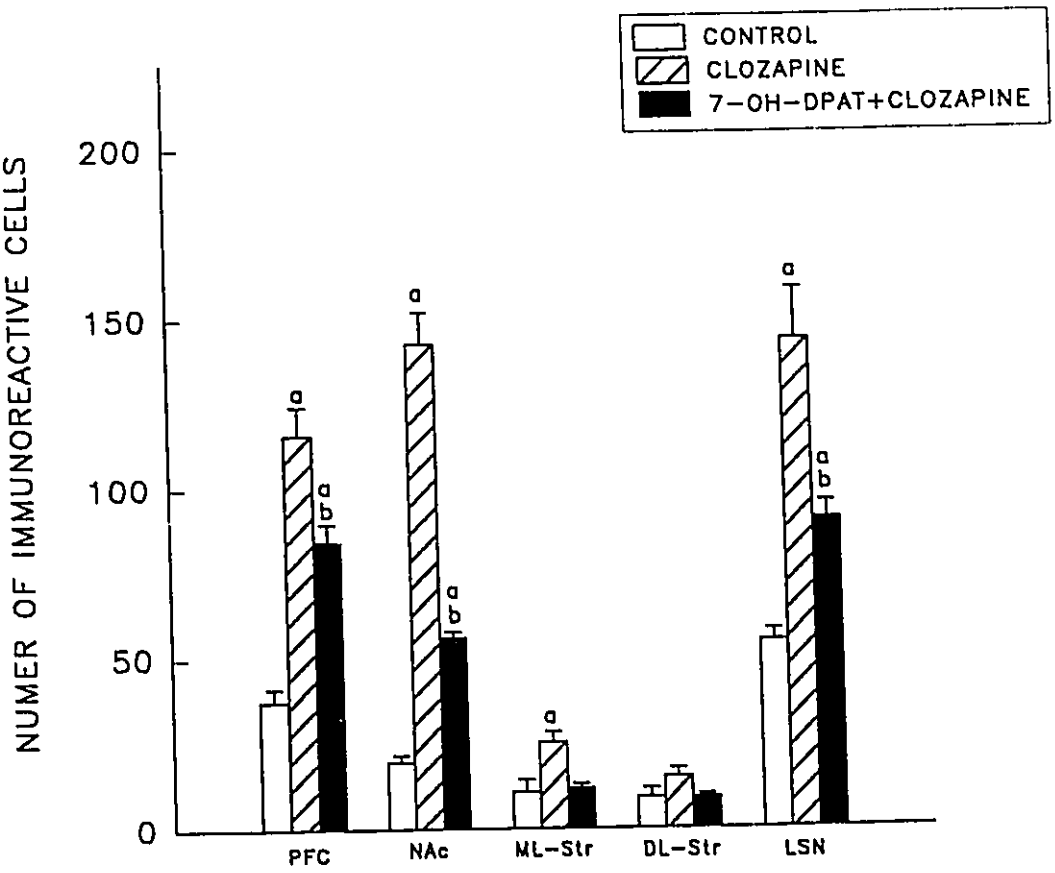


Fig. 10. Effects of 7-OH-DPAT pretreatment on clozapine induced-increases in Fos-like immunoreactivity in the forebrain. Three groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.) or 7-OH-DPAT (0.05 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from clozapine group ($p<0.01$).

B. Effects of 7-OH-DPAT on haloperidol-induced FLI and catalepsy

Haloperidol (0.1 mg/kg, s.c.) significantly elevated FLI in the NAc, DL-Str, PFC and LSN. Injection of 7-OH-DPAT (0.5 mg/kg, s.c.) 30 min prior to haloperidol (0.1 mg/kg, s.c.) reduced haloperidol-induced increases in FLI in NAc and DL-Str, by approximately 50% and 40%, respectively (Fig. 11, 13). 7-OH-DPAT (0.5 mg/kg, s.c.) did not reverse catalepsy induced by haloperidol (0.1 mg/kg, s.c.) (data not shown). Prior administration of a low dose of 7-OH-DPAT (0.05 mg/kg, s.c.) failed to reduce increases in FLI produced by haloperidol (0.1 mg/kg, s.c.) (Fig. 12).

C. Effects of quinpirole on clozapine-induced FLI

Administration of quinpirole (0.5 mg/kg, s.c.) reduced basal FLI in the NAc, ML-Str, DL-Str and LSN (Fig. 14). Administration of quinpirole (0.5 mg/kg) 30 min prior to clozapine (20 mg/kg, s.c.) reduced increases in FLI produced by this antipsychotic in all the regions examined (Fig. 14A). In the cases of the NAc and ML-Str, quinpirole (0.5 mg/kg, s.c.) produced a complete reversal of clozapine (20 mg/kg, s.c.) induced increases in FLI. Administration of quinpirole (1 mg/kg, s.c.) also produced a profound reduction of basal FLI in the NAc, ML-Str, DL-Str and LSN (Fig. 14B). Administration of quinpirole (1 mg/kg, s.c.) 30 min prior to clozapine (20 mg/kg, s.c.) also significantly reduced increases in FLI produced by this neuroleptic in the PFC, NAc, ML-Str and DL-Str (Fig. 14B).

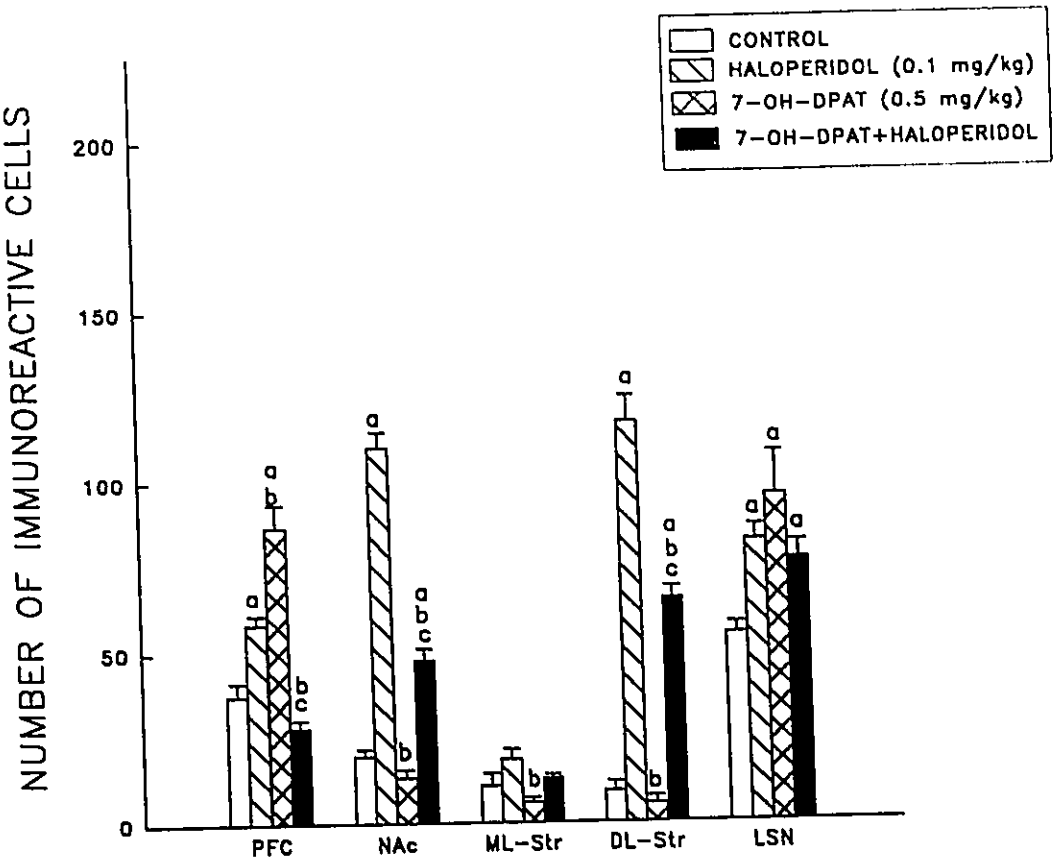


Fig. 11. Effects of 7-OH-DPAT pretreatment on haloperidol induced-increases in Fos-like immunoreactivity in the forebrain. Four groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.), 7-OH-DPAT (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.), or 7-OH-DPAT (0.5 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).
c, significantly different from 7-OH-DPAT group ($p<0.01$).

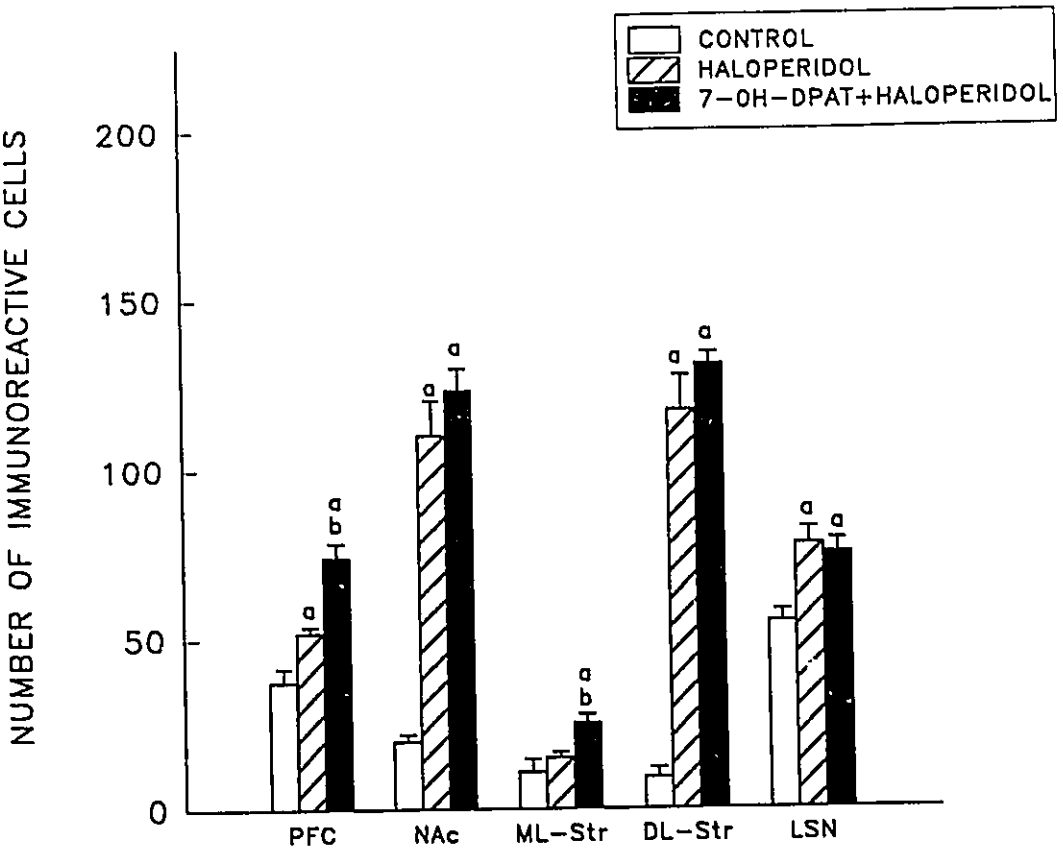


Fig. 12. Effects of 7-OH-DPAT pretreatment on haloperidol induced-increases in Fos-like immunoreactivity in the forebrain. Three groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.) or 7-OH-DPAT (0.05 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.). The first injection occurred 30 min prior to the second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

Fig. 13. Photomicrographs of the effects of single administration of 7-OH-DPAT (0.5 mg/kg, s.c.) prior to vehicle (1 ml/kg, s.c.)-, clozapine (20 mg/kg, s.c.) and haloperidol (0.1 mg/kg, s.c.)-induced FLI in the nucleus accumbens. In each experiment, four groups composed of 4 rats each, recieved one of the following:

A. saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.)

B. 7-OH-DPAT (0.5 mg/kg, s.c.) + vehicle (1 ml/kg, s.c.)

C. vehicle (1ml/kg, s.c.) + clozapine (20 mg/kg, s.c.)

D. 7-OH-DPAT (0.5 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.)

E. vehicle (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.)

F. 7-OH-DPAT (0.5 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.)

Scale bar = 100 μm

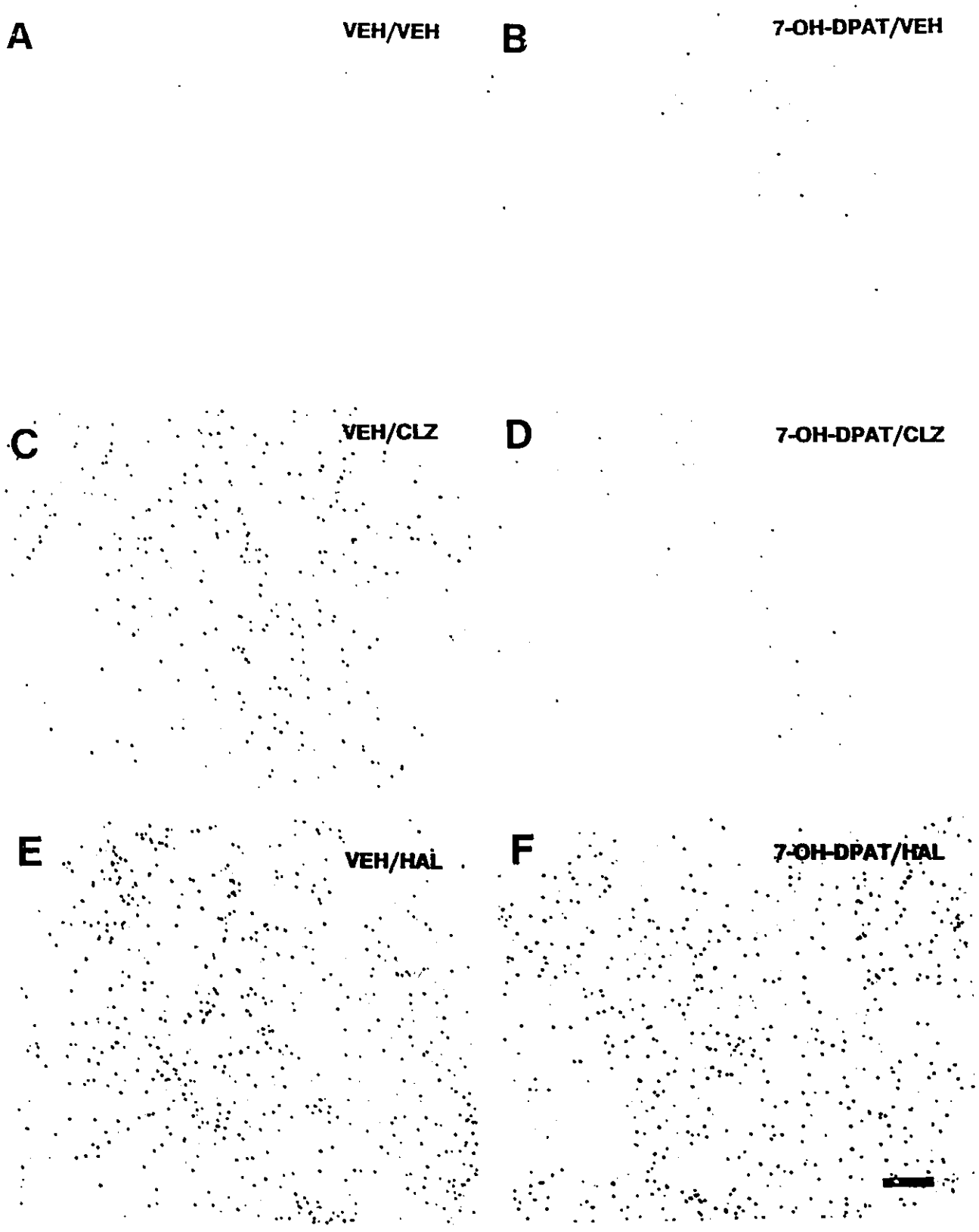

A
VEH/VEH
B
7-OH-DPAT/VEH
C
VEH/CLZ
D
7-OH-DPAT/CLZ
E
VEH/HAL
F
7-OH-DPAT/HAL

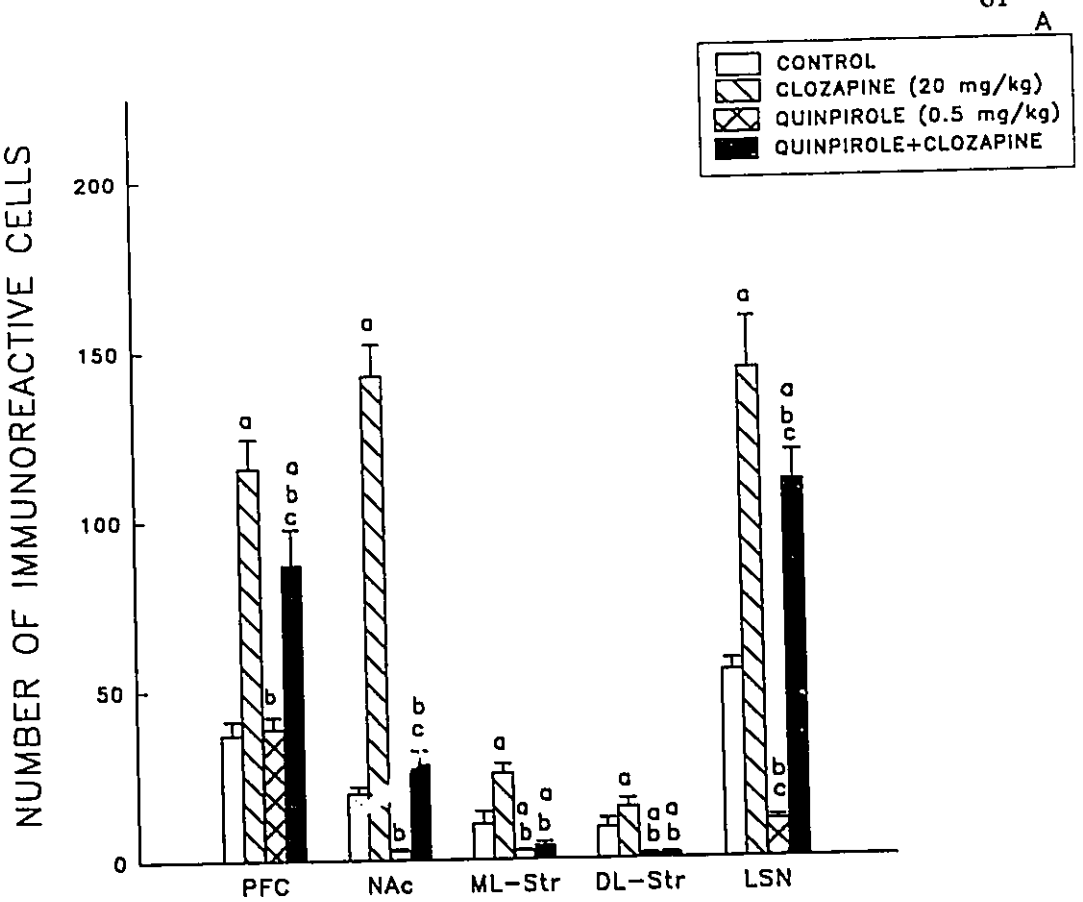
Fig. 14. Effects of different doses of quinpirole on Fos-like immunoreactivity induced by clozapine in the forebrain. In each experiment, four groups, composed of 4 rats each, received one of the following treatments:

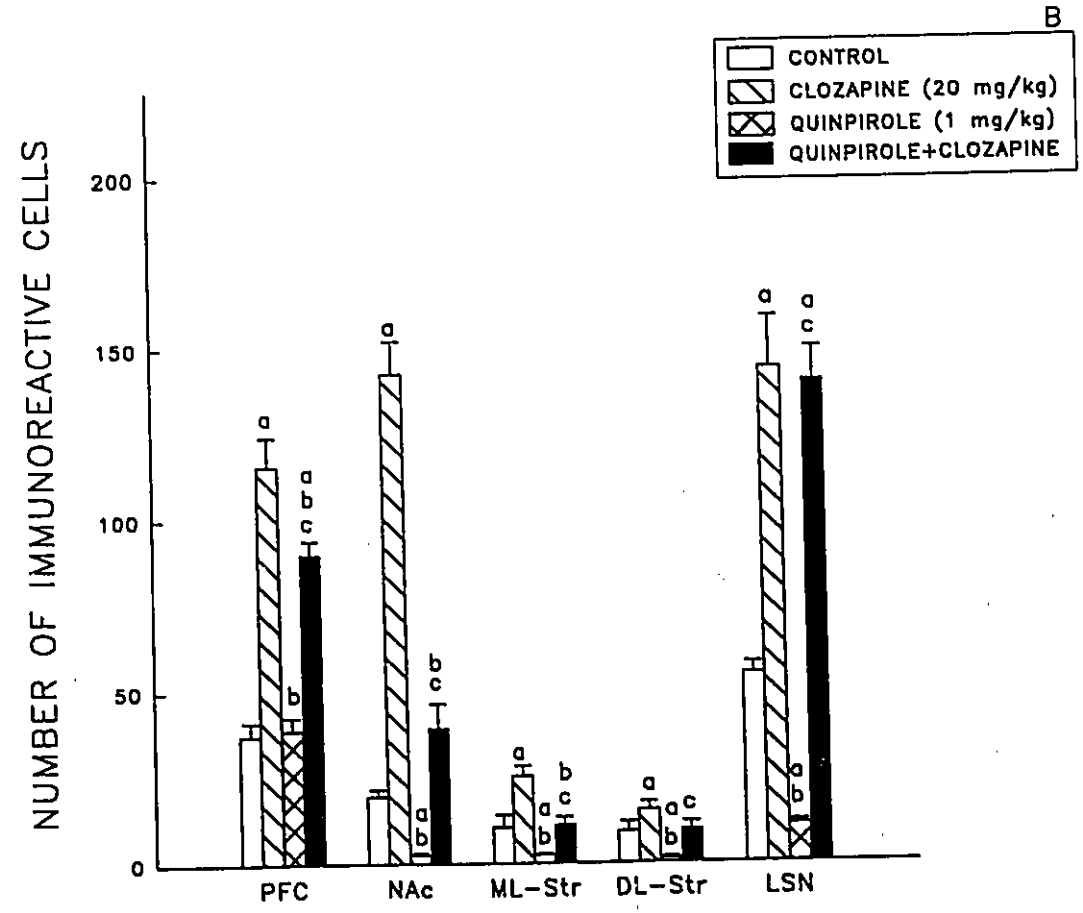
Panel **A**: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.), vehicle (1 ml/kg, s.c.) + quinpirole (0.5 mg/kg, s.c.), or quinpirole (0.5 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.).

Panel **B**: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.), vehicle (1 ml/kg, s.c.) + quinpirole (1 mg/kg, s.c.), or quinpirole (1 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.).

The first injection occurred 30 min prior to the second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from clozapine group ($p<0.01$).
c, significantly different from quinpirole group ($p<0.01$).


A
CONTROL
CLOZAPINE (20 mg/kg)
QUINPIROLE (0.5 mg/kg)
QUINPIROLE+CLOZAPINE
NUMBER OF IMMUNOREACTIVE CELLS
0
50
100
150
200
PFC
NAc
ML-Str
DL-Str
LSN


B
CONTROL
CLOZAPINE (20 mg/kg)
QUINPIROLE (1 mg/kg)
QUINPIROLE+CLOZAPINE
NUMBER OF IMMUNOREACTIVE CELLS
0
50
100
150
200
PFC
NAc
ML-Str
DL-Str
LSN

D. Effects of quinpirole on haloperidol-induced FLI and catalepsy

Prior administration of quinpirole (1 mg/kg, s.c.) has been reported to reverse the elevation striatal *c-fos* mRNA produced by haloperidol (2.5 mg/kg, s.c.) (Miller, 1990). Using these doses, the effect of quinpirole (0.5 mg/kg, s.c.) on FLI induced by haloperidol (2 mg/kg, s.c.) was examined first. At this dose, quinpirole failed to reduce either haloperidol-induced catalepsy or the number of neurons which displayed FLI in the DL-Str and NAc (Fig. 15A). Consequently, the effect of quinpirole (0.5 mg/kg, s.c.) on progressively lower doses of haloperidol were assessed next. Decreasing the haloperidol dose from 2 to 1 mg/kg (s.c.) failed to endow quinpirole (1.0 mg/kg, s.c.) with the ability to reverse increases in FLI produced by this antipsychotic (Fig. 15B). Similarly, reducing the haloperidol dose from 1 to 0.5 mg/kg (s.c.) failed to endow quinpirole (0.5 mg/kg, s.c.) with the ability to reduce haloperidol-induced FLI (Fig. 15C). Accordingly, the cataleptic response produced by 0.5 to 2.0 mg/kg of haloperidol was not reversed by prior administration of quinpirole (0.5 mg/kg, s.c.) (data not shown). However, prior administration of quinpirole (1 mg/kg) did reverse the increase in FLI in the PFC, NAc, DL-Str and LSN produced by a dose of 0.1 mg/kg of haloperidal (Fig. 15D). The cataleptic effect of haloperidol (0.1 mg/kg, s.c.) was also reversed by quinpirole (1 mg/kg, s.c.) (data not shown).

Fig. 15. Effects of different doses of quinpirole on Fos-like immunoreactivity induced by different doses of haloperidol in the forebrain. In each experiment, four groups, composed of 4 rats each, received one of the following:

Panel **A**: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (2 mg/kg, s.c.), vehicle (1 ml/kg, s.c.) + quinpirole (0.5 mg/kg, s.c.), or quinpirole (0.5 mg/kg, s.c.) + haloperidol (2 mg/kg, s.c.).

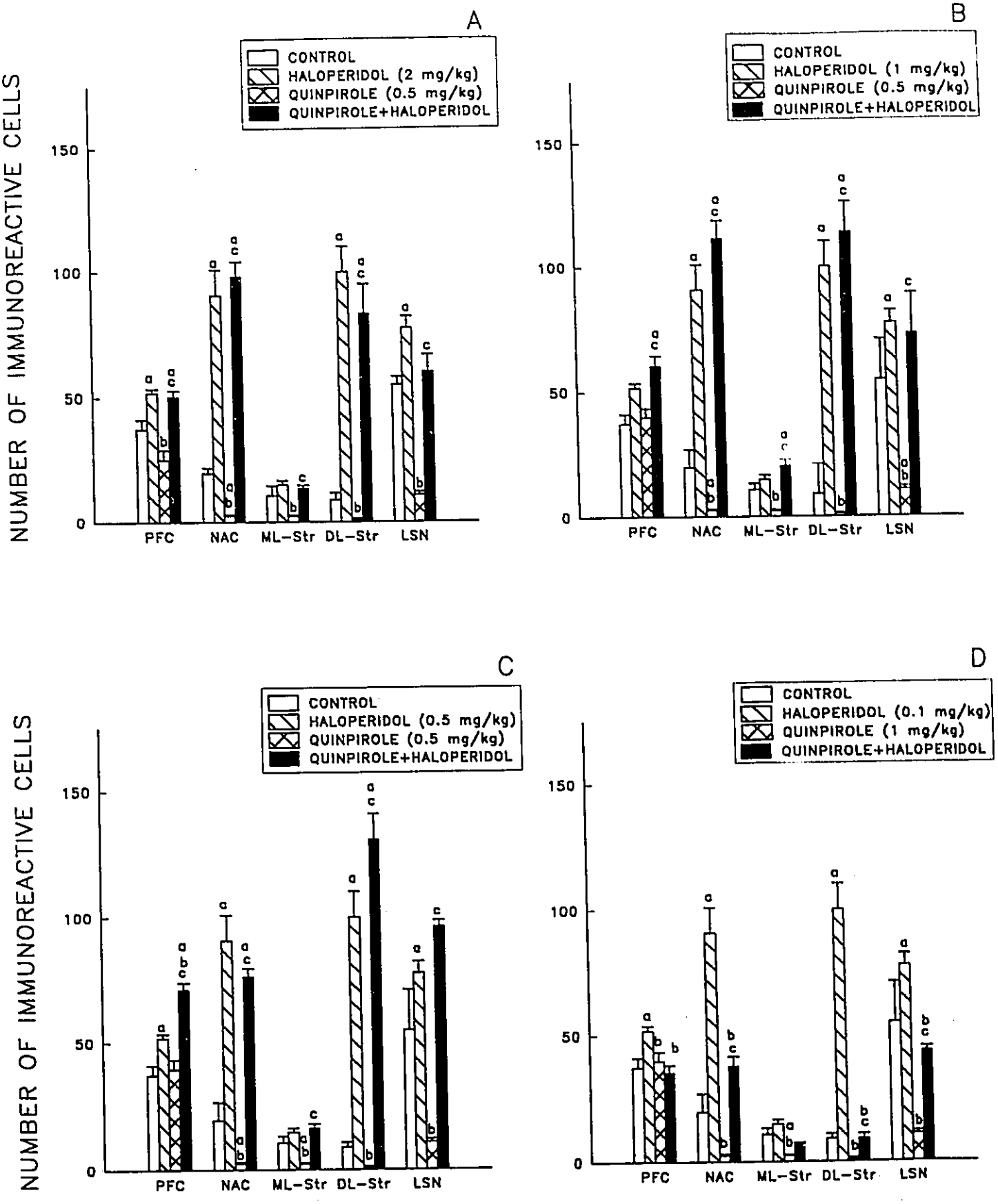
Panel **B**: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (1 mg/kg, s.c.), vehicle (1 ml/kg, s.c.) + quinpirole (0.5 mg/kg, s.c.), or quinpirole (0.5 mg/kg, s.c.) + haloperidol (1 mg/kg, s.c.).

Panel **C**: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (0.5 mg/kg, s.c.), vehicle (1 ml/kg, s.c.) + quinpirole (0.5 mg/kg, s.c.), or quinpirole (0.5 mg/kg, s.c.) + haloperidol (0.5 mg/kg, s.c.).

Panel **D**: saline (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), saline (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.), vehicle (1 ml/kg, s.c.) + quinpirole (1 mg/kg, s.c.), or quinpirole (1 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.).

The first injection occurred 30 min prior to second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).
c, significantly different from quinpirole group ($p<0.01$).


A
CONTROL
HALOPERIDOL (2 mg/kg)
QUINPIROLE (0.5 mg/kg)
QUINPIROLE+HALOPERIDOL
NUMBER OF IMMUNOREACTIVE CELLS
150
100
50
0
PFC
NAC
ML-Str
DL-Str
LSN
B
CONTROL
HALOPERIDOL (1 mg/kg)
QUINPIROLE (0.5 mg/kg)
QUINPIROLE+HALOPERIDOL
C
CONTROL
HALOPERIDOL (0.5 mg/kg)
QUINPIROLE (0.5 mg/kg)
QUINPIROLE+HALOPERIDOL
D
CONTROL
HALOPERIDOL (0.1 mg/kg)
QUINPIROLE (1 mg/kg)
QUINPIROLE+HALOPERIDOL

III. EFFECTS OF PRIOR ADMINISTRATION OF THE D_1-LIKE RECEPTOR ANTAGONIST SCH 23390 ON ANTIPSYCHOTIC-INDUCED FLI IN THE LIMBIC SYSTEM

The purpose of this experiment was to determine whether D_2-like receptor antagonist-induced FLI was altered by prior administration of the potent dopamine D_1-like receptor antagonist SCH 23390.

A. Effects of SCH 23390 on haloperidol-induced FLI

In agreement with previous work, SCH 23390 (0.1 mg/kg, s.c.) did not induce FLI in any tested regions in limbic system, whereas haloperidol (0.1 mg/kg, s.c.) significantly elevated FLI in the NAc, DL-Str, PFC and LSN (Fig. 16). Injection of SCH 23390 (0.1 mg/kg, s.c.) 30 min prior to haloperidol (0.1 mg/kg, s.c.) significantly reduced haloperidol-induced FLI in the NAc (Fig. 16). In contrast, haloperidol-induced increases in FLI in the DL-Str, PFC and LSN were unchanged by SCH 23390.

B. Effects of SCH 23390 on clozapine-induced FLI

The ability of clozapine (20 mg/kg, s.c.) to enhance FLI in the PFC, NAc and LSN was significantly reduced by prior administration of SCH 23390 (0.1 mg/kg, s.c.) (Fig. 17). Injection of SCH 23390 (0.1 mg/kg, s.c.) reduced, by 30%, increases in FLI induced by clozapine (20 mg/kg) in the PFC and LSN while SCH 23390 reduced clozapine-induced increases FLI in the NAc by 70%.

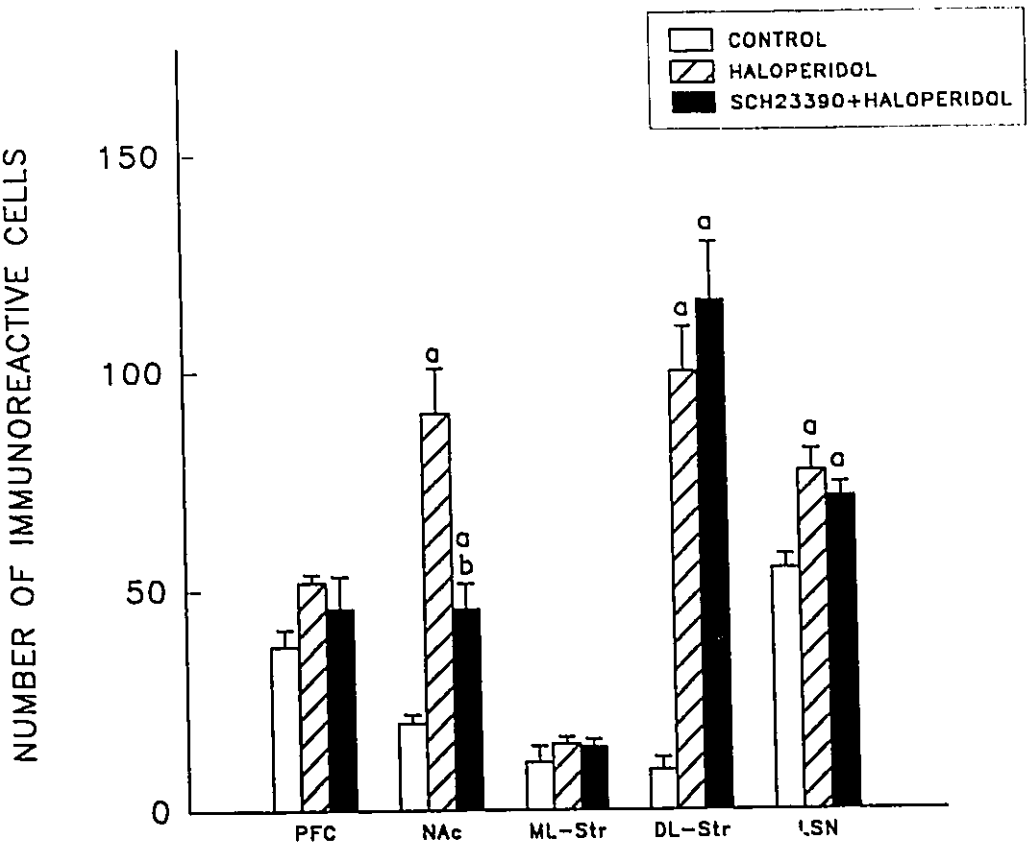


Fig. 16. Effects of SCH 23390 pretreatment on haloperidol induced-increases in Fos-like immunoreactivity in the forebrain. Three groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.), or SCH 23390 (0.1 mg/kg, s.c.) + haloperidol (0.1 mg/kg, s.c.). The first injection occurred 30 min prior to second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

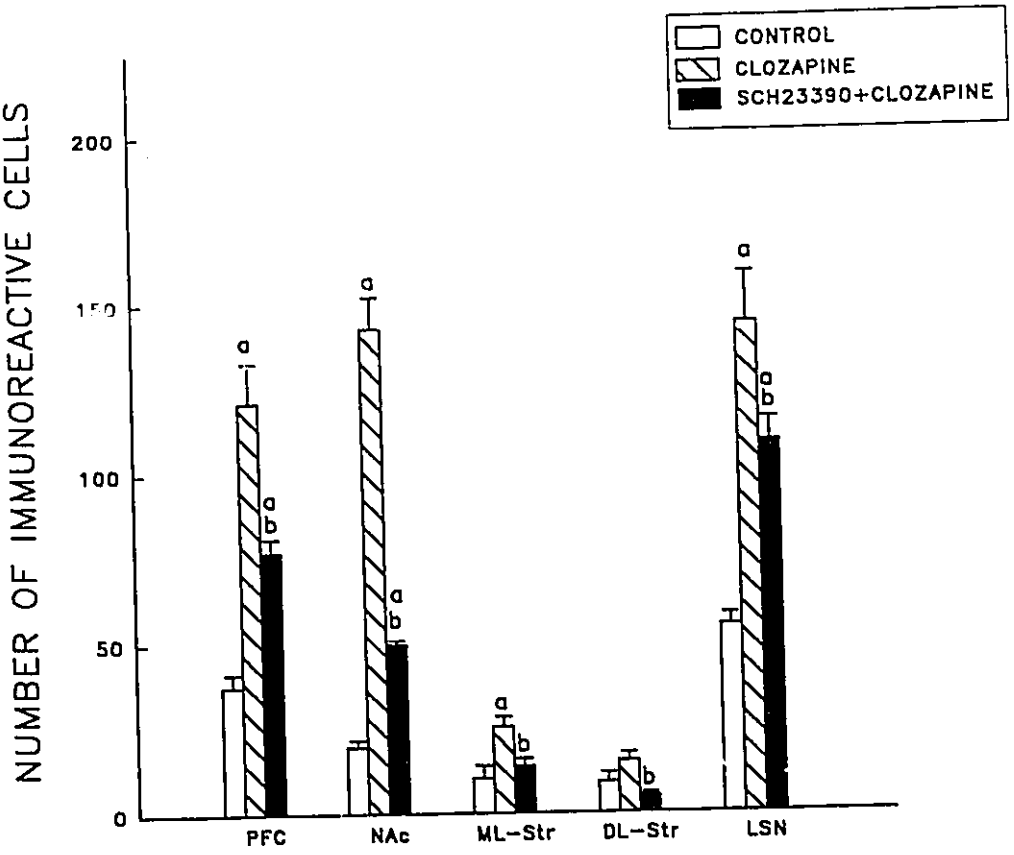


Fig. 17. Effects of SCH 23390 pretreatment on clozapine induced-increases in Fos-like immunoreactivity in the forebrain. Three groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + clozapine (20 mg/kg, s.c.) or SCH 23390 (0.1 mg/kg, s.c.) + clozapine (20 mg/kg, s.c.). The first injection occurred 30 min prior to second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from clozapine group ($p<0.01$).

C. Effects of SCH 23390 on UH 232-induced FLI

The D_2-like antagonist UH 232 (10 mg/kg, s.c.) significantly elevated FLI in the PFC, NAc, mediolateral and dorsolateral regions of the striatum (Fig. 18). Administration of SCH 23390 (0.1 mg/kg, s.c.) 30 min prior to UH 232 (10 mg/kg, s.c.) reduced markedly increases in FLI produced by UH 232 (10 mg/kg, s.c.) in the PFC, NAc, ML-Str and DL-Str (Fig. 18).

D. Effect of SCH 23390 on naphtamide-induced FLI

The selective D_3 receptor antagonist naphtamide (5 mg/kg, s.c.) selectively elevated FLI in the PFC and NAc (Fig. 19). Injection of SCH 23390 (0.1 mg/kg, s.c.) 30 min prior to administration of naphtamide (5 mg/kg, s.c.) significantly reversed increases in FLI produced by naphtamide (5 mg/kg) in the NAc (Fig. 19).

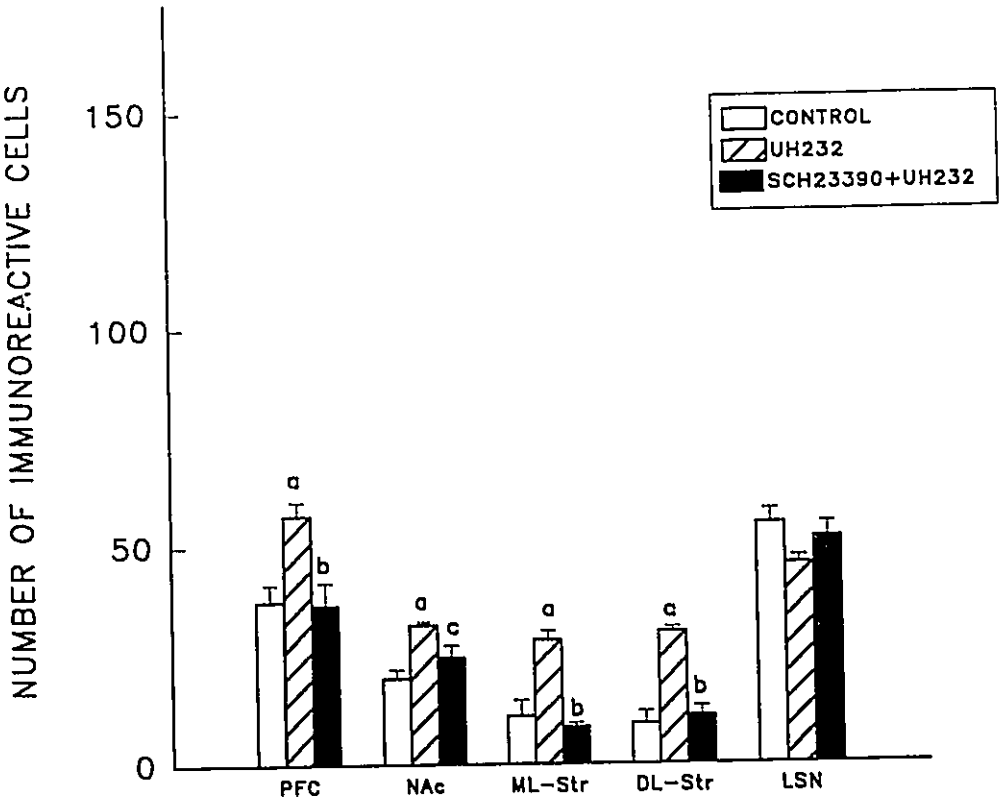


Fig. 18. Effects of SCH 23390 pretreatment on UH 232 induced-increases in Fos-like immunoreactivity in the forebrain. Three groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + UH 232 (10 mg/kg, s.c.) or SCH 23390 (0.1 mg/kg, s.c.) + UH 232 (10 mg/kg, s.c.). The second injection occurred 30 min prior to first one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from UH 232 group ($p<0.01$).
c, significantly different from UH 232 group ($p<0.05$).

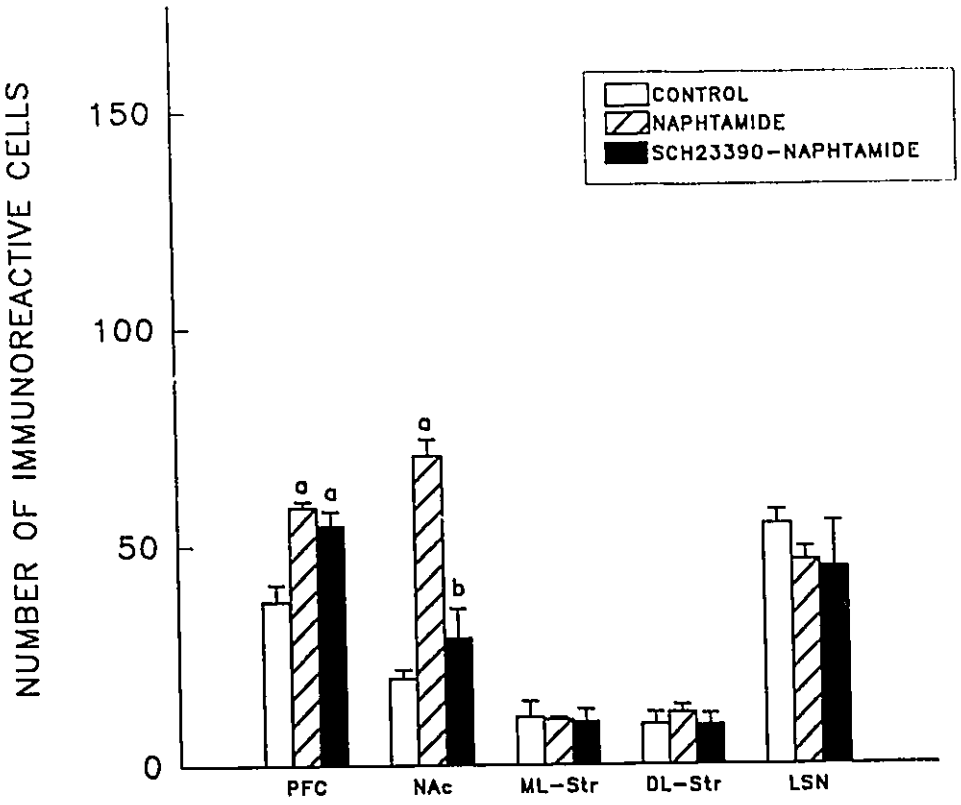


Fig. 19. Effects of SCH 23390 pretreatment on naphtamide induced-increases in Fos-like immunoreactivity in the forebrain. Three groups, composed of 4 rats each, received one of the following: distilled water (1 ml/kg, s.c.) + vehicle (1 ml/kg, s.c.), distilled water (1 ml/kg, s.c.) + naphtamide (5 mg/kg, s.c.) or SCH 23390 (0.1 mg/kg, s.c.) + naphtamide (5 mg/kg, s.c.). The first injection occurred 30 min prior to second one. The effects of these treatments on Fos-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 4 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from naphtamide group ($p<0.01$).

V. EFFECTS OF D_2-LIKE RECEPTOR ANTAGONIST ADMINISTRATION ON *c-fos* mRNA EXPRESSION IN THE FOREBRAIN

The purpose of this study was to determine the dose-dependent effects of haloperidol on *c-fos* expression in the striatum and whether there is a correspondence between these regions which express FLI and *c-fos* mRNA after haloperidol and clozapine administration.

A. Dose-dependent effects of haloperidol on the number of striatal neurons which expressed *c-fos* mRNA

The dose-response relationships for haloperidol-induced increases in the number of neurons which expressed *c-fos* mRNA in the NAc, ML-Str and DL-Str are shown in Fig. 20. Haloperidol produced a dose-dependent elevation of the number of neurons which expressed *c-fos* mRNA in these regions at doses from 0.025 mg/kg to 2 mg/kg (s.c.). Maximal increases were observed at a dose of 0.25 mg/kg (s.c.) in the nucleus accumbens, mediolateral and dorsolateral striatum. The EC_{50} dose for haloperidol-induced *c-fos* expression in all of these regions was approximately 0.09 mg/kg (s.c.) (Fig. 20).

B. Dose-dependent effects of haloperidol on cellular levels of *c-fos* mRNA expression

The dose-respone relationships for haloperidol elevated the amount of c-fos mRNA per cell in the nucleus accumbens, mediolateral striatum and dorsolateral striatum

are shown in Fig. 21, 22. Haloperidol produced a dose-dependent elevation of the amount of *c-fos* mRNA per cell in all of these regions indicating that maximal expression was observed at a dose of 2 mg/kg (s.c.) of haloperidol. The EC_{50} dose for haloperidol-expressed *c-fos* mRNA per cell in the NAc, ML-Str and DL-Str was approximately 0.09 mg/kg (s.c.).

C. Effects of haloperidol and clozapine on celiular levels of *c-fos* mRNA expression

A single injection of haloperidol (2 mg/kg, s.c.) produced a dramatic increase in the amount of *c-fos* mRNA per cell in the NAc, Ml-Str and DL-Str. In contrast, a single injection of clozapine (20 mg/kg, s.c.) elevated the amount of *c-fos* mRNA per cell selectively in limbic structures such as PFC, NAc and LSN (Fig. 23, 24)

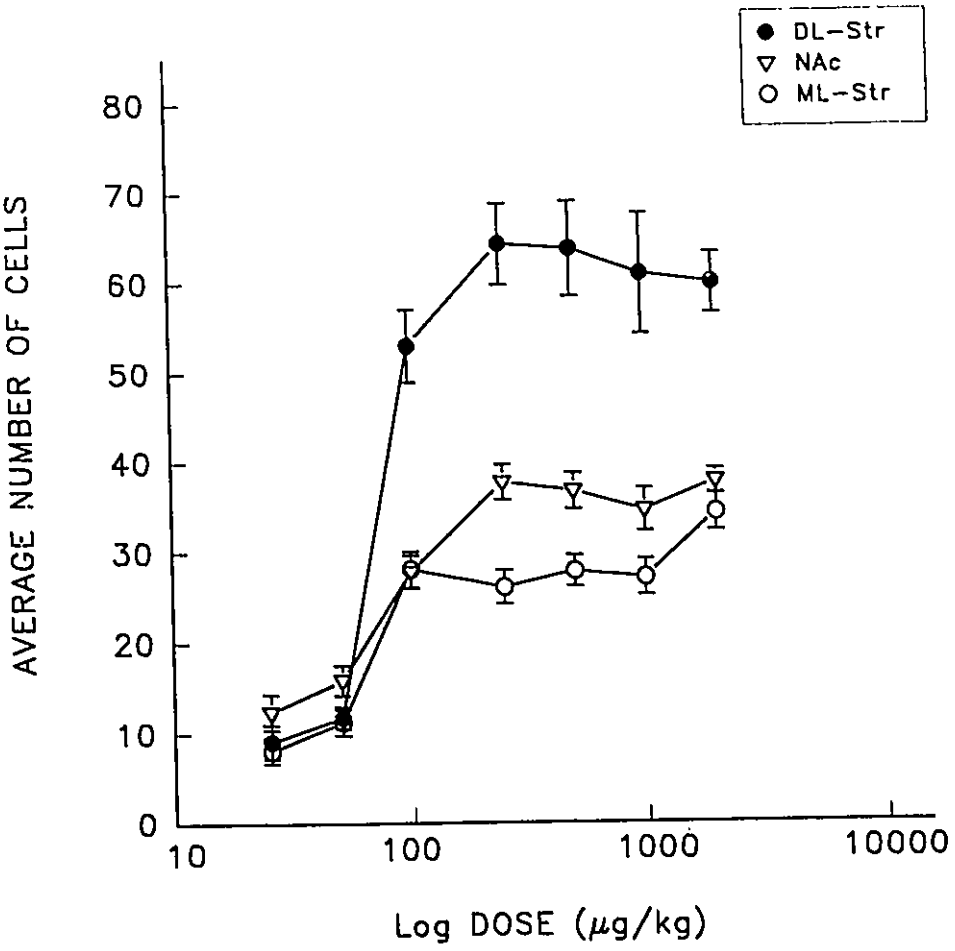


Fig. 20. Dose-dependent effects of haloperidol on the number of neurons which expressed *c-fos* mRNA. Seven groups, composed of 4 rats each, received one of the following doses of haloperidol: (0.025, 0.05, 0.1, 0.25, 0.5, 1, or 2 mg/kg, s.c.). The average number of neurons labelled in the NAc (nucleus accumbens), ML-Str (mediolateral striatum) and DL-Str (dorsolateral striatum) with an ^{35}S-oligonucleotide probe complimentary to *c-fos* mRNA was measured by quantitative image analysis.

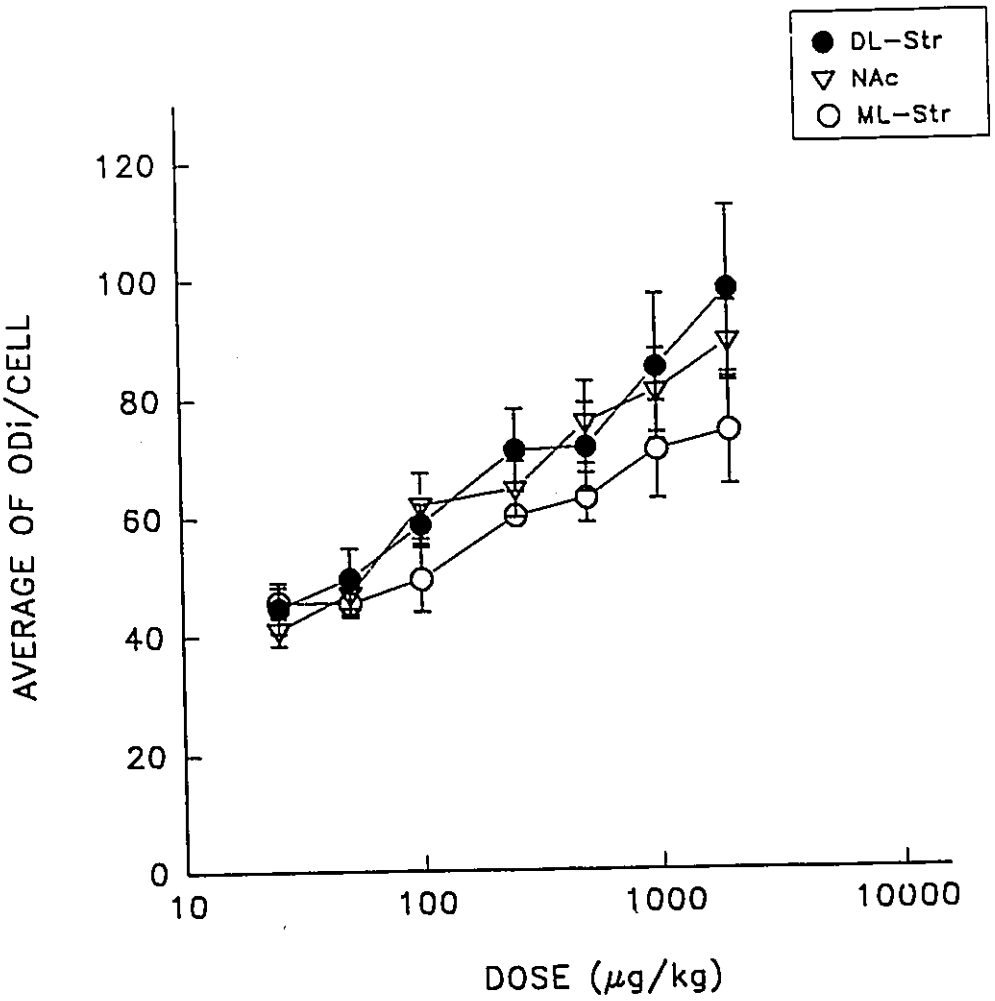
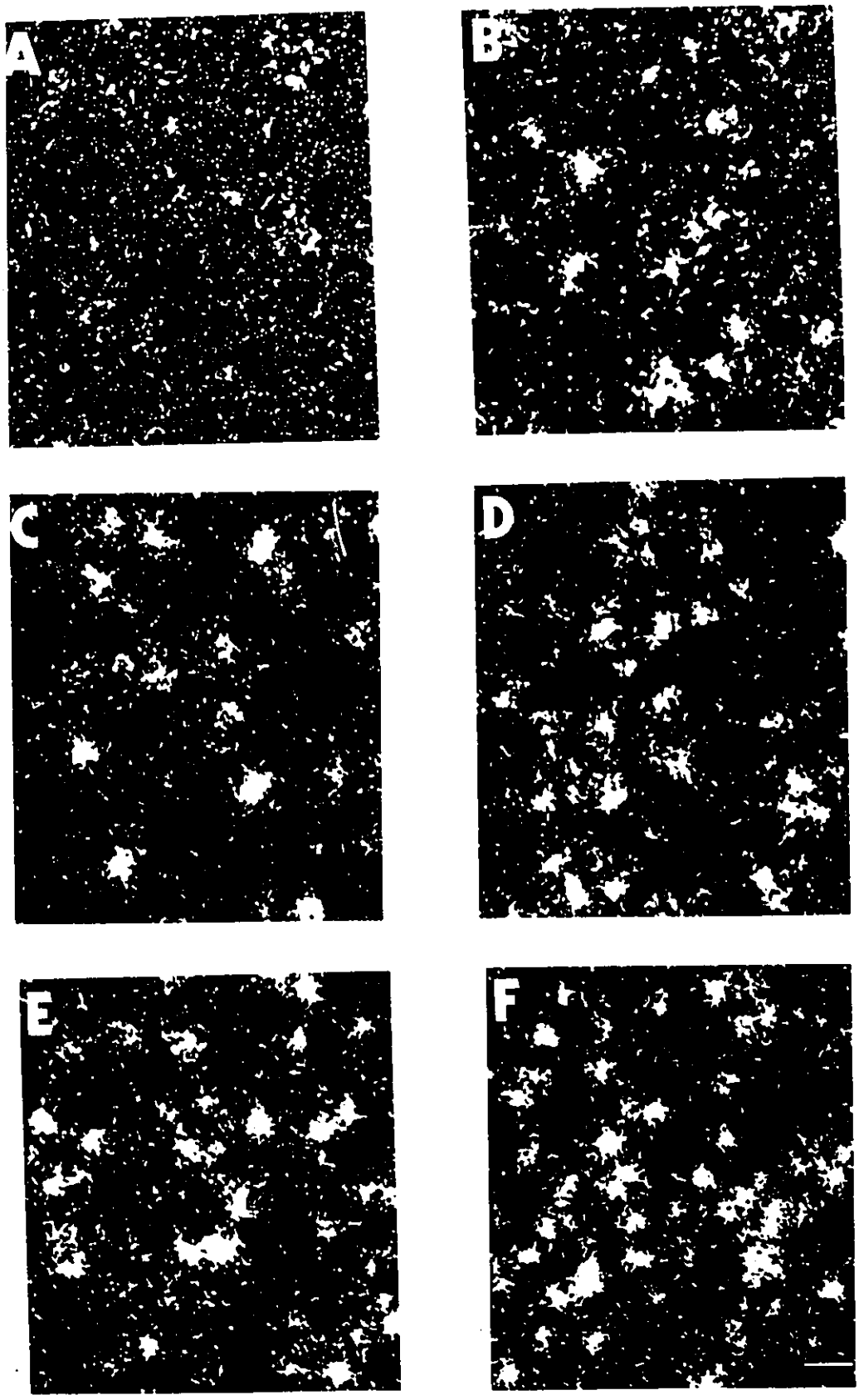


Fig. 21. Dose-dependent effects of haloperidol on cellular *c-fos* expression in the striatum. Seven groups, composed of 4 rats each, received one of the following doses of haloperidol: (0.025, 0.05, 0.1, 0.25, 0.5, 1, or 2 mg/kg, s.c.). The relative amount of *in situ* hybridization histochemistry labelling per neuron was measured as inverse optical density (ODi) in the NAc (nucleus accumbens), ML-Str (mediolateral striatum) and DL-Str (dorsolateral striatum) using an image analysis system.

Fig. 22. Dark-field illumination of ISHH labelling in the dorsolateral striatum representative of the effects of vehicle (1 ml/kg, s.c.) (A) and dose-response relationships for haloperidol (0.1 mg/kg, s.c.) (B), (0.25 mg/kg, s.c.) (C), (0.5 mg/kg, s.c.) (D), (1 mg/kg, s.c.) (E) and (2 mg/kg, s.c.) (F) on *c-fos* expression. Scale bar = 30 μm


A
B
C
D
E
F

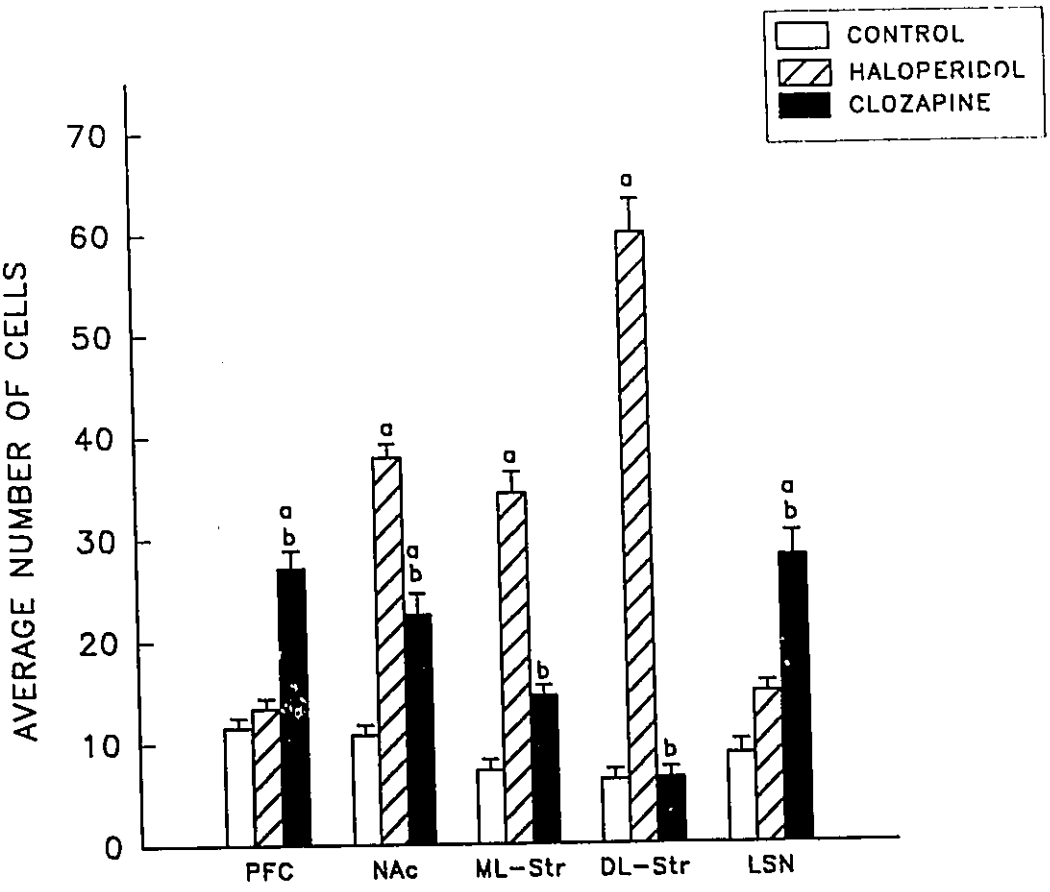


Fig. 23. Comparison of the effects of single vehicle (1 ml/kg, s.c.), haloperidol (2 mg/kg, s.c.) or clozapine (20 mg/kg, s.c.) injection on the number of neurons which expressed *c-fos* mRNA. The average number of neurons labelled in the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus) with an ^{35}S-oligonucleotide probe complimentary to *c-fos* mRNA was measured by quantitatve image analysis.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

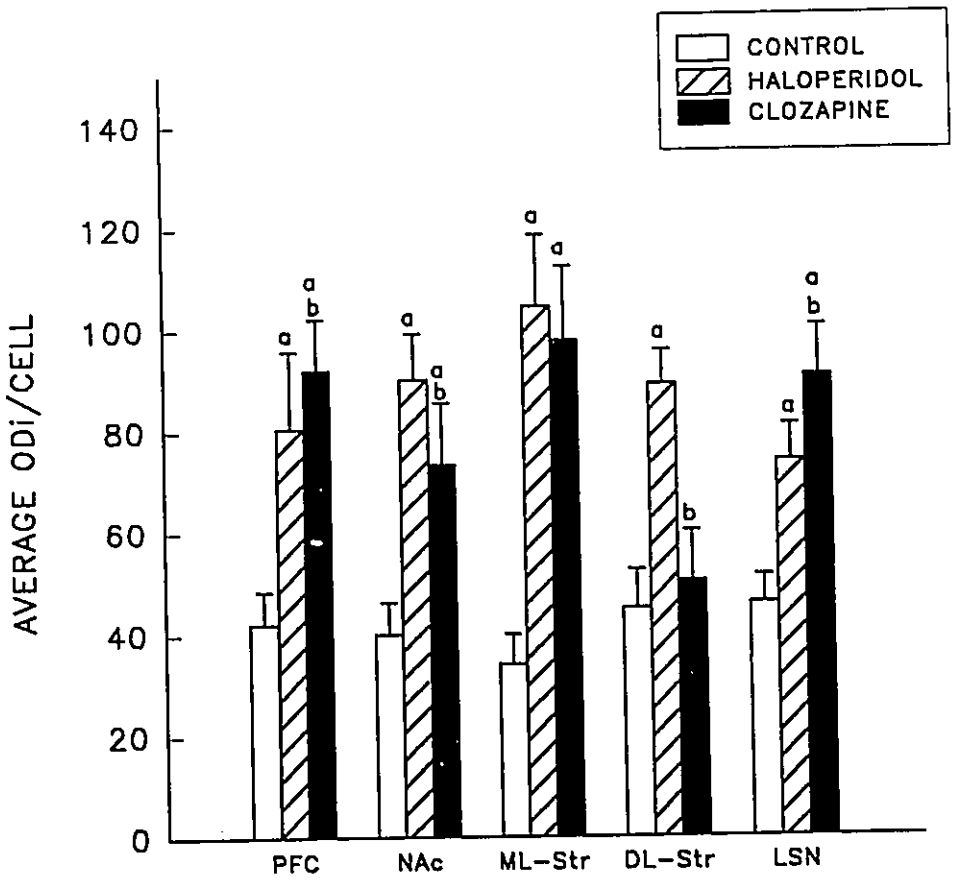


Fig. 24. Comparison of the effects of single vehicle (1 ml/kg, s.c.), haloperidol (2 mg/kg, s.c.) or clozapine (20 mg/kg, s.c.) injection on cellular *c-fos* expression. The relative amount of *in situ* hybridization histochemistry labelling per neuron was measured as inverse optical density (ODi) in the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus).

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

V. EFFECTS OF CHRONIC ANTIPSYCHOTIC ADMINISTRATION ON IMMEDIATE-EARLY GENE EXPRESSION IN THE FOREBRAIN

The goal of this study was to determine the effects of chronic clozapine and haloperidol administration on expression of the immediate-early gene products Fos, FosB, ΔFosB, Jun, and JunB in the forebrain.

A. Effects of chronic haloperidol and clozapine administration on Fos-like immunoreactivity

Basal levels of Fos-like immunoreactivity were very low in all regions examined (PFC, NAc, ML-Str, DL-Str and LSN) after 16 days of vehicle administration (1 ml/kg/day) (data not shown). Similarly, chronic administration of haloperidol (2 mg/kg, i.p.) and clozapine (20 mg/kg, i.p.), once daily for 16 days, did not induce FLI in any of these regions (data not shown).

B. Effects of chronic haloperidol and clozapine administration on FosB-like immunoreactivity

The FosB(N) and FosB(C) antibodies were used to assess the effects of chronic administration of antipsychotic drugs such as haloperidol and clozapine on FosB and ΔFosB expression in the forebrain. FosB(C)-like immunoreactivity was unaffected by chronic administration of vehicle (1 ml/kg, i.p.), haloperidol (2 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.), once daily for 16 days (data not shown). In contrast,

FosB(N)-like immunoreactivity was elevated by chronic administration of haloperidol (2 mg/kg, i.p.), in the NAc, ML-Str and DL-Str (Fig. 25, 26, 27, 28, 29, 30). Chronic administration of clozapine (20 mg/kg, i.p.), however, elevated FosB(N)-like immunoreactivity primarily in limbic structure such as the PFC, NAc and LSN. By comparison to haloperidol, ciozapine has weak effects on FosB(N)-like immunoreactivity in the striatum (Fig. 25, 26, 27, 28, 29, 30).

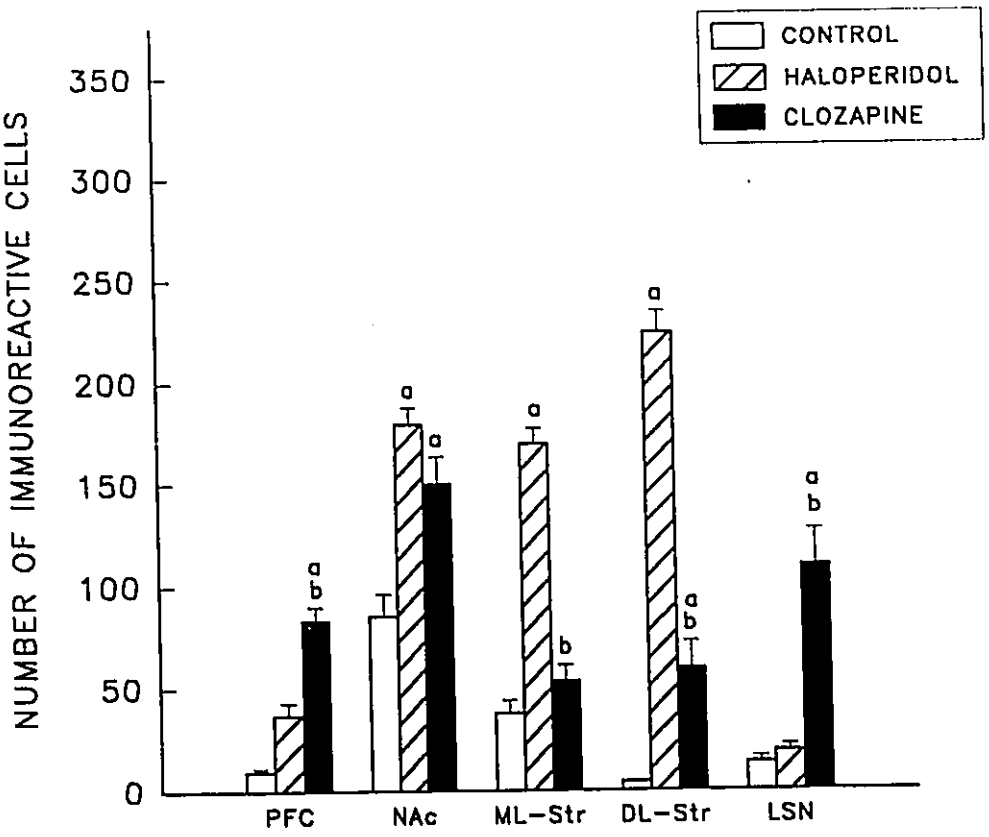


Fig. 25. Induction of FosB(N)-like immunoreactivity by chronic adminstration of clozapine and haloperidol in the forebrain. Three groups, composed of 5 rats each, received a single daily injection of one of the following for 16 days: vehicle (1 ml/kg, i.p.), haloperidol (2 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.). The effects of these treatments on FosB(N)-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 5 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

Fig. 26. Photomicrographs of ΔFosB-like immunoreactivity in the medial prefrontal cortex (PFC) and lateral septal nucleus (LSN) after chronic administration of vehicle (1 ml/kg/day, i.p. for 16 days) (A, PFC; D, LSN), haloperidol (2 mg/kg/day, i.p. for 16 days) (B, PFC; E, LSN), clozapine (20 mg/kg/day, i.p. for 16 days) (C, PFC; F, LSN). Scale bar = 100 μm.

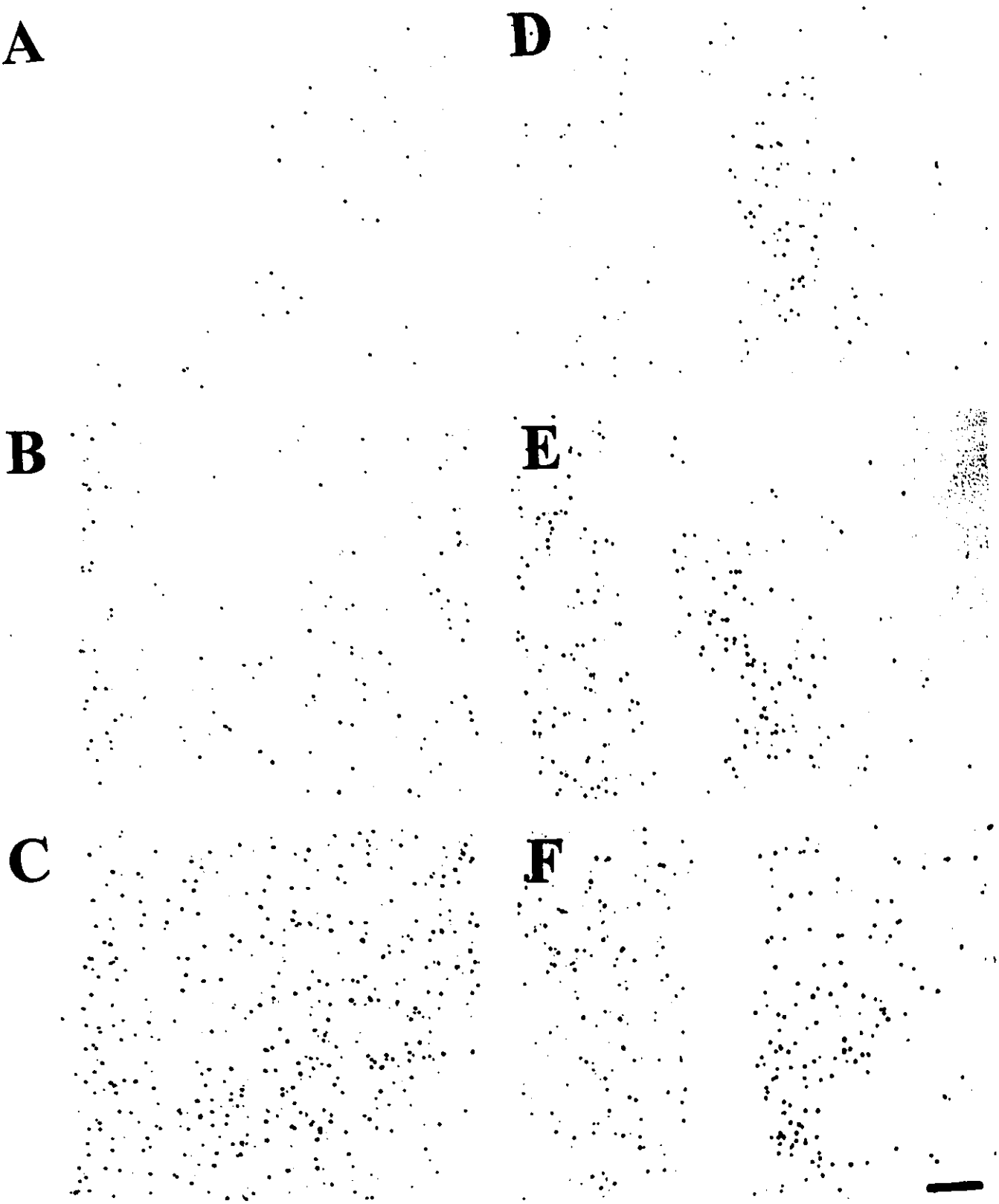
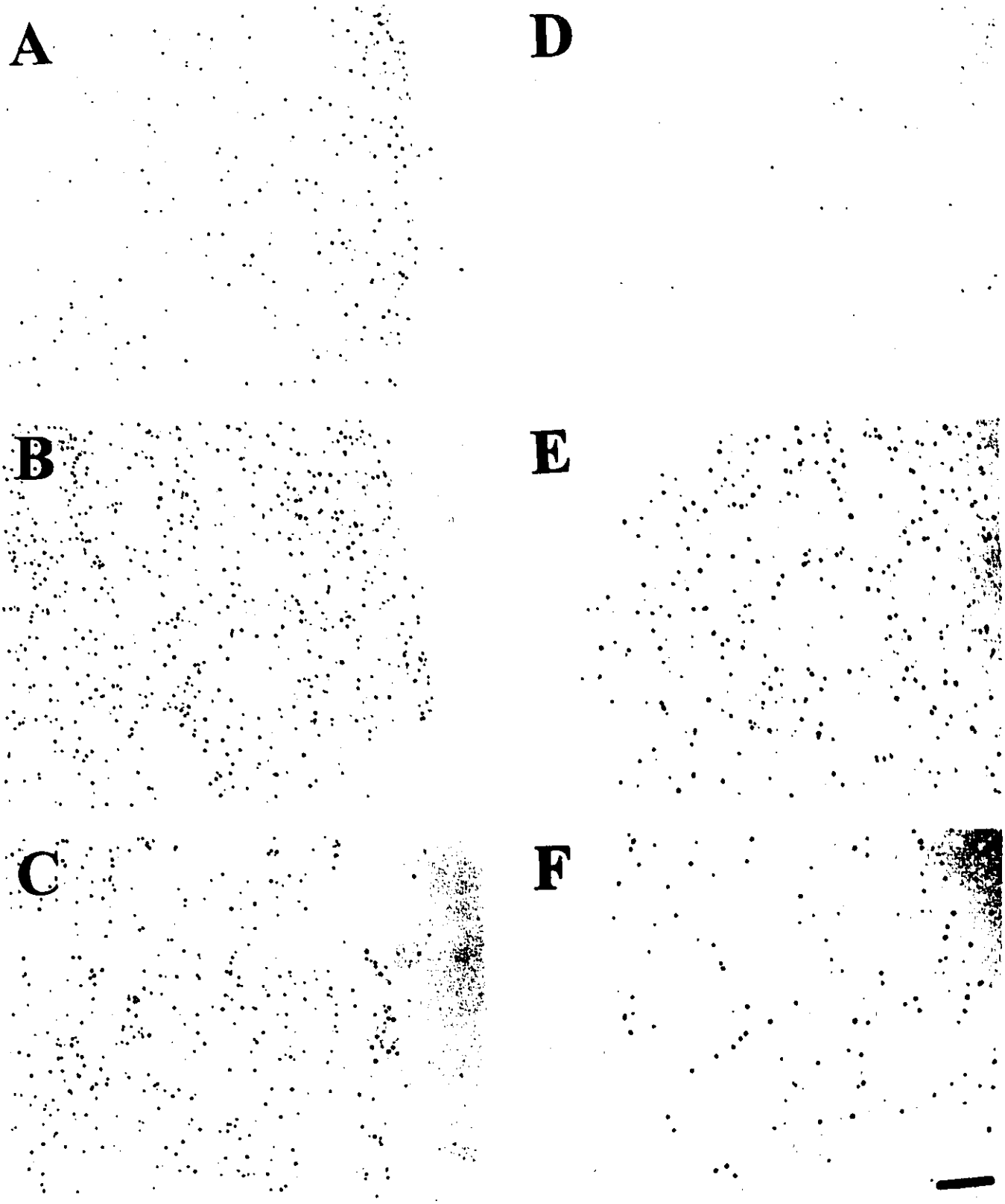

A
D
B
E
C
F

Fig. 27. Photomicrographs of ΔFosB-like immunoreactivity in the nucleus accumbens (NAc) and dorsolateral striatum (DL-Str) after chronic administration of vehicle (1 ml/kg/day, i.p. for 16 days) (A, NAc; D, DL-Str), haloperidol (2 mg/kg/day, i.p. for 16 days) (B, NAc; E, DL-Str), clozapine (20 mg/kg/day, i.p. for 16 days) (C, NAc; F, DL-Str). Scale bar = 100 μm.


A
D
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C
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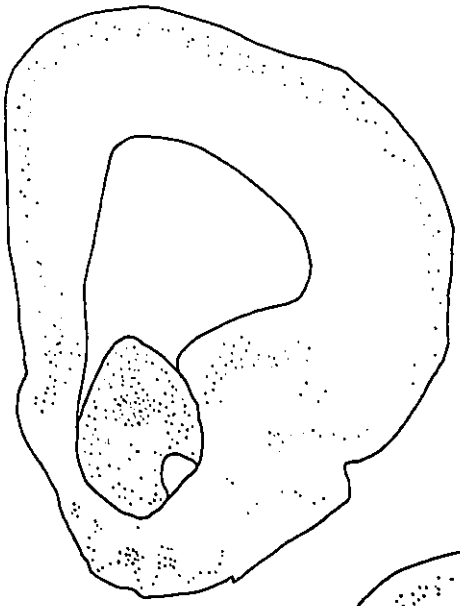
Fig. 28. Camera lucida drawings representative of the effects of chronic administration of vehicle (1 ml/kg/day, i.p. for 16 days), haloperidol (2 mg/kg/day, i.p. for 16 days), clozapine (20 mg/kg/day, i.p. for 16 days) on the distribution of ΔFosB-like positive neurons in the medial prefrontal cortex (PFC) and anterior nucleus accumbens (NAc). Each black dot represents a single ΔFosB-like positive nucleus. Sections correspond to an AP position approximately 2.7 mm from bregma according to the atlas of Paxinos and Watson.



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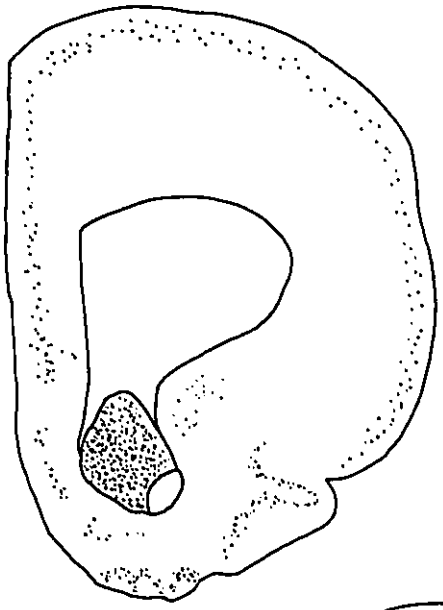


Haloperidol

Clozapine

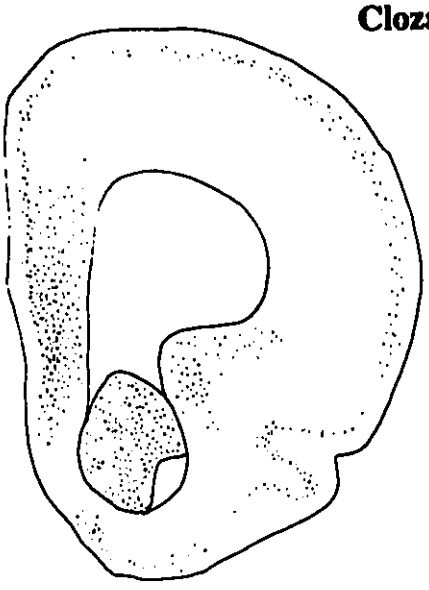


Fig. 29. Camera lucida drawings representative of the effects of chronic administration of vehicle (1 ml/kg/day, i.p. for 16 days), haloperidol (2 mg/kg/day, i.p. for 16 days), clozapine (20 mg/kg/day, i.p. for 16 days) on the distribution of ΔFosB-like positive neurons at the level of the caudal nucleus accumbens (NAc) and mid-striatum. Each black dot represents a single ΔFosB-like positive nucleus. Sections correspond to an AP position approximately 1.0 mm from bregma according to the atlas of Paxinos and Watson.

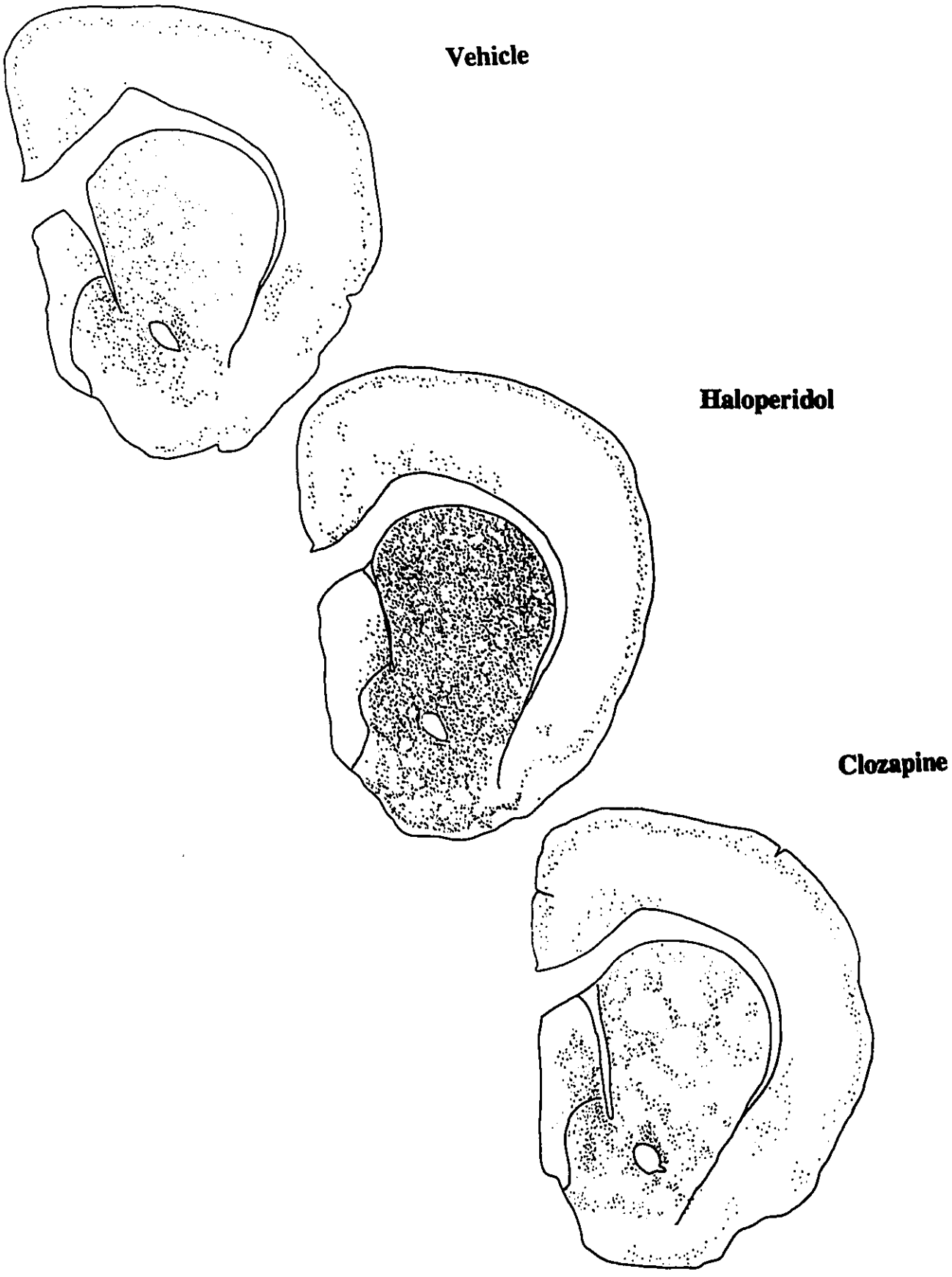
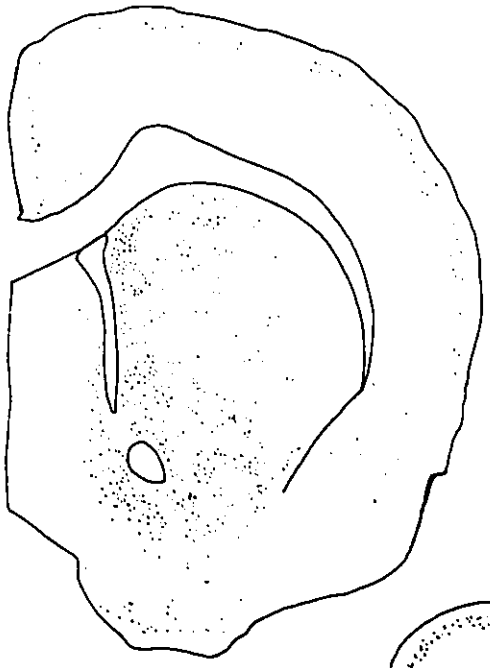
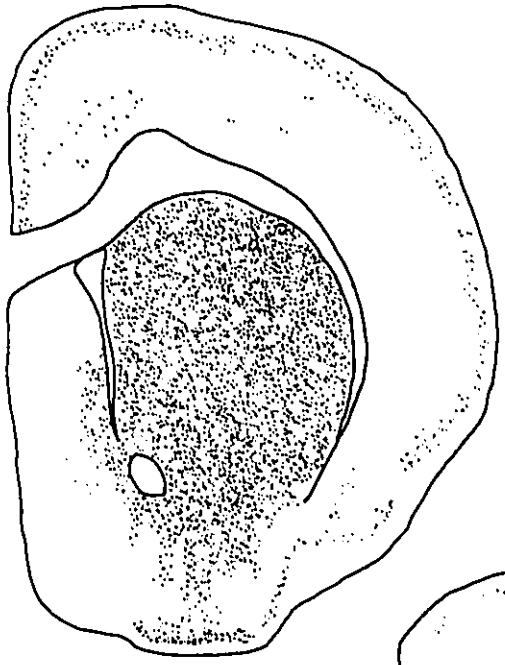

Vehicle
Haloperidol
Clozapine

Fig. 30. Camera lucida drawings representative of the effects of chronic administration of vehicle (1 ml/kg/day, i.p. for 16 days), haloperidol (2 mg/kg/day, i.p. for 16 days), clozapine (20 mg/kg/day, i.p. for 16 days) on the distribution of ΔFosB-like positive neurons at the level of the mid-striatum and septum (LSN). Each black dot represents a single ΔFosB-like positive nucleus. Sections correspond to an AP position approximately 0.1 mm from bregma according to the atlas of Paxinos and Watson.

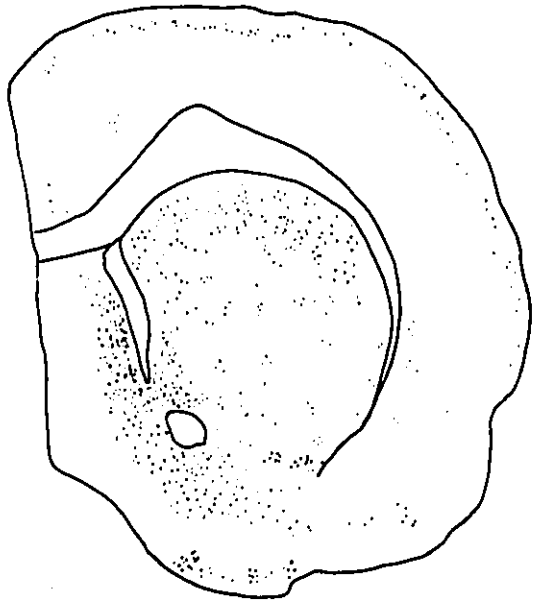


Vehicle

Haloperidol



Clozapine



C. Effects of chronic haloperidol and clozapine administration on Jun-like immunoreactivity

A large number of Jun-like immunoreactive neurons were detected in the NAc, ML-Str and DL-Str of vehicle treated animals (1 ml/kg/day) for 16 days (Fig. 31). By contrast basal Jun-like immunoreactivity was low in PFC and LSN. Chronic administration of haloperidol (2 mg/kg, i.p.) significantly elevated c-Jun-like immunoreactivity in the NAc, ML-Str and DL-Str. In contrast, chronic administration of clozapine (20 mg/kg, i.p.) did not elevate Jun-like immunoreactivity in the DL-Str and NAc. However, chronic clozapine administration significantly increased Jun-like immunoreactivity in the LSN.

D. Effects of chronic haloperidol and clozapine administration on JunB-like immunoreactivity

A few JunB-like immunoreactive neurons were detected in the PFC, NAc, ML-Str and LSN of vehicle treated animals (Fig. 32). Chronic administration of haloperidol (2 mg/kg, i.p.), once daily for 16 days, significantly increased JunB-like immunoreactivity in the NAc, ML-Str and DL-Str. The LSN was the only region which displayed JunB-like immunoreactivity after chronic clozapine administration (20 mg/kg, i.p.; one daily for 16 days).

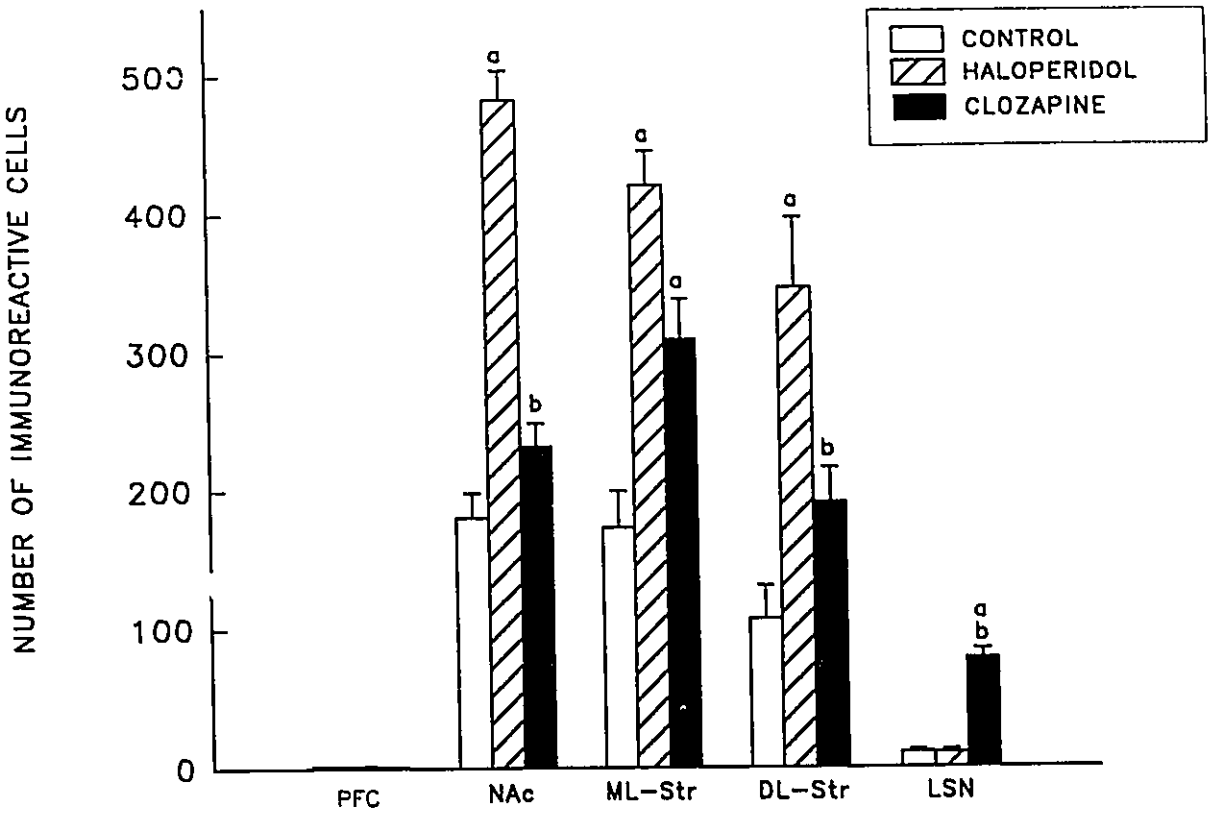


Fig. 31. Induction of Jun-like immunoreactivity by chronic adminstration of clozapine and haloperidol in the forebrain. Three groups, composed of 5 rats each, received a single daily injection of one of the following for 16 days: vehicle (1 ml/kg, i.p.), haloperidol (2 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.). The effects of these treatments on c-Jun-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 5 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

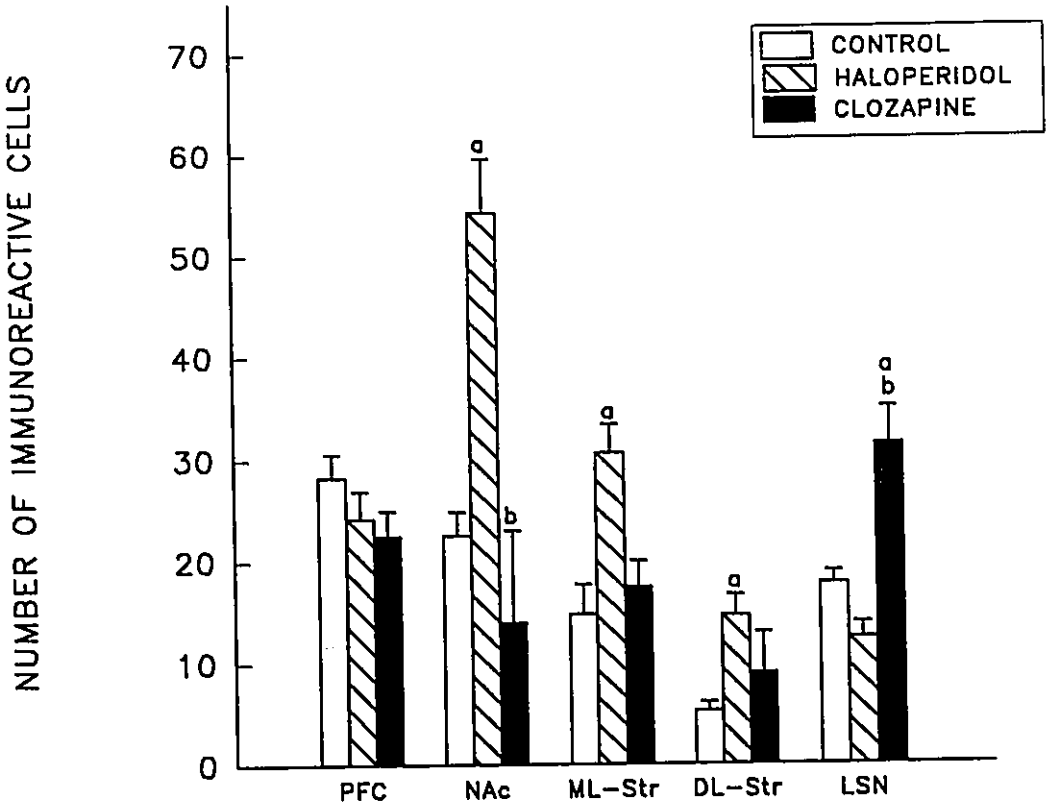


Fig. 32. Induction of JunB-like immunoreactivity by chronic adminstration of clozapine and haloperidol in the forebrain. Three groups, composed of 5 rats each, received a single daily injection of one of the following for 16 days: vehicle (1 ml/kg, i.p.), haloperidol (2 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.). The effects of these treatments on JunB-like immunoreactivity were quantified by counting the number of immunoreactive cells within a 600 x 880 μm square area of the PFC (prefrontal cortex), NAc (nucleus accumbens), ML-Str (mediolateral striatum), DL-Str (dorsolateral striatum) and LSN (lateral septal nucleus). Each bar represents the mean $\pm$ SEM of duplicate determinations performed on two sections from 5 animals.

Data were analyzed by ANOVA followed by Newman-Keuls test,
a, significantly different from control level ($p<0.01$).
b, significantly different from haloperidol group ($p<0.01$).

DISCUSSION

I. EFFECTS OF D_2-LIKE RECEPTOR ANTAGONIST ADMINISTRATION ON Fos-LIKE IMMUNOREACTIVITY AND *c-fos* mRNA EXPRESSION IN THE FOREBRAIN

A. Dose-dependent effects of haloperidol on catalepsy and FLI in the dorsolateral striatum

A previous report by Miller (1990), based on quantitative densitometry of Northern blots, indicated that the D_2-like receptor antagonist haloperidol (Christensen et al., 1984) produced a dose dependent increase in *c-fos* mRNA in the rat striatum. Maximal induction was observed at a dose of 2.5 mg/kg (s.c.) while increases of 10, 60 and 87% were obtained with 0.1, 0.5, and 1 mg/kg (s.c.) of haloperidol, respectively. In order to determine if there was a similar dose-response relationship for haloperidol-induced FLI, we examined the effects of several doses of haloperidol on FLI in the dorsolateral striatum. Furthermore, since the dorsolateral striatum has been implicated in the production of haloperidol-induced catalepsy, we also compared the dose-response relationships for haloperidol-induced catalepsy and FLI in the dorsolateral striatum.

Haloperidol produced a dose-dependent increase in FLI in the dorsolateral striatum. However, the EC_{50} (0.03 mg/kg, s.c.) and E_{max} (0.1 mg/kg, s.c.) doses for haloperidol-induced FLI were much lower than those reported for haloperidol-induced increases in *c-fos* mRNA levels by Miller (1990). Consistent with previous reports

(Klemm, 1985), haloperidol also induced catalepsy in a dose-dependent manner. Maximal induction of catalepsy was observed at a dose of 0.05 mg/kg (s.c.) of haloperidol while the EC_{50} dose was 0.03 mg/kg (s.c.). These results suggest that the dose-response relationships for haloperidol-induced FLI in the dorsolateral striatum and catalepsy are similar. Since Fos is considered to be a marker of neuronal activity, this raises the possibility that neuronal activation in the dorsolateral striatum may mediate haloperidol-induced catalepsy.

Retrograde tracing studies have established that haloperidol-induced FLI is located predominantly in striatal neurons which project to the globus pallidus termed striatopallidal neurons (Robertson et al., 1992). D_2 receptors are preferentially located on striatopallidal neurons and thought to tonically inhibit these neurons (Gerfen et al., 1990; Pan and Walter, 1988). Neuroleptics which block these dopamine receptors are therefore thought to produce an excessive stimulation of striatopallidal neurons. The overactivity of striatopallidal neurons caused by D_2 receptor antagonists has been proposed to contribute to the hypokinetic state and rigidity (catalepsy) produced by neuroleptics (Albin et al., 1989). Consequently, the strong relationship between haloperidol-induced catalepsy and FLI in the dorsolateral striatum is consistent with the suggestion that an overactive striatopallidal pathway may mediate catalepsy.

Several lines of evidence indicate that the dorsolateral striatum regulates movement. Single cell recording studies have demonstrated that neuronal firing in the dorsolateral striatum occurs during forelimb reaching, limb manipulation and electrical stimulation of sensory or motor cortices (Hirata et al., 1984; Carelli et al., 1991).

Furthermore, destruction of the dorsolateral striatum interferes with normal motor control and somatosensory orientation (Pisa, 1988; Sabol et al., 1985). More recently, Robertson et al. (1994) examined the effects of 17 compounds considered to be either typical or atypical antypsychotics on FLI in different regions of the forebrain. Antipsychotics with a clearly documented liability for producing EPS in humans or catalepsy in rats elevated FLI in the dorsolateral striatum (Robertson et al., 1994). In contrast, compounds that are unlikely to produce EPS or catalepsy did not induce FLI in the dorsolateral striatum. These results are consistent with the present findings and implicate the dorsolateral striatum in catalepsy.

B. Dose dependent effects of haloperidol on *c-fos* mRNA levels in the dorsolateral striatum

In comparison to the dose response relationship for haloperidol-induced increases in striatal *c-fos* mRNA levels reported by Miller (1990), haloperidol had significantly different dose-dependent effects on FLI in the dorsolateral striatum. One possible explanation for these discrepant findings is that changes in FLI were quantified in the present study by simply counting the number of immunoreactive neurons. This method does not permit the quantification of changes in *c-fos* expression that occur within single neurons. However, using *in situ* hybridization histochemistry (ISHH) it is possible to measure changes in *c-fos* expression at the cellular level. ISHH was therefore employed to determine the dose-dependent effects of haloperidol on *c-fos* mRNA levels at the cellular level.

Haloperidol produced a dose dependent increase in cellular *c-fos* expression (OD_i per cell) in both the dorsal and ventral striatum, the EC_{50} dose was 0.1 mg/kg (s.c.) while the E_{max} dose was 2 mg/kg (s.c.) (Fig. 21). These values are more in line with the results of Miller (1990) in which the EC_{50} and E_{max} doses for haloperidol-induced *c-fos* expression were about 0.3 and 2.5 mg/kg, respectively.

Fig. 20. shows the dose-dependent effects of haloperidol on the number of neurons which expressed *c-fos* mRNA in the ventral and dorsal striatum, the EC_{50} dose was 0.1 mg/kg (s.c.) while the E_{max} was 0.25 mg/kg (s.c.) of haloperidol for both of these regions. These values are very similar to those obtained for haloperidol-induced FLI. Taken together, these findings suggest that the majority of striatal neurons capable of expressing haloperidol-induced *c-fos* mRNA and FLI do so after low doses of haloperidol. However, they also indicate that low doses of haloperidol only produce a partial activation of these neurons and that higher doses are required to produce maximal increases in *c-fos* expression.

Previous work has demonstrated that haloperidol produces greater increases in FLI in the dorsolateral striatum than medial striatum (Dragunow et al., 1990; Robertson and Fibiger, 1992). In agreement with this finding, haloperidol produced a large increase in the number of neurons which expressed *c-fos* mRNA in the dorsolateral than medial striatum.

C. Dose-dependent effects of haloperidol on FLI in the ventral and mediolateral striatum

In addition to the dorsolateral striatum, haloperidol produced dose-dependent increases in FLI in the medial and ventral aspects of the striatum (Fig. 7). In the nucleus accumbens, the EC_{50} dose for haloperidol-induced FLI was 0.05 mg/kg (s.c.) while the E_{max} dose was 0.5 mg/kg (s.c.). Haloperidol also induced FLI in a dose-dependent manner in the mediolateral striatum. Maximal induction of FLI in this area was produced by a dose of 0.1 mg/kg (s.c.) of haloperidol while the EC_{50} dose was 0.03 mg/kg (s.c.). Consequently, the dose-response relationships for haloperidol-induced FLI in the medial and ventral striatum were similar.

Electrophysiological and behavioral data indicate that the medial and dorsolateral striatum are functionally distinct. For example, sensory and motor cortices project predominantly to the dorsolateral striatum, whereas association cortices project principally to the medial striatum (Donoghue et al., 1986; McGeorge et al., 1989). Lesions of the medial striatum produce cognitive deficits (Divac et al., 1978) while lesions of the dorsolateral striatum cause motor impairments. In contrast, the medial striatum has more in common with the nucleus accumbens, a major component of ventral striatum. For instance, the nucleus accumbens and mediolateral striatum share many of the same connections with both receiving afferents from the amygdala, association cortices and ventral tegmental dopamine neurons (Carter and Fibiger, 1977; Beckstead et al., 1979; Gerfen et al., 1987; McGeorge et al., 1989). Like the medial striatum, the nucleus accumbens has been implicated in affective aspects of behaviour (Le Moel and

Simon, 1991). Moreover, the medial striatum and nucleus accumbens are the only regions in which all antipsychotics elevate *c-fos* expression (Robertson et al., 1994). Taken together, these findings suggest that the medial striatum and nucleus accumbens may be sites of antipsychotic drug actions.

Retrograde tracing studies have established that haloperidol-induced FLI in the nucleus accumbens is located in neurons that project to the ventral pallidum (Robertson and Jian, 1995). This suggests that the activation of accumbopallidal neurons may contribute to the therapeutic effects of haloperidol.

II. COMPARISON OF THE RECEPTOR MECHANISMS RESPONSIBLE FOR HALOPERIDOL- AND CLOZAPINE-INDUCED Fos-LIKE IMMUNOREACTIVITY IN THE FOREBRAIN

A. Effects of prior administration of D_2-like receptor agonists on antipsychotic-induced FLI in the forebrain

Several studies have shown that typical and atypical antipsychotics have regionally different effects on *c-fos* expression in the forebrain; e.g. haloperidol induces *c-fos* expression in the nucleus accumbens, dorsolateral striatum while clozapine increases *c-fos* expression selectively in the limbic regions such as prefrontal cortex, nucleus accumbens, mediolateral striatum and lateral septal nucleus (Deutch et al., 1991; Nguyen et al., 1992; Robertson and Fibiger, 1992; Robertson et al., 1994). These findings have

provided a neuroanatomical basis for both the beneficial and adverse effects of these antipsychotics. However, because clozapine and haloperidol interact with a wide variety of neurotransmitter systems, the pharmacological basis for their clinical effects is unclear.

Meltzer et al. (1989) have proposed that clozapine's unique clinical profile may be related to its greater affinity for $5HT_2$ serotonin than D_2 dopamine receptors. However, Baldessarini et al. (1992) have argued that antagonism of α_1 and α_2 noradrenergic receptors may also contribute to clozapine's atypical profile. In order to gain insight into the role that serotonergic and adrenergic systems play in clozapine's CNS actions, Guo et al. (1995) examined the effects of depleting forebrain serotonin and norepinephrine levels on the ability of clozapine to elevate FLI in the prefrontal cortex, nucleus accumbens and lateral septal nucleus. These studies showed that prior lesions of serotonergic and noradrenergic projections to the telencephalon failed to reduce the ability of clozapine to elevate FLI in the prefrontal cortex, nucleus accumbens and lateral septal nucleus (Guo et al., 1995). However, clozapine-induced FLI was competitively reduced in these structures by prior administration of D_2-like receptor agonists (Guo et al., 1995). Thus, D_2-like receptor antagonism may contribute to clozapine's ability to elevate FLI in the prefrontal cortex, nucleus accumbens and lateral septal nucleus.

Several lines of evidence indicate that haloperidol-induced *c-fos* expression in the striatum is also mediated by dopamine D_2 receptor blockade. First, prior administration of the D_2-like receptor agonist, quinpirole, reduces haloperidol-induced *c-fos* mRNA in the striatum (Miller, 1990). Second, the regional distributions of haloperidol-induced

FLI and D_2 receptors in the striatum are very similar (Robertson and Fibiger, 1992). Third, destruction of midbrain dopaminergic neurons with 6-OHDA, which severely depletes forebrain dopamine level abolishes, reduces the ability of haloperidol to elevate FLI in the nucleus accumbens and dorsolateral striatum (Robertson and Fibiger, 1992). Similarly, clozapine-induced FLI in the nucleus accumbens is decreased by 6-OHDA lesions of the mesencephalon dopamine pathway (Robertson and Fibiger, 1992). Thus, haloperidol-induced FLI in the striatum and nucleus accumbens as well as clozapine-induced FLI in the nucleus accumbens are dopamine dependent.

While D_2-like receptor blockade is thought to mediate the induction of FLI by clozapine and haloperidol in the nucleus accumbens, the distinct patterns of FLI produced by these compounds in the nucleus accumbens suggests that they may block distinct dopamine receptor subtypes (Robertson and Fibiger, 1992). Three D_2-like receptor subtypes have been identified by molecular cloning studies termed D_2, D_3 and D_4 (Bunzow et al., 1988; Sokoloff et al., 1990; Van Tol et al., 1991). Although all of these receptors share a high affinity for antipsychotics, their anatomical distributions are different. For example, D_2 receptor mRNA is found in all regions of the brain that receive dopaminergic innervation, whereas D_3 receptor mRNA is concentrated in limbic areas of the brain such as the ventral striatum (nucleus accumbens, islands of Calleja, olfactory tubercle) implicated in the control of emotional aspects of behaviour (Sokoloff et al., 1990; Lévesque et al., 1992). This has led to the suggestion that D_3 receptor blockade may contribute to the antipsychotic actions of neuroleptics.

The distribution of clozapine (20 mg/kg, s.c.)-induced FLI and *c-fos* expression closely matches that for the D_3 receptor suggesting that preferential D_3 receptor blockade is responsible for clozapine-induced FLI. In contrast, haloperidol (0.1 mg/kg, s.c.) significantly increases the number of neurons that display FLI and *c-fos* expression in regions which express the D_2 receptor. Given that D_3 receptor blockade may mediate clozapine-induced FLI while D_2 receptor antagonism may be responsible for haloperidol-induced FLI, it was hypothesized that clozapine-induced FLI should be more sensitive than haloperidol-induced FLI to competitive reduction by prior administration of either quinpirole or 7-OH-DPAT which both have greater affinity for the D_3 than D_2 receptor.

Ligand binding studies have shown that quinpirole has approximately equal affinity for the D_3 and D_4 receptor and about 100-fold lower affinity for the D_2 receptor (Gingrich et al. 1993). Furthermore, Miller (1990) reported that *c-fos* mRNA induced by haloperidol (2.5 mg/kg, s.c.) in the striatum was reversed by prior administration of quinpirole (1 mg/kg, s.c.) suggesting that this strategy could be used to determine whether D_2-like receptors mediate antipsychotic-induced FLI. Using these doses as a starting point, the effects of prior quinpirole (0.5 mg/kg, s.c.) administration on haloperidol (2 mg/kg, s.c.)-induced FLI and catalepsy were examined. Quinpirole (0.5 mg/kg, s.c.) did not reduce haloperidol (2 mg/kg, s.c.)-induced catalepsy or FLI in the dorsolateral striatum. Using progressively higher doses of quinpirole and lower doses of haloperidol, it was found that haloperidol-induced catalepsy and FLI could only be reversed if a high dose of quinpirole (1 mg/kg, s.c.) was administrated prior to a low dose of haloperidol (0.1 mg/kg, s.c.). In contrast, clozapine (20 mg/kg, s.c.) induced

FLI in the prefrontal cortex, nucleus accumbens and lateral septal nucleus was significantly reversed by prior administration of quinpirole at doses of either 0.5 mg/kg (s.c.) or 1 mg/kg (s.c.). Thus, even though a 200 fold higher dose of clozapine was used, clozapine-induced increases in forebrain FLI were more considerably sensitive to reversal by quinpirole than those produced by haloperidol.

The ability of 7-OH-DPAT (0.05 mg/kg, 0.5 mg/kg, s.c.) to reverse clozapine- and haloperidol-induced FLI was also compared. Administration of 7-OH-DPAT (0.05 mg/kg or 0.5 mg/kg, s.c.) prior to clozapine (20 mg/kg, s.c.) completely blocked increases in FLI produced by this neuroleptic in the nucleus accumbens, mediolateral striatum and partially reversed clozapine (20 mg/kg, s.c.)-induced increases in FLI in the prefrontal cortex and lateral septal nucleus. Consistent with Guo et al. (1995), 7-OH-DPAT (0.5 mg/kg, s.c.) completely reversed clozapine (20 mg/kg, s.c.) induced FLI in the islands of Calleja which contains D_3 but not D_2 receptors (Lévesque et al., 1993). In contrast, prior treatment with the highest dose of 7-OH-DPAT (0.5 mg/kg, s.c.) only partially reversed increases in FLI in the nucleus accumbens produced by haloperidol (0.1 mg/kg, s.c.). Similarly, haloperidol-induced FLI in the dorsolateral striatum were only partly reduced by 7-OH-DPAT (0.5 mg/kg, s.c.). Consistent with the hypothesis that haloperidol-induced increases in FLI in the dorsolateral striatum may define a neuronal population that mediates the cataleptic effect of haloperidol, 7-OH-DPAT (0.5 mg/kg, s.c.) also failed to reduce haloperidol (0.1 mg/kg, s.c.)-induced catalepsy.

Additional support for the notion that D_3 receptor blockade may be more relevant than D_2 receptor blockade for clozapine's antipsychotic effects comes from the study of

D_2 and D_3 dopamine receptor mRNA levels in rodent brains after chronic administration of clozapine and haloperidol. Buckland et al. (1992) demonstrated that whole brain levels of both D_2 and D_3 receptor mRNAs are elevated 2-4 fold by administration of haloperidol (3 mg/kg/day) for 32 days. These increases were interpreted as an adaptive response secondary to the antagonism of D_2 and D_3 receptors. In contrast, chronic treatment with clozapine (30 mg/kg/day) for 4 days was sufficient to increase whole brain levels of D_3 receptor mRNA by 5 fold (Buckland et al., 1992). Thus, clozapine induced a more rapid and larger elevation of D_3 receptor mRNA than haloperidol suggesting that it produces a more immediate and complete blockade of the D_3 receptor than haloperidol. Moreover, D_2 receptor mRNA levels were not enhanced by 32 days of clozapine administration (Buckland et al., 1992). The effects of chronic clozapine and haloperidol administration on D_2 and D_3 mRNA levels are consistent with the present results which suggest that clozapine-induced FLI is mediated largely by D_3 receptor blockade whereas haloperidol-induced FLI is mediated principally by the antagonism of the D_2 receptor.

The high affinity of clozapine for the D_4 receptor led to the proposal that D_4 receptor blockade may be responsible for its unique therapeutic profile. However, the concentration of D_4 receptor mRNA in rat brain is very low, it is only detectable by PCR amplification in the frontal cortex and olfactory tubercle (O'Malley et al., 1992). Furthermore, *in situ* hybridization histochemistry indicates that D_4 mRNA is present in the striatum in only a few scattered cells (O'Malley et al., 1992). Hence, while clozapine has high affinity for this receptor (Van Tol et al., 1991), its very low

abundance in rat brain strongly suggests that D_4 receptor blockade contributes little to clozapine-induced FLI.

Clozapine is unique in that it reduces both the negative and positive symptoms of schizophrenia without producing EPS. On the basis of imaging studies, Weinberger (1988) has proposed that negative symptoms in schizophrenia might be due to hypoactivity in the prefrontal cortex. If this proposal is correct, then clozapine may improve negative symptoms by enhancing activity in the prefrontal cortex. Indeed, clozapine-induced increases in FLI in the prefrontal cortex are suggestive of such an action.

The reversal studies also showed that the relatively selective D_3 agonist, 7-OH-DPAT, significantly reduced clozapine-induced FLI in the prefrontal cortex, a region that contains D_3 receptors. Thus, the unique ability of clozapine to reduce the negative symptoms of schizophrenia may be mediated in part by D_3 receptor blockade in the prefrontal cortex. Moreover, since the D_3 receptor is not present in the dorsolateral striatum, an area which regulates movement, D_3 receptor antagonism may also account for clozapine's low propensity to induce EPS.

B. Effects of the selective D_3 antagonists UH 232 and naphtamide on FLI in the forebrain

Radioligand binding studies have shown that UH 232 has about a 2-3 fold greater affinity for D_3 than D_2 receptors (Sokoloff et al., 1992) while naphtamide has a 20 times greater affinity for D_3 than D_2 receptors (Griffon et al., 1995). A single administration

of UH 232 (10 mg/kg, s.c.) increased levels of FLI in the prefrontal cortex, nucleus accumbens, mediolateral and dorsolateral striatum. Because UH 232 (10 mg/kg, s.c.) has only a 2-3 greater affinity for D_3 than D_2 receptors, the ability of this compound to increase FLI in the dorsolateral striatum may be related to its significant D_2 receptor affinity. In contrast, naphtamide (5 mg/kg, s.c.) increased FLI in the prefrontal cortex and nucleus accumbens but not the medial and dorsolateral striatum. The distributions of FLI produced by naphtamide in the limbic structure matches well that produced by clozapine. Since naphtamide has good selectivity for the D_3 receptor, this finding suggests that clozapine's ability to selectively elevate FLI in the limbic system may be mediated by the D_3 receptor. However, like UH 232, clozapine has only slightly greater affinity for the D_3 than D_2 receptor indicating that D_3 receptor blockade cannot account completely for its unique effects on FLI in the forebrain.

C. Effects of prior administration of the D_1-like receptor antagonist SCH 23390 on antipsychotic-induced FLI

Pharmacological findings indicate that psychostimulants such as amphetamine and cocaine increase synaptic levels of dopamine and cause behavioral activation which can be blocked by both dopamine D_1 and D_2 antagonists (Christensen et al., 1984). Administration of amphetamine and cocaine at doses that cause behavioral activation also induces IEGs such as *c-fos*, *zif*268 and *jun*B in the striatum of neurologically intact animals (Graybiel et al., 1990). These increases are thought to be mediated by the ability of stimulants to potently elevate extracellular dopamine levels in the striatum,

nucleus accumbens and prefrontal cortex leading to enhanced dopamine receptor activation. Indeed, these increases are blocked by the selective D_1 receptor antagonist SCH 23390 (Graybiel et al., 1990). Neuroleptics can also elevate dopamine release because they block inhibitory D_2 autoreceptors on dopamine neurons (Assunta Imperate and Gaetano Di Chiara, 1985; Assunata Imperato and Gaetano Di Chiara, 1988). This raises the possibility that D_1 receptor activation may also contribute to antipsychotic-induced increases in FLI. In order to address this proposal, the ability of the selective D_1 receptor antagonist SCH 23390 (Iorio et al., 1983) to reverse clozapine-, haloperidol-, UH 232- and naphtamide-induced FLI were compared. Administration of SCH 23390 (0.1 mg/kg, s.c.) prior to clozapine (20 mg/kg, s.c.) attenuated FLI produced by this neuroleptic in the prefrontal cortex, nucleus accumbens and lateral septal nucleus. Similarly, SCH 23390 (0.1 mg/kg, s.c.) reduced UH 232 (10 mg/kg, s.c.)-induced FLI in the prefrontal cortex, nucleus accumbens, mediolateral and dorsolateral striatum. However, prior administration of SCH 23390 (0.1 mg/kg, s.c.) reduced naphtamide (5 mg/kg, s.c.)-induced FLI only in the nucleus accumbens. Lastly, SCH 23390 (0.1 mg/kg, s.c.) diminished haloperidol (0.1 mg/kg, s.c.)-induced increases in FLI in the nucleus accumbens. These results indicate that D_1 receptor activation contributes to the ability of these neuroleptics to elevate FLI in the forebrain. Given that stimulant-induced increases in FLI are potently blocked by SCH 23390, it would appear that neuroleptic-induced increases in extracellular dopamine levels also contribute to the ability of antipsychotics to enhance FLI in several forebrain structures.

III. EFFECTS OF CHRONIC ANTIPSYCHOTIC ADMINISTRATION ON IMMEDIATE-EARLY GENE EXPRESSION IN THE FOREBRAIN

In agreement with the hypothesis that the nucleus accumbens is a potential site for antipsychotic action (Robertson et al., 1994), chronic administration of clozapine (20 mg/kg, i.p.) and haloperidol (2 mg/kg, i.p.) examined in the present study elevated different immediate early-genes (IEGs) (ΔFosB, JunB, Jun) in this structure. Although we have not addressed the receptor mechanisms which mediate antipsychotic-induced IEG expression in the nucleus accumbens, the ability of D_2-like, but not D_1-like, receptor antagonists to elevate accumbal FLI suggests that D_2-like receptor blockade may also be responsible for neuroleptic-induced IEG in this region (Robertson and Fibiger, 1992). Molecular cloning studies have identified three D_2-like dopamine receptors termed D_2, D_3 and D_4 (for excellent reviews see Sibley and Monsma, 1992; Gingrich and Caron, 1993). While recent evidence suggests that all of these dopamine receptor subtypes may be involved in the mediation of antipsychotic-induced IEG expression (Guo et al., 1995), determination of the precise role played by each member of the D_2-like receptor family will require the development of highly selective antagonists.

Similar to the acute effects of haloperidol on Fos-, JunB- and Zif268-like immunoreactivity in the dorsolateral striatum (Robertson and Fibiger, 1992; MacGibbon et al., 1994), chronic haloperidol administration produced a dramatic elevation of Jun-, JunB- and ΔFosB-like immunoreactivity in this striatal sector. Since the dorsolateral striatum is involved in the regulation of movement (Pisa, 1988; Carelli and West, 1991)

and IEGs encode known transcriptional regulating factors, it has been proposed that haloperidol-induced IEG expression may promote long lasting changes in striatal neuronal gene expression that contribute to the development of EPS such as tardive dyskinesia. In agreement with previous work, previous studies in this laboratory have shown that chronic haloperidol administration produces a rapid reduction in the acute FLI response to a single haloperidol injection (data not shown). Indeed, a variety of treatments which acutely increase *c-fos* and *zif*268 expression rapidly lose this ability with repeated exposure (Hope et al., 1994; Rosen et al., 1994; Coppens et al., 1995). Given that haloperidol must be administered chronically in order to generate tardive dyskinesia, it is unlikely that these IEGs are involved in the production of this EPS. In contrast, the prolonged elevation of striatal Jun-, JunB- and ΔFosB-like immunoreactivity produced by repeated haloperidol administration suggests that these IEGs product may be more likely to participate in those intracellular events responsible for the development of tardive dyskinesia. Consistent with this proposal, chronic administration of the atypical neuroleptic clozapine, which is unlikely to produce EPS, had weak effects on Jun, JunB- and ΔFosB-like immunoreactivity in the dorsolateral striatum.

The predominant striatal cell type is the medium spiny projection neuron which accounts for approximately 90-95% of the neuronal population in this structure. Medium spiny neurons have been classified into two subtypes based on the target region innervated by their axons: approximately half project to the substantia nigra pars reticulata while the other half project to the globus pallidus. While we have not determined the connectional character of neurons that display haloperidol-induced IEG

expression in the present study, two lines of evidence suggest that chronic haloperidol administration elevates IEGs expression in striatal neurons that project to the globus pallidus. Firstly, acute haloperidol administration has been shown to selectively increase FLI in striatopallidal neurons (Robertson et al., 1992). Secondly, depletion of striatal dopamine by destruction of the nigrostriatal pathway enhances ΔFosB-like immunoreactivity principally in striatopallidal neurons (Doucet et al., 1995). Hence, like chronic haloperidol administration, striatal dopamine depletion is thought to increase ΔFosB expression by reducing the tonic activation of D_2-like dopamine receptor in the striatum. Given these mechanistic similarities between denervation- and haloperidol-induced increases in ΔFosB-like immunoreactivity, it is probable that chronic haloperidol administration also elevates Jun, JunB and ΔFosB-like immunoreactivity principally in striatopallidal neurons.

Chronic haloperidol administration also enhanced the number of Jun-, JunB- and ΔFosB-like immunoreactive neurons detected in the medial aspect of the striatum. This striatal region shares many connections in common with the nucleus accumbens and has been implicated in complex behaviours such as cognition (Carter and Fibiger, 1977; Divac et al., 1978; Donoghue and Herkenham, 1986; McGeorge and Fuall, 1989). These features along with the finding that all antipsychotic drugs elevate FLI in the medial striatum has led to the suggestion that this region may be a locus of antipsychotic activity (Robertson et al., 1994). Hence, the ability of chronic haloperidol administration to elevate expression of these IEGs in the medial striatum is consistent with this hypothesis. Similarly, chronic clozapine administration elevates jun-like

immunoreactivity in the mediolateral striatum. Although clozapine did not increase JunB- and ΔFosB-like immunoreactivity in the medial striatum, the high levels of basal JunB and ΔFosB-like immunoreactivity detected in this region may have obscured any weak effects of these compounds on these IEGs expression.

Unlike haloperidol, chronic clozapine administration elevated ΔFosB-like immunoreactivity, but not Jun- and JunB-like immunoreactivity, in the medial aspect of the prefrontal cortex . Similarly, acute administration of clozapine, but not haloperidol, increases FLI in the medial prefrontal cortex (Robertson et al., 1994). Assuming that increased expression of these IEGs is indicative of enhanced neuronal activity, it is tempting to speculate that repeated clozapine administration produces a persistent activation of the medial prefrontal cortex. *In vivo* imaging studies suggest that a reduction in neuronal activity in the prefrontal cortex may underly the symptoms of schizophrenia (Berman et al., 1986; Franzen and Ingvar, 1974). In particular, Weinberger (1988) has hypothesized that hypofrontality may be responsible for the negative or deficit symptoms of schizophrenia against which clozapine is uniquely effective (Kane et al., 1988; Meltzer et al., 1989). If this proposal is correct, then clozapine may improve negative symptoms by enhancing activity in the prefrontal cortex. Indeed, clozapine-induced increases in ΔFosB-like immunoreactivity in the prefrontal cortex are suggestive of such an action.

The marked increase in the number of Jun-, JunB- and ΔFos-like immunoreactive neurons in the lateral septal nucleus produced by clozapine activity suggests that this important component of the limbic system may also mediate some of the therapeutic

actions of neuroleptics. This nucleus is extensively interconnected with other limbic structures including the nucleus accumbens, hippocampus, amygdala and hypothalamus, and has been implicated in a variety of motivated and emotional behaviours (Brodal, 1981; Krettek and Price, 1978; Swanson and Cowan, 1979; Thomas, 1988). Moreover, with regard to schizophrenia, it is of considerable interest that electrophysiological abnormalities in this part of the brain have been correlated with acute psychotic episodes (Heath, 1975; 1977).

Taken together, chronic administration of both clozapine and haloperidol produce the same neuroanatomical patterns of ΔFosB-like immunoreactivity, but not Jun- and JunB- like immunoreactivity, as that previously seen for FLI after acute haloperidol and clozapine treatments. Acute treatment of clozapine (20 mg/g, s.c.)-induced increases in FLI in the prefrontal cortex, nucleus accumbens and lateral septal nucleus while single injection of haloperidol (2 mg/kg, s.c.)-induced increases in FLI in the nucleus accumbens, mediolateral striatum and dorsolateral striatum (Robertson and Fibiger, 1992). Similarly, chronic clozapine (20 mg/kg/day for 16 days)-induced increases in ΔFosB-like immunoreactivity in the prefrontal cortex, nucleus accumbens and lateral septal nucleus while, chronic haloperidol (2 mg/kg/day for 16 days)-induced increases in ΔFosB-like immunoreactivity in the nucleus accumbens, mediolateral and dorsolateral striatum.

In summary, the present results indicate that immunohistochemical detection of ΔFosB-like immunoreactivity can be used to identify neurons activated by chronic neuroleptic administration. Given that these neuronal populations may mediate the

antipsychotic effects of neuroleptics, determining the connectional and neurochemical character of neurons that display ΔFosB-like immunoreactivity after repeated neuroleptic administration may yield insights into the cellular basis of antipsychotic drug action.

FUTURE WORK

My studies indicate that the immediate-early gene product ΔFosB is a putative activity marker that may be used to identify neurons stimulated by chronic antipsychotic administration. Repeated administration of haloperidol (2 mg/kg/day) for 16 days produced a dramatic and homogeneous elevation of neurons which displayed ΔFosB-like immunoreactivity throughout the entire striatum, i.e. ventral, medial and dorsolateral aspects. Chronic haloperidol administration did not enhance ΔFosB-like immunoreactivity in the prefrontal cortex and lateral septal nucleus. In contrast, chronic administration of clozapine (20 mg/kg/day) for 16 days increased ΔFosB-like immunoreactivity not only in the ventral striatum but also in the prefrontal cortex and lateral septal nucleus. However, this compound had weak effects on ΔFosB-like immunoreactivity in the dorsolateral striatum. Since the dorsolateral striatum plays a key role in regulating movement and ΔFosB is a transcriptional regulating factor, it is tempting to speculate that haloperidol-induced increases in ΔFosB may promote changes in striatal gene expression that contribute to the development of extrapyramidal side effects. Consequently, the unique ability of clozapine to reduce the symptoms of schizophrenia without generating extrapyramidal side effects may be mediated by a preferential action on limbic structures such as the prefrontal cortex, ventral striatum and lateral septal nucleus.

A logical extension of these studies would be to determine the neurochemical and connectional character of neurons which display ΔFosB-like immunoreactivity after chronic haloperidol and clozapine administration. Determining whether clozapine and

haloperidol activate the same neurons is a particularly important question because it addresses whether these antipsychotics share a common mechanism of action. Given that all of the brain regions which display antipsychotic-induced increases in ΔFosB-like immunoreactivity contain a wide variety of neuropeptides that are located in distinct neuronal populations, antibodies that selectively recognize neurotensin, enkephalin, substance P, neuropeptide Y or cholecystokinin could be used to double label neurons that contain ΔFosB-like immunoreactivity after administration of clozapine (20 mg/kg/day) or haloperidol (2 mg/kg/day) for 16 days. In this way, to determine if it would be possible that clozapine elevates ΔFosB-like immunoreactivity in neurons that are neurochemically distinct from those which express haloperidol-induced ΔFosB-like immunoreactivity. In order to confirm findings from these immunohistochemical studies, a second approach to double label neurons that display ΔFosB-like immunoreactivity would be used. This would entail combining ΔFosB immunohistochemistry with the detection of neuropeptide mRNA by *in situ* hybridization histochemistry. After tissue sections had been processed for ΔFosB-like immunoreactivity, cDNA probes that selectively recognize mRNA species encoding neurotensin, enkephalin, substance P, dynorphin, neuropeptide Y or cholecystokinin would be used to determine the neurochemical identity of neurons that express antipsychotic-induced ΔFosB-like immunoreactivity. These two double labelling techniques could provide complementary data that would permit me to ascertain the neurochemical nature of ΔFosB neurons with greater confidence.

Since dysfunctional connectivity of the prefrontal and limbic cortices is a proposed model of schizophrenic pathophysiology, the unique ability of clozapine to increase ΔFosB expression in the prefrontal cortex suggests that some of their therapeutic effects may be related to an action on this structure. Elucidating the connectional character of neurons that display ΔFosB-like immunoreactivity in the prefrontal cortex may therefore identify specific neuronal populations which contribute to the antipsychotic activities of these neuroleptics. This could be done by combining immunohistochemical detection of ΔFosB with retrograde tract tracing techniques. Distinct output pathways of the prefrontal cortex could be identified by injection of the retrograde tracer Fluoro-Gold into the appropriate projection target site. Separate groups of animals would receive unilateral Fluoro-Gold injections into either the striatum, mediodorsal thalamus, frontal cortex or dorsal spinal cord in order to retrogradely label corticostriatal, corticothalamic, corticocortical and corticospinal neurons, respectively. These animals would then be treated chronically with either clozapine (20 mg/kg/day) or haloperidol (2 mg/kg/day) for 16 days to induce ΔFosB-like immunoreactivity in the prefrontal cortex. Overlap between neurons that contain antipsychotic-induced ΔFosB-like immunoreactivity and Fluoro-Gold would be assessed in sections from the prefrontal cortex using a fluorescence microscope equipped with an image analysis system.

By comparing the neurochemical and connectional character of neurons which express ΔFosB-like immunoreactivity after chronic haloperidol and clozapine administration, these studies would provide valuable insights into the cellular basis for clozapine's and haloperidol's therapeutic profile.

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