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NEUROLEPTICS INCREASE *C-FOS* EXPRESSION IN THE FOREBRAIN: CONTRASTING EFFECTS OF HALOPERIDOL AND CLOZAPINE

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Abstract—The mechanisms by which the atypical neuroleptic clozapine produces its therapeutic effects in the treatment of schizophrenia without causing the extrapyramidal side effects that are characteristic of most antipsychotic drugs remain unclear. Recently, a single injection of the typical antipsychotic haloperidol has been shown to increase *c-fos* expression in the striatum [Dragunow *et al.* (1990) *Neuroscience* **37**, 287–294]. *C-fos* is a proto-oncogene that encodes a 55,000 mol. wt phosphoprotein, Fos, which is thought to assist in the regulation of "target genes" containing an AP-1 binding site. Because a wide variety of physiological and pharmacological stimuli increase *c-fos* expression, it has been proposed that Fos immunohistochemistry might be useful in mapping functional pathways in the central nervous system. The present experiments examined some potential neuroanatomical differences in the actions of clozapine and haloperidol by comparing their effects on *c-fos* expression in the medial prefrontal cortex, nucleus accumbens, striatum and lateral septum. The effects of the selective dopamine receptor antagonists SCH 23390 (D_1) and raclopride (D_2) were also examined.

Haloperidol (0.5, 1 mg/kg) and raclopride (1, 2 mg/kg) produced large increases in the number of Fos-containing neurons in the striatum and nucleus accumbens. SCH 23390 (0.5, 1 mg/kg) reduced the number of Fos-positive neurons in the nucleus accumbens and striatum, and had no effect in the other regions. Neither haloperidol nor raclopride increased the number of Fos-positive neurons in the medial prefrontal cortex. Haloperidol, but not raclopride, produced a modest increase in *c-fos* expression in the lateral septal nucleus. Clozapine (10, 20 mg/kg) was without effect in the striatum; however, it significantly increased the number of Fos-positive neurons in the nucleus accumbens, medial prefrontal cortex and lateral septal nucleus. Destruction of mesotelencephalic dopaminergic neurons with 6-hydroxydopamine abolished the increase in Fos expression in the nucleus accumbens and striatum produced by haloperidol and raclopride, and also blocked the clozapine-induced increase in the nucleus accumbens. However, the inductive effects of clozapine and haloperidol on *c-fos* expression in the lateral septal nucleus and of clozapine in the medial prefrontal cortex were not affected by the 6-hydroxydopamine lesions.

These results suggest that clozapine's unique therapeutic profile may be related to its failure to induce Fos in the striatum as well as its idiosyncratic actions in the lateral septum and medial prefrontal cortex. The effects of clozapine in these latter regions do not appear to be mediated by dopaminergic mechanisms.

proto-oncogene *c-fos* encodes a 55,000 mol. wt sphoprotein (Fos) which, after translation in the oplasm, re-enters the nucleus and binds to NA.[36,51] Cell culture studies have demonstrated that is one of two components which form the AP-1 nscriptional activating factor.[36] The second com-ent of AP-1 is Jun, the 39,000 mol. wt product of proto-oncogene *c-Jun*, which binds to Fos by ins of five leucine residues common to both ecules.[36] Some neuropeptide genes in the basal glia and limbic system contain consensus sequences which could be activated by AP-1.[19,36,56] The ability of a host of growth factors, neurotransmitters, drugs and physiological manipulations to increase Fos expression in the CNS suggests that *c-fos* induction can occur as a consequence of synaptic activation.[16,17,20,23,28,34–37,46,47,49,50] Indeed, these studies suggest that increased Fos immunoreactivity is generally associated with increased metabolic demand on a neuron. This has led to the proposal that Fos immunohistochemistry might be used in a manner similar to 2-deoxyglucose to map functional pathways in the CNS.[23,50]

The introduction of chlorpromazine in 1952 revolutionized the treatment of schizophrenia and led to the subsequent development of a large number of neuroleptic agents.[8,10] There is considerable evidence that D_2 dopamine receptor blockade is associated with the antipsychotic effects of these drugs.[52] Since schizophrenia is thought to be a disturbance of the limbic system, it has been suggested that antagonism of D_2 receptors in limbic structures, particularly in the nucleus accumbens (NAc), might mediate the

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reviations: CRB, Cambridge Research Biochemicals; DAB, 3,3′-diaminobenzidine; EPS, extrapyramidal side effects; LH, lateral hypothalamus; L. STR, lateral striatum; LY 171555, 4,4*a*,5,6,7,8,8*a*,9-octahydro-5-*n*-propyl-2H-pyrazolo-3,4-*g*-quinoline; M. STR, medial striatum; NAc, nucleus accumbens; 6-OHDA, 6-hydroxydopamine; PBS, phosphate-buffered saline; PFC, medial prefrontal cortex; SCH 23390, (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol; YM 09151-2, *cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-(methylamino)benzamide.

therapeutic actions of neuroleptics.[52] Similarly, the extrapyramidal side effects (EPS) of classical neuroleptics such as chlorpromazine and haloperidol are thought to reflect D_2 receptor blockade in the striatum.[52] Clozapine has been referred to as an atypical neuroleptic because it is highly effective in the treatment of schizophrenia[14,26,31] but has a low propensity to induce EPS.[8,10,14,15,26] Radioligand binding studies suggest that preferential blockade of D_2 receptors in the NAc may be responsible for this unusual profile.[2,8,10,15,26] However, electrophysiological results have failed to provide functional support for this proposal because both clozapine and haloperidol increase the single unit activity of striatal and NAc neurons of paralysed rats.[44,45]

It has recently been reported that haloperidol greatly increases the number of Fos-immunoreactive neurons in the striatum.[17] The selective D_2 receptor antagonist *cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-(methylamino)benzamide (YM 09151–2) had similar actions while the selective D_1 receptor antagonist (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SCH 23390) was without effect.[17] Related studies have demonstrated that the selective D_2 agonist 4,4*a*,5,6,7,8,8*a*,9-octahydro-5-*n*-propyl-2H-pyrazolo-3,4-*g*-quinoline (LY 17155) attenuates the stimulant effect of haloperidol on striatal *c-fos* expression.[34] These results suggest that haloperidol-induced increases in Fos-positive neurons in the striatum are related to the ability of this compound to block D_2 receptors in this structure. They also raise the possibilities (1) that this approach may be useful in identifying the brain regions that are targets for neuroleptic drugs, and (2) that there may be differences between typical and atypical neuroleptics with respect to the distribution of Fos-activated neurons. The present experiments were designed to address these questions by examining the effects of clozapine, haloperidol, raclopride (a selective D_2 antagonist[40]), and SCH 23390 (a selective D_1 antagonist[24]) on the number and anatomical distribution of Fos-positive neurons in the forebrain, particularly in the medial prefrontal cortex, nucleus accumbens, striatum and lateral septum. In addition, in order to determine the role of dopamine in these responses, the effects of unilateral lesions of the mesotelencephalic dopamine system on neuroleptic-induced *c-fos* expression were investigated.

EXPERIMENTAL PROCEDURES

Drugs

Haloperidol (McNeil Pharmaceutical; Stouffville, Canada) and clozapine (Sandoz; Dorval, Canada) were dissolved in 40 μl of 20% acetic acid and brought to final volume with distilled water. SCH 23390 (Schering Corporation; Bloomfield, NJ) and raclopride (Astra; Mississauga, Canada) were dissolved in distilled water.

Protocol for drug studies

All experiments were performed on male Wistar rats (300–450 g). Injections were made subcutaneously (s.c.) in the neck. Eleven groups, composed of three to five rats each, received one of the following treatments: water (1 ml/kg), vehicle (1 ml/kg; 40 μl of 20% acetic acid in 1 ml of distilled water), clozapine (10, 20, 30 mg/kg; dissolved in 1 ml of vehicle), SCH 23390 (0.5, 1.0 mg/kg; dissolved in 1 ml of water), raclopride (1, 2 mg/kg; dissolved in 1 ml of water), and haloperidol (0.5, 1.0 mg/kg; dissolved in 1 ml of vehicle). Two hours after the injection, all of the animals were deeply anaesthetized with pentobarbital (100 mg/kg, i.p.) and perfused with saline (200 ml) followed by 200 ml of 4% paraformaldehyde in phosphate-buffered (0.1 M) saline. Each brain was removed immediately after perfusion and placed in fresh fixative for at least 12 h.

6-Hydroxydopamine lesions

Rats weighing 300–325 g were injected with desmethylimipramine (25 mg/kg, i.p.) and then anaesthetized 30 min later with sodium pentobarbital (50 mg/kg, i.p.). Unilateral lesions of the ascending mesotelencephalic dopaminergic projection were made at the level of the lateral hypothalamus (LH) by injection of 11.4 μg of 6-hydroxydopamine HBr (6-OHDA; Sigma) dissolved in 4 μl of saline containing 0.05% ascorbic acid. The solution was injected over 10 min into the left LH at the coordinates AP 4.0, ML 1.3 and DV 1.6 from interaural zero according to the atlas of Paxinos and Watson.[41] Behavioural screening was carried out four weeks after surgery; only animals that rotated at least nine times/min after an injection of apomorphine HCl (0.2 mg/kg, s.c.) were used in the subsequent experiments.

Fos immunohistochemistry

After the postfixative period, 30-μm sections were cut from each brain using a Vibratome. Antisera from two suppliers were used to detect Fos. The majority of exper-

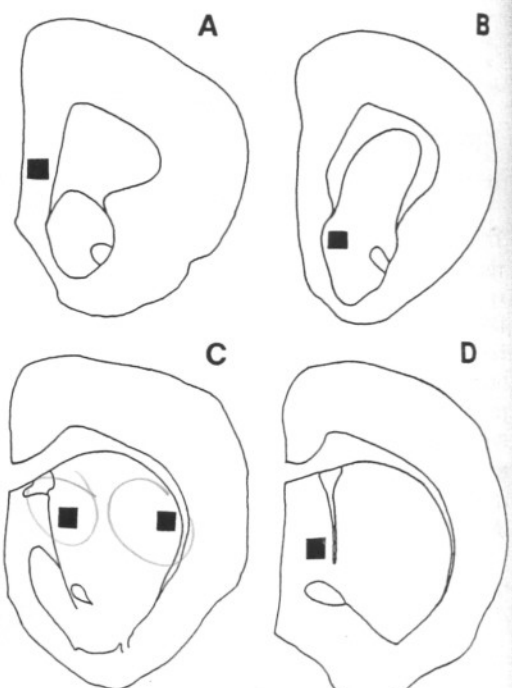

Fig. 1. Camera lucida drawings of representative sections used for the counting of Fos-positive neurons in the medial prefrontal cortex (PFC) (A), NAc (B), medial striatum (M. STR) and lateral striatum (L. STR) (C) and lateral septal nucleus (D). Boxed regions indicate the 520 μm^2 sampled areas.

ents were performed using a sheep polyclonal antibody ambridge Research Biochemicals, CRB OA-11-823) di- ed against residues 2–16 of the N-terminal region of the molecule. A second sheep polyclonal antibody (Serotec, PA 53), which also recognizes amino acids 2–16 of the erminal region of Fos, was used to verify results obtained the CRB antibody. The antibodies produced similar sults.

Sections were washed three times with 0.02 M phosphate- ffered saline (PBS) and then incubated in PBS containing % hydrogen peroxide for 10 min to block endogenous roxidase activity. Sections were then washed three times PBS and incubated in PBS containing 0.3% Triton-X, % azide and Fos primary antisera (diluted 1: 2000) for h. The sections were then washed three times with PBS d incubated with a biotinylated rabbit anti-sheep sec- dary antibody (Vector Laboratories; diluted 1: 500) for h. The sections were washed three times with PBS and ubated for 1 h with PBS containing 0.3% Triton-X and % avidin–biotinylated horseradish peroxidase complex ector Laboratories). After three washes in PBS the ctions were rinsed in 0.1 M acetate buffer, pH 6.0. e reaction was visualized using a glucose oxidase– -diaminobenzidine (DAB)–nickel method described viously.[53] The reaction was terminated by washing in BS, and the sections were mounted on chrome–alum ated slides, dehydrated and prepared for microscopic ervation.

Background staining was found to decrease with reated use of both Fos antibodies. Consequently, 20-μl liquots of the reconstituted CRB or Serotec antiserum were ntinely washed for 48 h at room temperature with ap- ximately eight to 10 fixed sections in 10 ml of PBS. To ntrol for the specificity of immunoreactivity, some of the ctions were incubated with Fos antisera which had been reabsorbed with Fos peptide (CRB OP-11-3210). Pre- sorption of the CRB and Serotec antibodies with the -terminal antigenic sequence eliminated Fos immuno- activity. In addition, omission of the primary antibody m the immunohistochemical procedure blocked Fos munoreactivity.

unting of labelled cells

Using the CRB antibody, drug-induced changes in *c-fos* pression were quantified by counting the number of s-positive nuclei in the medial prefrontal cortex, nucleus cumbens, medial and lateral striatum, and the lateral ptum. The number of Fos-positive nuclei was counted hin a 520 × 520 μm grid placed over each of these regions x100 magnification. Camera lucida drawings illustrating e sampled area (dark squares) and AP position of sections d for cell counts in the medial prefrontal cortex (PFC) l, NAc (B), medial (M. STR) and lateral (L. STR) iatum (C) and septum (D) are shown in Fig. 1. A one-way alysis of variance was performed on the cell count data r each dose and the corresponding vehicle control. If e ANOVA was significant, multiple comparisons re performed using Tukey's test. Camera lucida awings were prepared from tissue sections from five trocaudal levels through the NAc and striatum viewed at 2 magnification.

RESULTS

tribution of Fos-positive neurons

The forebrain was initially scanned for the presence Fos-positive neurons under the control and drug atment conditions. Depending on the treatment dition, Fos-containing neurons were restricted to following structures: anterior olfactory nucleus, dial prefrontal (cingulate) cortex, insular cortex, piriform cortex, nucleus accumbens, striatum, lateral septal nucleus (ventral and intermediate aspects), the Islands of Calleja, the caudal portion of the horizontal limb of the diagonal band, and the septo-hypothalamic nucleus. Fos-positive neurons were not consistently observed in any other regions back to the level of Fig. 17 of Paxinos and Watson.[41] The drug treatments had their most pronounced effects in the PFC, striatum, NAc and lateral septal nucleus and these regions were therefore assessed quantitatively.

Effects of haloperidol, clozapine, raclopride, and SCH 23390 *on Fos immunoreactivity in the medial prefrontal cortex*

Clozapine produced a dose-dependent increase in the number of Fos-positive neurons in the PFC (Table 1, Figs 2–4). In contrast, haloperidol (Table 1, Figs 2–4), SCH 23390 and raclopride (Table 2, Figs 2–4) did not influence *c-fos* expression in the PFC.

Effects of haloperidol, clozapine, raclopride, and SCH 23390 *on Fos immunoreactivity in the nucleus accumbens*

Haloperidol and raclopride increased the number of Fos-positive nuclei detected in the NAc (Tables 1 and 2, Figs 2–5). In contrast, SCH 23390 reduced the number of Fos-immunoreactive neurons in the NAc relative to vehicle controls (Table 2, Figs 2–5). Raclopride and haloperidol produced strikingly similar patterns in the distribution of Fos-containing neurons within the NAc. Fos-positive neurons were concentrated in patches throughout the rostrocaudal extent of the NAc (Figs 2–5). In coronal sections through the middle portion of the NAc, both haloperidol and raclopride produced a band of Fos-positive nuclei which extended from the dorsomedial to ventrolateral NAc (Fig. 4). This region has been reported to contain the highest density of D_2 receptors in the NAc.[1]

Clozapine also increased *c-fos* expression in the NAc in a dose-dependent fashion (Table 1, Figs 2–5). However, the distribution of Fos-immunoreactive neurons after clozapine was distinctly different from that produced by haloperidol. Clozapine produced a homogeneous increase in Fos immunoreactivity in the anterior NAc (Figs 2 and 3), while at more caudal levels the activated neurons were concentrated in the medial NAc (Figs 4 and 5).

Effects of haloperidol, clozapine, raclopride, and SCH 23390 *on Fos immunoreactivity in the striatum*

There were clear differences in the striatum between the effects of clozapine and haloperidol on *c-fos* expression. While both haloperidol and raclopride markedly increased the number of Fos-immunoreactive neurons in this structure (Tables 1 and 2, Figs 3–7), clozapine had no effect either at 10 or 20 mg/kg and produced only a small increase at 30 mg/kg (Table 1, Figs 3–7). The selective D_1 antag-

Table 1. Effects of clozapine and haloperidol on the average number (mean ± S.E.M.) of Fos-positive neurons within a 520 μm^2 area of the medial prefrontal cortex, nucleus accumbens, medial striatum, lateral striatum, and lateral septal nucleus

	Vehicle (1 ml/kg)	Clozapine (mg/kg)			Haloperidol (mg/kg)	
		10	20	30	0.5	1.0
PFC	33 ± 4.9	42 ± 1.6	52.5 ± 8.3*	74 ± 2.7*	47.5 ± 3.6	39 ± 2.1
NAc	21 ± 2.4	66 ± 9*	62 ± 10*	87.7 ± 9.6*	115 ± 19*	93 ± 10*
M. STR	2.4 ± 0.4	4 ± 1	4.5 ± 1.9	9.2 ± 1.9*	27.6 ± 2.6*	20 ± 4*
L. STR	0.6 ± 0.2	1 ± 1	2.5 ± 1.7	6.7 ± 3.8	96 ± 13*	110 ± 8*
Septum	27.9 ± 2.6	95 ± 6.9*	101 ± 6.5*	119 ± 7.5*	47.3 ± 3.9*	49.3 ± 5*

Asterisks (*) indicate statistically significant differences from the vehicle control ($P < 0.05$).

Table 2. Effects of SCH 23390 and raclopride on the average number (mean ± S.E.M.) of Fos-positive neurons within a 520 μm^2 area of the prefrontal cortex, nucleus accumbens, medial striatum, lateral striatum, and the lateral septal nucleus

	Water (1 mg/kg)	SCH 23390 (mg/kg)		Raclopride (mg/kg)	
		0.5	1.0	1.0	2.0
PFC	33.7 ± 2.9	35.7 ± 2.3	42 ± 1.6	23 ± 5	26 ± 3
NAc	20.5 ± 1.6	8.7 ± 0.7*	8.3 ± 3.1*	117 ± 2*	105 ± 9*
M. STR	3.5 ± 0.4	0*	0*	22.6 ± 6*	35 ± 3*
L. STR	0.5 ± 0.3	0	0	101 ± 16*	112 ± 19*
Septum	30.2 ± 2.6	41 ± 6.1	43.7 ± 4.7	16 ± 2.9	27 ± 4.4

Abbreviations as in Table 1. Asterisks (*) indicate statistically significant differences from the water control ($P < 0.05$).

onist decreased Fos immunoreactivity in the striatum (Table 2, Figs 3–7).

Effects of haloperidol, clozapine, raclopride, and SCH 23390 on Fos immunoreactivity in the lateral septal nucleus

Clozapine produced a marked increase in *c-fos* expression in the lateral septal nucleus (Table 1, Fig. 6). These increases were confined to the ventral and intermediate aspects of the nucleus. More posteriorly, they were observed in the septohypothalamic nucleus[41] and the caudal aspect of the horizontal limb of the diagonal band. Haloperidol produced a small but statistically significant increase in *c-fos* expression in the lateral septal nucleus (Table 1, Fig. 6). Raclopride and SCH 23390 did not increase Fos immunoreactivity in the septum (Table 2, Fig. 6).

Effects of unilateral 6-hydroxydopamine lesions on neuroleptic-induced Fos immunoreactivity

Unilateral 6-OHDA lesions of the mesotelencephalic dopaminergic projection abolished the increase in Fos immunoreactivity in the lateral striatum (Table 3) and NAc (Table 3) produced by both haloperidol and raclopride. The 6-OHDA lesions also prevented the increase in *c-fos* expression in the NAc produced by clozapine. However, the 6-OHDA lesions failed to influence the clozapine- and haloperidol-induced increases in the number of Fos-positive neurons in the ipsilateral septum and the clozapine-induced increases in the PFC (Table 3).

DISCUSSION

In agreement with previous work, the present experiments demonstrated that D_2 but not D_1 receptor antagonists increase *c-fos* expression in the striatum.[17] In the anterior striatum, haloperidol- and raclopride-induced Fos immunoreactivity was pronounced in the dorsolateral region (Fig. 4). In the middle portion, Fos-positive neurons were concentrated in the lateral rim (Fig. 5), and in the caudal striatum they were situated ventrally (Fig. 6). This neuroanatomical pattern of D_2 antagonist-induced Fos immunoreactivity correlates with the distribution of striatal D_2 receptors.[5,9,25,30,33,39] D_2 dopamine receptors have also been shown to have a rostrocaudal gradient.[9,25,33] Similarly, the number of Fos-immunoreactive neurons after haloperidol and raclopride was greatest in the rostral striatum and declined caudally.[17] The close topographical relationship between D_2 antagonist-induced Fos immunoreactivity and D_2 receptors suggests that haloperidol and raclopride may increase Fos in those striatal neurons that most strongly express D_2 receptors. The reduction in the haloperidol- and raclopride-induced increases in Fos immunoreactivity in the striatum after 6-OHDA lesions further suggests that dopaminergic mechanisms mediate the effects of these drugs on *c-fos* expression in this structure. In contrast to haloperidol and raclopride, clozapine only produced a small increase in the number of Fos-positive neurons in the striatum which was only statistically significant at the highest dose. Radioligand binding studies have indicated that

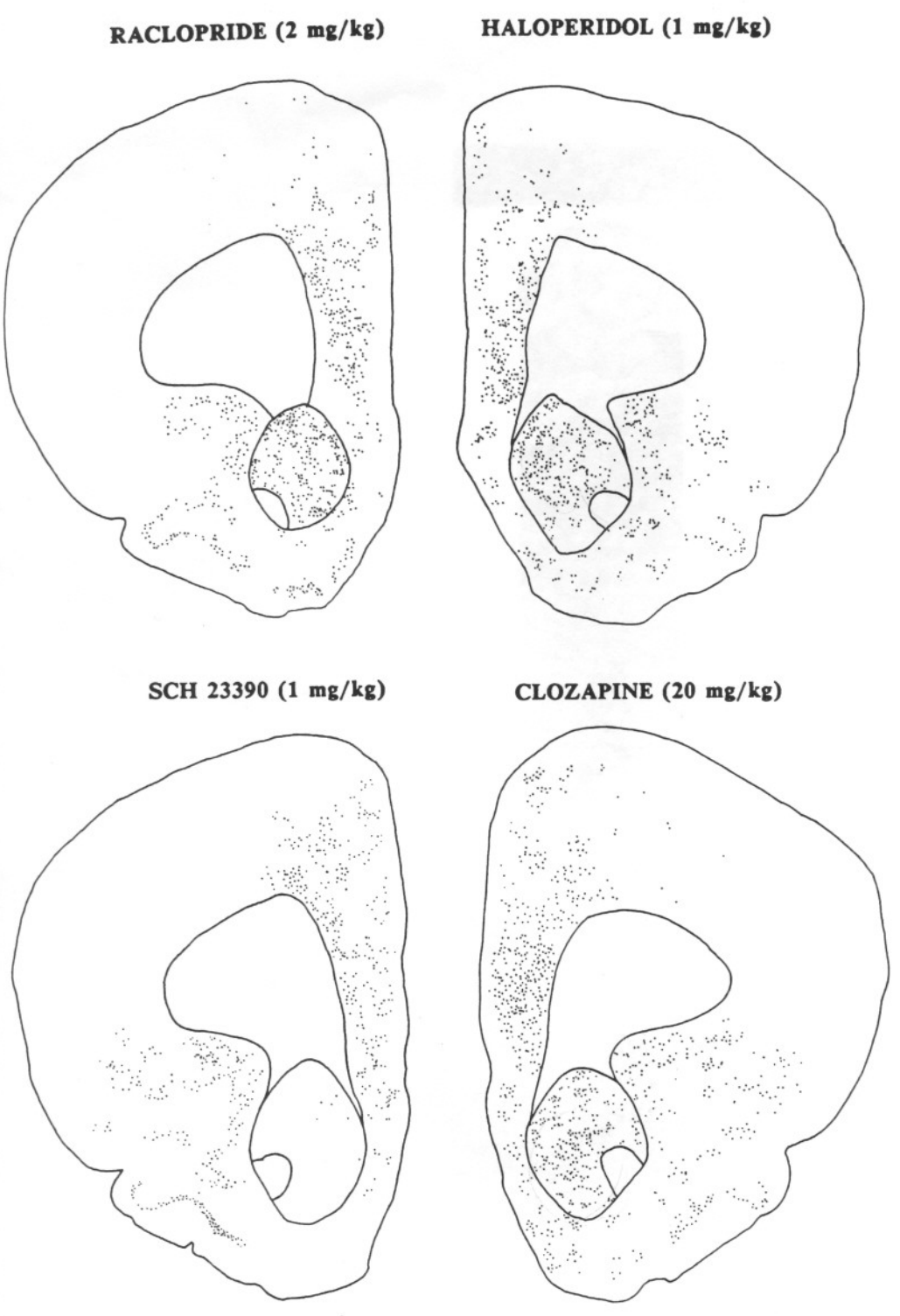

Fig. 2. Camera lucida drawings representative of the effects of an injection of raclopride, haloperidol, SCH 23390, and clozapine on the distribution of Fos-positive neurons in the anterior NAc and PFC. Each black dot in this and subsequent figures represents a single Fos-positive nucleus. Sections correspond to an AP position approximately 2.7 mm from bregma according to the atlas of Paxinos and Watson.[41]

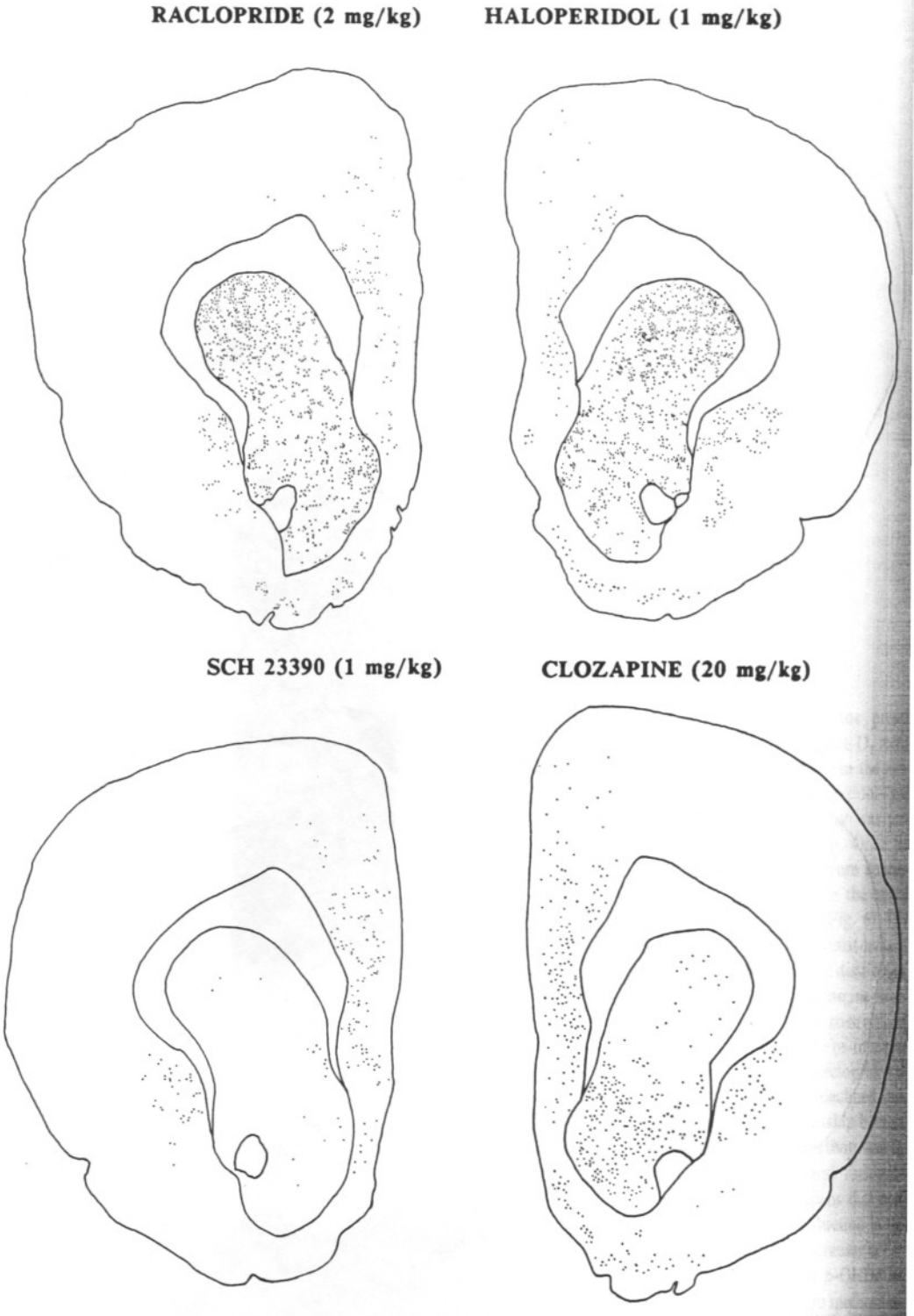

Fig. 3. Camera lucida drawings representative of the effects of an injection of raclopride, haloperidol, SCH 23390, and clozapine on the distribution of Fos-positive neurons in mid-NAc, anterior striatum and caudal PFC. Sections correspond to an AP position approximately 2.1 mm from bregma according to the atlas of Paxinos and Watson.[41]

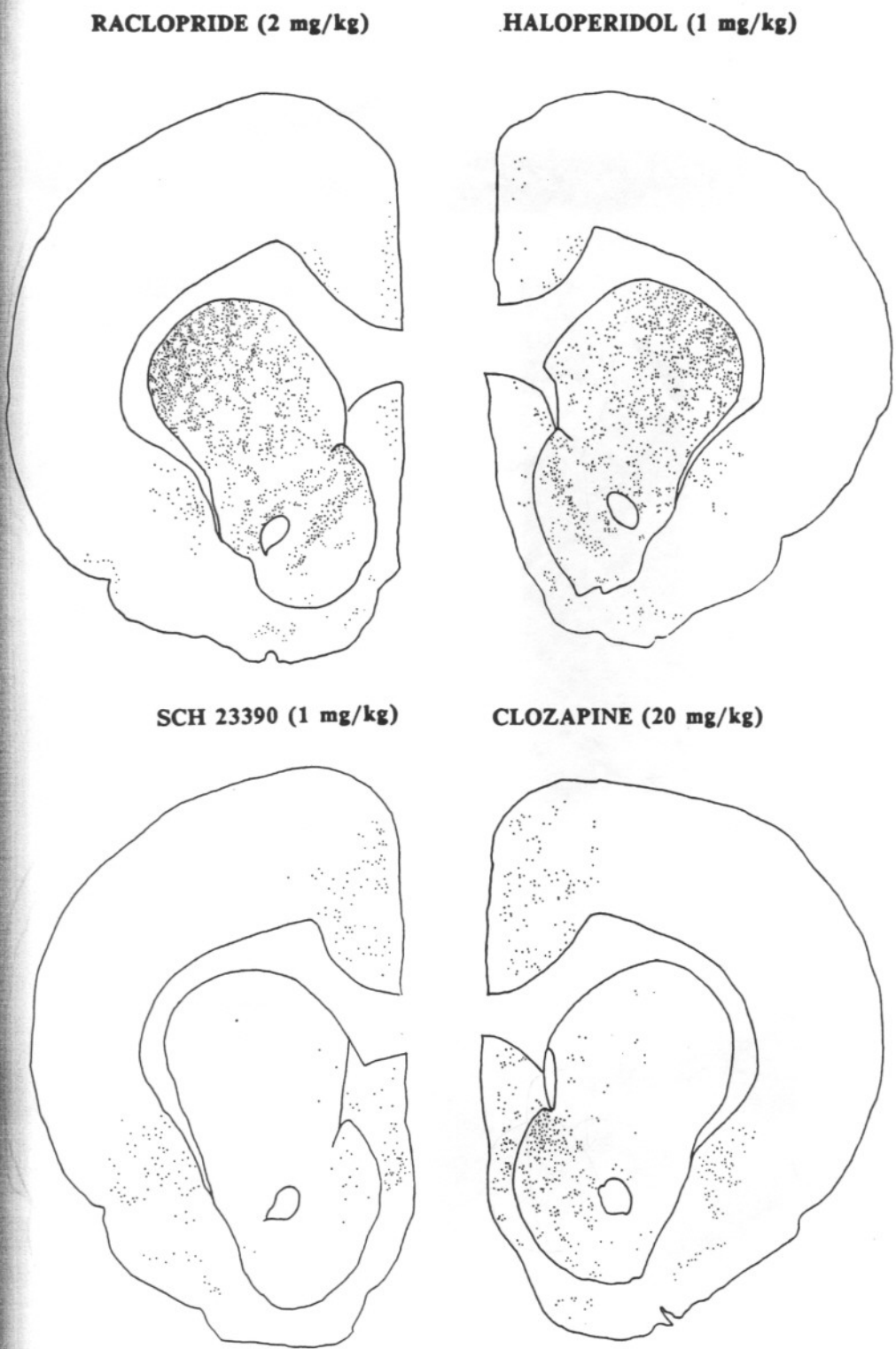

Fig. 4. Camera lucida drawings representative of the effects of an injection of raclopride, haloperidol, SCH 23390, and clozapine on the distribution of Fos-positive neurons in the mid-NAc and anterior striatum. Sections correspond to an AP position approximately 1.7 mm from bregma according to the atlas of Paxinos and Watson.[41]

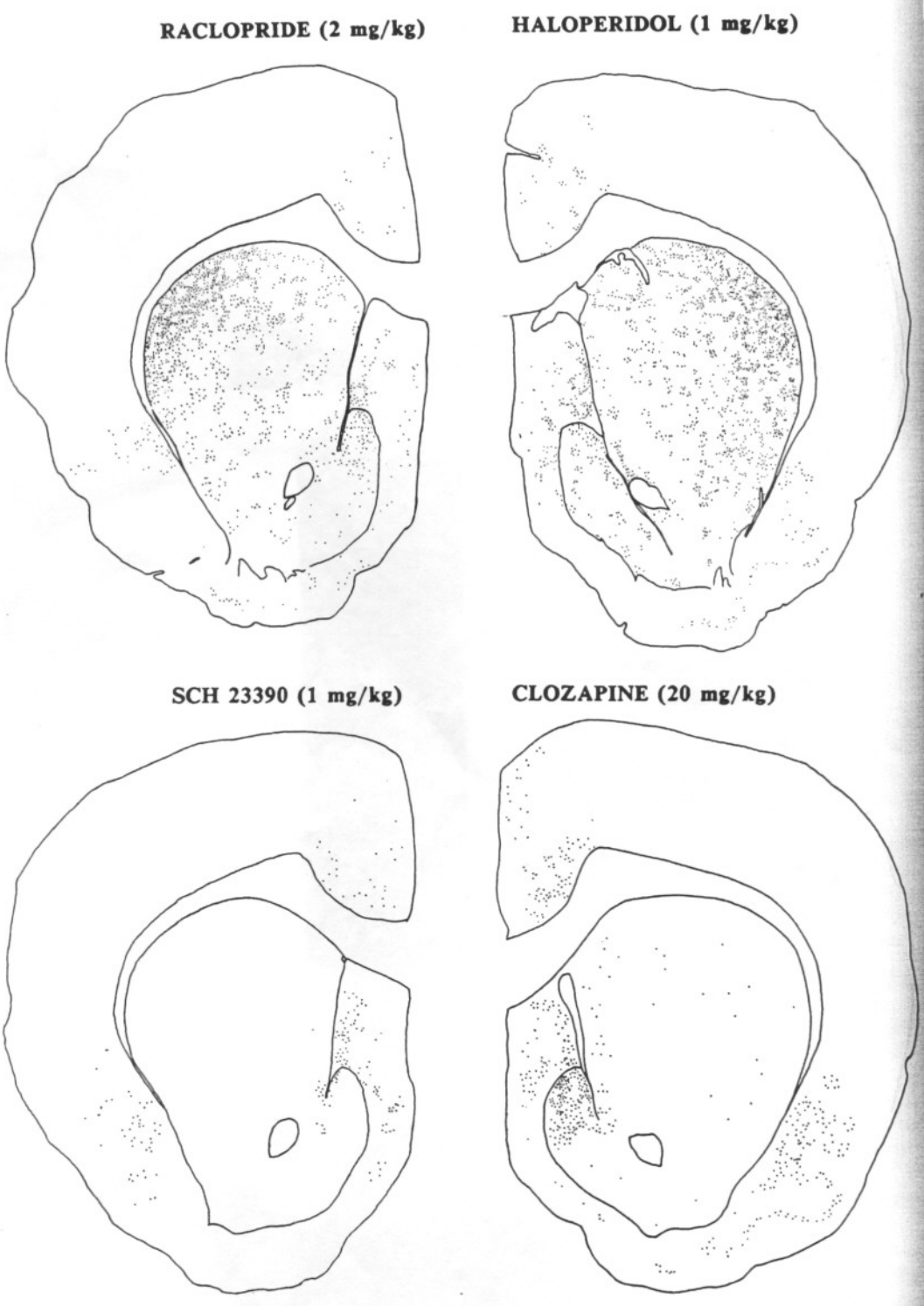

Fig. 5. Camera lucida drawings representative of the effects of an injection of raclopride, haloperidol, SCH 23390, and clozapine on the distribution of Fos-positive neurons at the level of the caudal NAc and mid-striatum. Sections correspond to an AP position approximately 1.0 mm from bregma according to the atlas of Paxinos and Watson.[41]

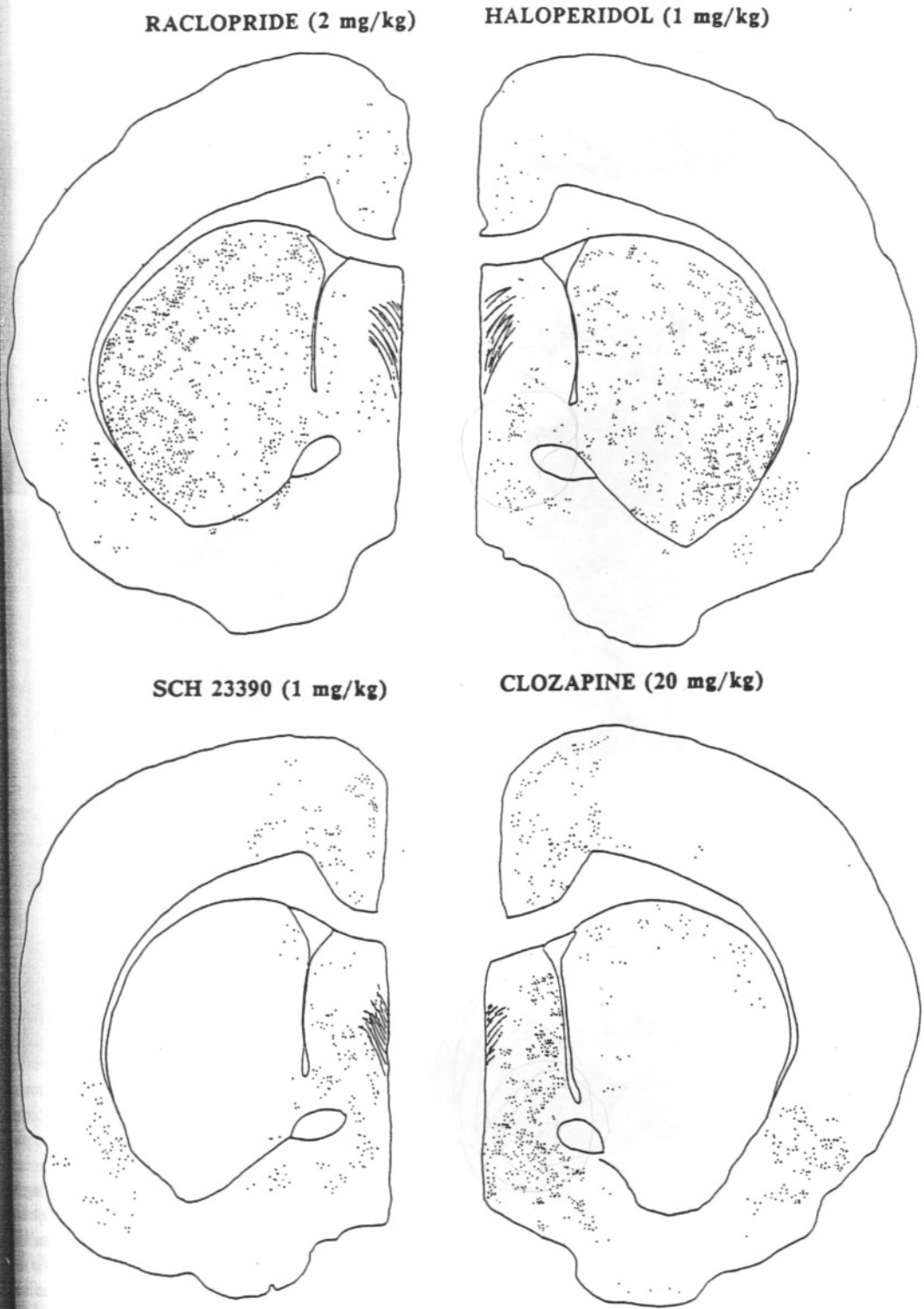

Fig. 6. Camera lucida drawings representative of the effects of an injection of raclopride, haloperidol, SCH 23390, and clozapine on the distribution of Fos-positive nuclei in the mid-striatum and septum. Sections correspond to an AP position approximately 0.1 mm from bregma according to the atlas of Paxinos and Watson.[41]

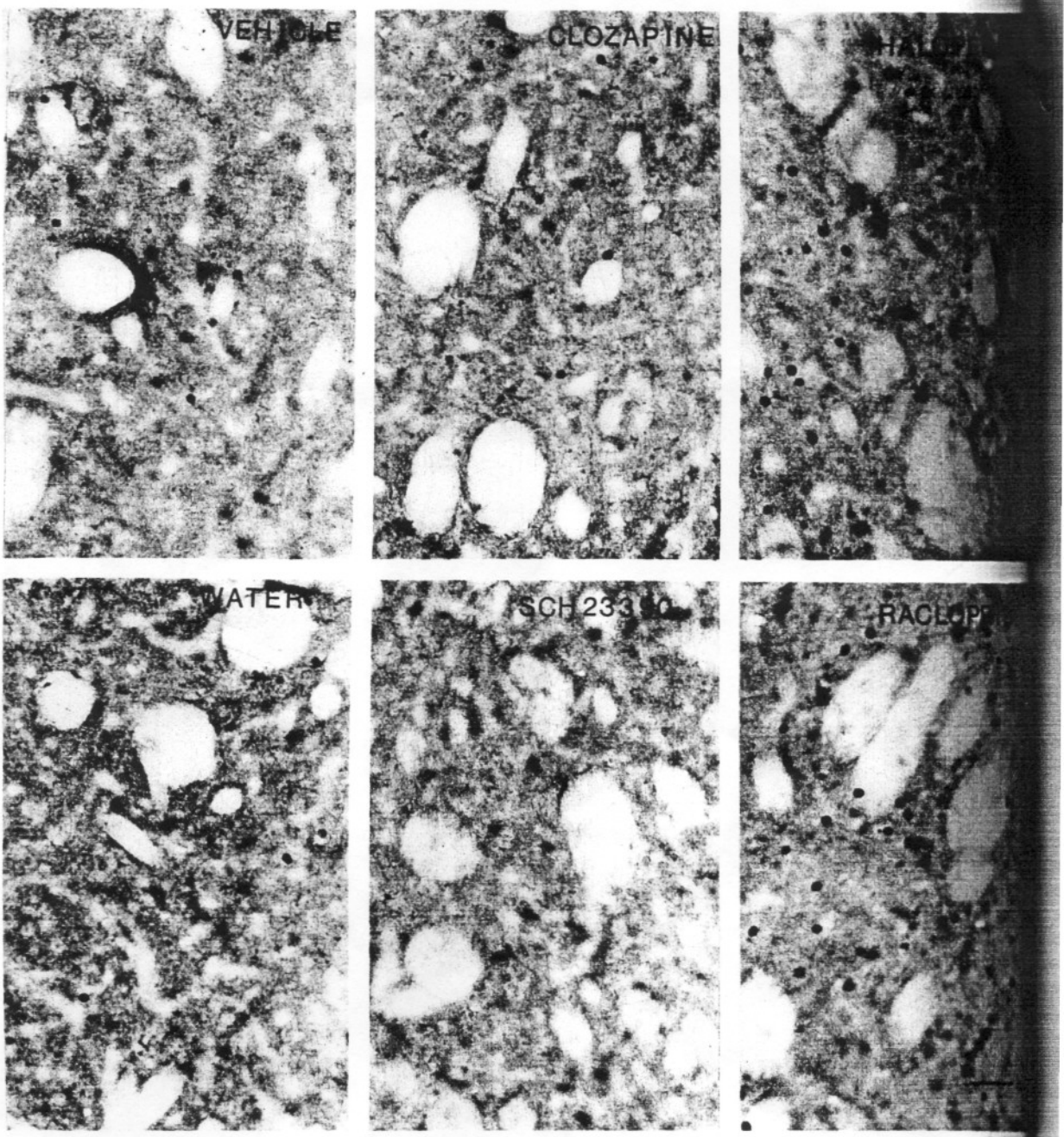

Fig. 7. Photomicrographs of Fos immunoreactivity in the medial aspect of the striatum 2 h after injection of vehicle (1 ml/kg), clozapine (20 mg/kg), haloperidol (1 mg/kg), water (1 ml/kg), SCH 23390 (1 mg/kg), and raclopride (2 mg/kg). Scale bar = 100 μm.

clozapine weakly displaces [^{3}H]spiroperidol from striatal D_2 sites.[2,8,10,27,39] A low affinity for D_2 receptors may therefore be responsible for the small number of Fos-positive neurons in the striatum after clozapine. The very limited capacity of clozapine to elevate Fos immunoreactivity in striatal neurons provides a potential neuroanatomical basis for the low incidence of EPS produced by this compound.

The fact that clozapine increased the number of Fos-positive neurons in the NAc is consistent with reports of a preferential action of this drug on mesolimbic systems.[2,8,10,13,21] Clozapine has a significantly greater ability to displace [^{3}H]spiroperidol from the NAc than it does from the striatum.[2,8,10,27] Hence, the dose-dependent increase in *c-fos* expression in the NAc after clozapine may be due to its higher affinity for D_2 receptors in this limbic region. The fact that the 6-OHDA lesions reduced clozapine-induced *c-fos* expression in the NAc is consistent with this hypothesis. Haloperidol and raclopride also increased Fos immunoreactivity in the NAc in a 6-OHDA lesion-sensitive manner. However, the

Table 3. Effect of 6-hydroxydopamine lesions of the mesotelencephalic dopamine system on the number of Fos-positive neurons in a 520 μm^2 area after clozapine (20 mg/kg), haloperidol (1 mg/kg), and raclopride (2 mg/kg) in the ipsilateral and contralateral, medial prefrontal cortex, nucleus accumbens, lateral striatum, and lateral septal nucleus

	Clozapine		Haloperidol		Raclopride	
	Intact	Lesioned	Intact	Lesioned	Intact	Lesioned
PFC	86.5 ± 6.2	71.5 ± 11.1	37.4 ± 5.2	35 ± 4.5	ND	ND
NAc	78 ± 8.5	21 ± 1*	105 ± 10	29 ± 7*	109 ± 14	42 ± 3*
L. STR	9 ± 3	4 ± 1	60 ± 13	6 ± 1*	60 ± 6	7 ± 3*
Septum	108 ± 12	101 ± 14	50 ± 3.2	46.1 ± 2.5	ND	ND

Asterisks (*) indicate statistically significant differences between the intact and lesioned sides ($P < 0.05$); ND, not determined.

distribution of activated neurons was different from that produced by clozapine. The former compounds produced small patches of Fos immunoreactivity throughout the rostrocaudal extent of the NAc. In contrast, clozapine-induced Fos immunoreactivity was distributed homogeneously in the anterior NAc but was limited to neurons in the medial aspect of this nucleus at more caudal levels. The different distributions of Fos-immunoreactive neurons produced by these drugs suggests that clozapine-induced Fos may not be related to D_2 receptor blockade, and may occur in different populations of NAc neurons than those activated by haloperidol and raclopride. The failure of the selective D_1 antagonist SCH 23390 to increase Fos immunoreactivity in the NAc indicates that D_1 blockade was not responsible for the clozapine-induced *c-fos* expression in the NAc. Together, these observations raise the possibility that clozapine increases Fos immunoreactivity in the NAc by blocking dopamine receptors that are pharmacologically distinct from the D_1 or D_2 subtype. 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.[56] The D_3 receptor is concentrated in limbic regions such as the NAc and has been suggested to be a potential target for antipsychotic actions.[56]

6-OHDA lesions blocked the ability of haloperidol and raclopride to increase *c-fos* expression in the striatum and NAc. This finding suggests that dopamine may tonically inhibit a population of striatal neurons which express the D_2 receptor. Recent *in situ* hybridization results have demonstrated that D_2 receptors are expressed predominantly by striatal neurons that utilize GABA and enkephalin as neurotransmitters and that project to the globus pallidus.[5-7,18,29] Chronic administration of haloperidol increases enkephalin mRNA in these striatal neurons,[3,21,29,48] while chronic clozapine produces only a small increase in enkephalin expression in the striatum.[3,21] These findings, together with the topographical correspondence between the distribution of haloperidol-induced Fos immunoreactivity and D_2 receptors in the striatum, suggest that this Fos may be located in enkephalinergic neurons. This raises the possibility that the elevated *c-fos* expression observed here may serve to increase the transcription of enkephalin mRNA in striatopallidal neurons. However, morphine, which has been reported to reduce striatal proenkephalin content,[56] also increases *c-fos* expression in the striatum.[12] If morphine-induced Fos is also located in striatopallidal neurons then there must be a complex relationship between *c-fos* expression and proenkephalin transcription.

Unlike haloperidol, clozapine increased the number of Fos-positive neurons in the PFC. Destruction of the dopaminergic innervation of the PFC did not influence this response. Neither selective D_1 nor D_2 antagonists increase Fos immunoreactivity in the PFC (Tables 1 and 2). It seems unlikely, therefore, that dopaminergic mechanisms are involved in clozapine-induced changes in *c-fos* expression in the PFC. Clozapine has a high affinity for 5-hydroxytryptamine$_2$ receptors which are found in relatively large numbers in the PFC.[2,39,43] There is electrophysiological evidence that iontophoretically applied clozapine, but not haloperidol, can reduce the inhibitory actions of the 5-hydroxytryptamine receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [(±)-DOI] on single unit activity in the PFC.[4] Hence, elevated Fos immunoreactivity in the PFC after clozapine may have been mediated by the effects of 5-hydroxytryptamine receptor blockade on PFC neurons. These results are also consistent with the proposal that 5-hydroxytryptamine$_2$/D_2 affinity ratios for atypical antipsychotics are higher than they are for typical antipsychotics.[32] Recently, clozapine has also been reported to have a high affinity for 5-hydroxytryptamine$_{1C}$ sites,[11] and these are also located in the PFC.[22] 5-Hydroxytryptamine$_{1C}$ receptors in the PFC must also, therefore, be considered as potential mediators of the effects of clozapine on the expression of *c-fos* in the PFC.

Clozapine greatly, and haloperidol slightly, increased the number of Fos-immunoreactive neurons in the lateral septal nucleus and this occurred primarily in the ventral and to a lesser extent intermediate regions as defined by Paxinos and Watson.[41] As was the case for the PFC, this increase was not affected by 6-OHDA lesions. However, in contrast to the PFC, there are few 5-hydroxytryptamine$_2$ receptors in the lateral septum;[43] instead, this region contains a

high concentration of 5-hydroxytryptamine$_1$ receptors, perhaps of the 5-hydroxytryptamine$_{1A}$ subtype.[42] Clozapine and haloperidol do not appear to block 5-hydroxytryptamine$_{1A}$ receptors.[38] Therefore, it seems unlikely that the clozapine- and haloperidol-induced increases in Fos-positive neurons in the lateral septal nucleus were mediated by either 5-hydroxytryptamine$_2$ or 5-hydroxytryptamine$_{1A}$ receptors. It remains possible, of course, that clozapine and haloperidol produced their effects in the lateral septum (and the PFC) via non-serotonergic mechanisms.

CONCLUSIONS

In summary, the present experiments demonstrate that clozapine, haloperidol, raclopride, and SCH 23390 have different effects on Fos immunoreactivity in the PFC, NAc, striatum, and lateral septum. Clozapine increased the number of Fos-positive nuclei in all of these regions except for the striatum. It is tempting to speculate that clozapine's unique therapeutic profile may be related to its distinctive patterns of *c-fos* activation observed here. For example, the failure of clozapine to produce a large increase in Fos-positive neurons in the striatum may be related to the lack of EPS produced by this compound. However, in view of the fact that in some neurons there may not be a strong correlation between electrophysiological activity and *c-fos* expression,[23,36,46] this interpretation must be advanced with caution. Of even greater potential significance is the potent and relatively selective effects that clozapine had on *c-fos* expression in the medial prefrontal cortex and lateral septal nucleus. One of the most intriguing clinical observations with clozapine is that in addition to reducing the positive symptoms in schizophrenia (delusions, hallucinations, thought disorder), it is also uniquely effective in treating the negative symptoms of this syndrome (e.g. poverty of speech, flatness of affect, apathy, decreased spontaneous movement.[26,31] The present findings provide a potential neuroanatomical framework within which to view these clinical observations. Specifically, they raise the interesting possibility that clozapine's unique therapeutic actions on negative symptoms in schizophrenia may be related to its distinctive effects in regions such as the medial prefrontal cortex and lateral septal nucleus. As more atypical neuroleptic agents become available and are characterized clinically, this will become a testable hypothesis.

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