Differential induction of neurotensin and c-fos gene expression by typical versus atypical antipsychotics

(caudate-putamen/nucleus accumbens shell/extrapyramidal side effects/mRNA)

KALPANA M. MERCHANT* AND DANIEL M. DORSA

Geriatric Research, Education and Clinical Center, Seattle Veterans Administration Medical Center, Seattle, WA 98108; and Departments of Psychiatry and Behavioral Sciences and Pharmacology, University of Washington, Seattle, WA 98195

Communicated by Susan E. Leeman, December 7, 1992

ABSTRACT Precise neural mechanisms underlying the pathophysiology and pharmacotherapy of psychotic disorders remain largely unknown. Present studies investigated the effects of various antipsychotic drugs on expression of the gene encoding the purported endogenous antipsychotic-like peptide neurotensin (NT) in striatal regions of the rat brain. The results demonstrate that several clinically efficacious antipsychotic drugs selectively and specifically increase expression of NT/neuromedin N (NT/N) mRNA in the shell of the nucleus accumbens, a region of the forebrain associated with limbic systems. On the other hand, only typical antipsychotics that cause a high incidence of acute motor side effects increased the expression of NT/N mRNA in the dorsolateral striatum, an extrapyramidal region primarily involved in motor control. In addition, it appears that distinct mechanisms may be involved in the effects of antipsychotics on NT/N gene expression in the dorsolateral striatum versus the accumbal shell. Thus neuroleptic-induced increases in NT/N mRNA expression in the dorsolateral striatum were preceded by a rapid and transient activation of c-fos mRNA, whereas none of the antipsychotics affected c-fos mRNA expression in the accumbal shell. The anatomical characteristics of NT/N gene expression induced by typical versus atypical antipsychotics raise the possibility that increased activity of specific NT neurons may contribute to the therapeutic effects (NT neurons in the accumbal shell) or motor side effects (NT neurons in the dorsolateral striatum) of these drugs.

Antipsychotic drugs are a group of chemically diverse compounds that are used in the treatment of schizophrenia and other psychotic disorders. Clinical use of several of these drugs termed typical antipsychotics or neuroleptics (e.g., haloperidol and fluphenazine) is accompanied by a wide spectrum of acute and chronic motor side effects such as dystonias and tardive dyskinesia, respectively. These side effects are thought to be mediated by extrapyramidal motor systems in the basal ganglia. The symptoms are often severe and disabling and frequently lead to patient noncompliance. Therefore, recent search for new antipsychotic agents has focused on the so-called ‘atypical’ antipsychotics, drugs that do not cause extrapyramidal motor side effects (EPS). Clozapine is a prototype of atypical antipsychotic drugs whose clinical efficacy is equal to or greater than that of typical antipsychotics such as haloperidol but has a much lower incidence of EPS (1). However, treatment with clozapine results in 1-2% incidence of agranulocytosis (2) and hence the search for new atypical antipsychotics continues.

Development of new therapeutic antipsychotic agents with fewer EPS has been difficult partly because precise neurochemical mechanisms underlying the etiology of schizophrenia and the pharmacological effects of antipsychotic drugs remain unclear (3). A role of dopamine D2 receptors is indicated in the observation that the potency of antipsychotic drugs correlates well with their ability to block the D2 receptors (4, 5). However, attempts to identify abnormalities in D2 receptor expression in drug-naive schizophrenic patients have been inconclusive (6, 7). Recent studies therefore have attempted to identify other postsynaptic transmitter systems targeted by antipsychotic drugs that may be involved in the etiology and/or pharmacotherapy of schizophrenia.

We have examined the interaction of antipsychotic drugs with a peptidergic transmitter, neurotensin (NT), which was isolated first from bovine hypothalamus (8) and has heterogeneous distribution in mammalian brain. NT interacts closely with nigrostriatal and mesolimbic dopamine systems (for reviews, see refs. 9-11). The pharmacological profile of this peptide in rats resembles that of clinically used antipsychotic drugs such as haloperidol and it has been proposed that NT may be an endogenous neuroleptic-like compound (12). Moreover, a potential role for central NT systems in the etiology/pharmacotherapy of schizophrenia is suggested by the observations that NT concentration in the cerebrospinal fluid of some schizophrenic patients is lower than that of controls. Treatment of these patients with antipsychotic drugs is associated with normalization of NT levels to control values (13). Various antipsychotic drugs have been shown to increase NT immunoreactivity in striatal regions of the rat brain (14-18), and this effect appears to be mediated via blockade of dopamine D2 receptors (19, 20). Our studies of cellular and molecular mechanisms underlying haloperidol-induced increases in NT peptide content indicate that an increase in the transcription of the NT/neuromedin N (NT/N) gene is, at least in part, responsible for the increased peptide content following acute treatment with haloperidol (21, 22). Hence, we hypothesized that alteration in NT/N gene transcription induced by antipsychotic drugs may underlie some of the pharmacological effects of this class of drugs.

Our previous studies (22) demonstrated that following acute treatment with haloperidol, the largest increase (1000%) in NT/N gene expression occurs in the dorsolateral region of the striatum (DLSt). Since this region is thought to be part of the basal ganglia circuitry involved in regulation of motor output, the question was raised whether atypical antipsychotics that do not cause EPS would increase NT/N mRNA expression in this region. It was observed that acute administration of clozapine did not affect NT/N gene transcription in the DLSt. On the other hand, haloperidol and clozapine significantly increased NT/N mRNA expression in the shell sector of the nucleus accumbens, a predominantly

Abbreviations: NT, neurtensin; NT/N, NT/neuromedin N; EPS, extrapyramidal motor side effects; DLSt, dorsolateral striatum.

*To whom reprint requests should be addressed: GRECC (182 B), Seattle Veterans Administration Medical Center, 1660 South Columbian Way, Seattle, WA 98108.
limbic region (23–26) and a likely locus of antipsychotic drug effects. The differential responses of limbic and extrapyramidal NT neurons to acute haloperidol and clozapine observed led us to suggest the following hypothesis. Antipsychotic drug-induced increases in NT/N gene expression in the DLSt may be functionally related to their acute EPS (such as dystonias), whereas their effects on NT neurons in the accumbal shell may contribute to the antipsychotic effects of these drugs. We have attempted to provide further indirect support for this hypothesis in the studies described here.

In the first set of experiments we compared the acute effects of haloperidol and clozapine on striatal NT/N gene expression with those induced by acute treatment with other typical and atypical antipsychotic drugs. Fluphenazine, a neuroleptic that is chemically distinct from haloperidol but induces EPS similar to haloperidol was selected as another representative of the typical class. On the other hand, remoxipride, a relatively new antipsychotic that appears to be as efficacious as haloperidol but shows a much lower incidence of acute EPS (27) was used as an atypical antipsychotic compound. Finally, the effects of thioridazine were studied since it appears to have a significantly lower propensity to induce acute dystonias in humans and possesses a somewhat atypical profile in animal studies (28).

In addition, we have studied potential cellular and molecular mechanisms by which the transcription of the gene encoding NT may be regulated by antipsychotic drugs. Specifically, we evaluated the expression of c-fos mRNA in striatal neurons following acute treatment of animals with the typical and atypical antipsychotic drugs described above.

MATERIALS AND METHODS

Animals and Drug Treatment. Adult male Sprague–Dawley rats (200–250 g; Simonsen Laboratories, Gilroy, CA) were housed two or three per cage in a temperature-controlled environment with a 12-hr light/dark cycle and were given free access to standard laboratory chow and water. Rats were given a single i.p. dose of haloperidol (1 mg/kg), fluphenazine (0.5 mg/kg), clozapine (20 mg/kg), remoxipride (1.25 mg/kg), thioridazine (10 mg/kg), or vehicle in a volume of 1 ml/kg. These doses represent about 5–10 x ED50 for blockade of apomorphine-induced hyperactivity in rats (28). Several pilot studies were carried out to determine the time course of drug-induced alterations in NT/N and c-fos mRNA expression. Incidentally, expression of c-fos mRNA preceded that of NT/N mRNA. The data shown here represent the time point at which peak effects of each drug were observed (1 hr for c-fos mRNA and 3–7 hr for NT/N mRNA).

In a second set of studies, the role of concurrent anticholinergic and/or dopamine D1 receptor blocking ability of some antipsychotic drugs on NT/N and c-fos mRNA expression was examined. Animals were given a single i.p. dose of atropine (2 mg/kg) or dopamine D1 receptor antagonist, SCH 23390 (10 mg/kg), 30 min before an acute dose of haloperidol (1 mg/kg) or saline. Finally, to examine the specificity of NT/N mRNA responses to drugs with antipsychotic efficacy, effects of agonists selective for dopamine D1 and D2 receptor subtypes, SKF 38393 (9 mg/kg) and quinpirole (5 mg/kg), respectively, were examined.

Rats were sacrificed by decapitation between 12:00 noon and 3:00 p.m. Brains were rapidly removed, frozen on dry ice, divided sagittally into two halves, and stored at −80°C until processed for in situ hybridization histochemistry.

In Situ Hybridization. Details of the in situ hybridization methods have been published (22). Hybridization was carried out for 16–18 hr using saturating concentrations of 35S-labeled probes. For the NT/N mRNA, an antisense RNA probe complementary to exon 4 of the NT/N gene was used. Hybridization to c-fos mRNA was carried out using an oligonucleotide probe complementary to nucleotides 270–319 of the rat c-fos cDNA.

Autoradiography. DLSt signal. Slides were apposed to Hyperfilm-βmax (Amersham) for 3–5 days and films were developed in Kodak D-19 solution. Brain regions were autoradiographically digitized according to the atlas of Konig and Klippel (29). Autoradiograms were digitized with a Drexel's unix-based microcomputer image analysis system. Optical density (OD) in the same region of the DLSt of each image was determined at a single level (Bregma 1 mm) using three brain sections per animal. Additionally, background signal of each brain section was determined by measuring the OD of deep cortical layers adjacent to the corpus collosum since we have not observed specific hybridization to NT/N or c-fos mRNA in this region. The specific hybridization signal in each DLSt was obtained by subtraction of the background OD value from the total OD value (in the DLSt) of each section.

Nucleus accumbens signal. Slides were coated with Kodak NTB2 nuclear tract emulsion (diluted 1:1 with 0.6 M ammonium acetate), air-dried in the dark for 2 hr, and exposed for 7–14 days. After developing the emulsion the sections were counterstained in 0.1% (wt/vol) cresyl violet acetate and anatomically matched. Three sections from each animal were used for analysis using a computer-assisted image analysis system. A labeled cell was defined as one that had at least 6 times (for c-fos oligonucleotide probe) or 10 times (for NT/N cRNA probe) the background autoradiographic grain density. Additionally, a significantly positive cell was confirmed using bright-field microscopy. Because of the high abundance of cells labeled for NT/N mRNA in the accumbal shell, the average number of hybridization-positive cells in a fixed area of this region was determined. On the other hand, very few c-fos mRNA-expressing cells were observed in the shell sector of the nucleus accumbens. Hence, all c-fos mRNA-labeled cells in this region were counted. Additionally, the number of autoradiographic grains over the labeled cells was determined as an index of the amount of mRNA per cell.

Statistics. Data are presented as mean ± SEM for each treatment group; the number of animals per group was five or six. Differences between means were analyzed using ANOVA. Following a significant difference in variance, Scheffe’s test was applied to identify groups differing significantly from the control values. Differences were considered significant if the probability that they were zero was <5%.

RESULTS

Effects of Various Antipsychotic Drugs on NT/N mRNA Expression. Acute administration of all antipsychotic drugs tested, regardless of their propensity to induce EPS, increased the expression of NT/N mRNA in the shell of the nucleus accumbens (Fig. 1). The primary effect of these drugs appeared to involve enhancement of the level of NT/N mRNA in individual cells constituting the NT/N gene (as assessed by the number of grains per cell) rather than recruitment of additional cells (Figs. 1 and 2). Thus the average number of autoradiographic grains per cell increased by 75–100% following acute treatment with these drugs. These increases were evident at 3–7 hr after drug treatments. The increased expression of the NT/N gene in the accumbal shell appears to be specific for the antipsychotic drugs since acute treatment with muscarinic antagonist (atropine), dopamine D1 receptor antagonist (SCH 23390), or selective dopamine receptor agonists (SKF 38393 for D1-like, quinpirole for D2-like receptors) did not alter NT/N mRNA expression in this region (Table 1).

In contrast to the nucleus accumbens, NT/N gene expression in the DLSt displayed some distinct characteristics.
First, this region showed comparatively little basal expression of NT/N mRNA. Additionally, an increase in the NT/N mRNA content of the dorsal striatum was induced only by the typical antipsychotics haloperidol and fluphenazine and not by clozapine, remoxipride, or thioridazine (Figs. 1 and 2). Finally, compared to the accumbal changes, the neuroleptics appeared to enhance NT/N gene expression in the DLSt with significant rapidity since the increases were evident as early as 30 min following drug treatment and appeared to peak between 3 and 7 hr.

**Fig. 1.** Detection of NT/N mRNA expression in the nucleus accumbens shell and DLSt by in situ hybridization histochemistry. Rats (n = 6 per group) were treated with a single dose of saline (C) or haloperidol (H, 1 mg/kg), fluphenazine (Fl, 0.5 mg/kg), clozapine (Cl, 20 mg/kg), remoxipride (R, 1.25 mg/kg), or thioridazine (T, 10 mg/kg) and expression of NT/N mRNA was examined by in situ hybridization at 1, 3, or 7 hr following treatment. Sections shown represent the time point at which peak increases in NT/N mRNA expression were observed (3 or 7 hr following drug administration). (Upper) High-magnification, dark-field photomicrographs through the accumbal shell. Note the increase in the number of grains per cell in the accumbal shell following treatment with all antipsychotic drugs as compared to the control. (Lower) Film autoradiograms demonstrate that only the neuroleptics (haloperidol and fluphenazine) but not the atypical antipsychotics (clozapine, remoxipride, or thioridazine) increased NT/N labeling in the DLSt (indicated by arrowheads).

**Fig. 2.** Quantification of NT/N hybridization signal in the nucleus accumbens shell (A) and DLSt (B). Anatomically matched sections (three per animal) were used to quantify the hybridization signal using computer-assisted image analysis systems. Analysis of hybridization signal in the accumbens shell using dark-field photomicroscopy revealed that the drug treatments affected primarily the grain density of labeled cells and not the number of labeled cells. Alterations in the DLSt were assessed by measuring the background-subtracted OD of film autoradiograms from each group. (Insets) Schematic depictions of the regions of the nucleus accumbens and the caudate-putamen sampled for the analysis. Data were analyzed by ANOVA followed by Scheffe’s test and are presented as % of control to facilitate comparisons between groups. *, P < 0.01 (n = 6 per group) compared to control.

**Effects of Various Antipsychotic Drugs on c-fos mRNA Expression.** Our previous studies had indicated that haloperidol-induced increases in NT/N mRNA levels in the DLSt neurons may result from an increase in the transcription of the NT/N gene (22). Presence of a consensus AP-1 sequence in the promoter region of the NT/N gene and its functional involvement in regulating the transcription of this gene in PC12 cells (30) led us to examine the antipsychotic drug-induced changes in the expression of the immediate early gene c-fos. Interestingly, expression of c-fos mRNA in the DLSt after antipsychotic drug treatment followed the same pattern as that seen with NT/N gene expression in this region. Thus, only the typical antipsychotics haloperidol and fluphenazine increased the expression of c-fos mRNA in the DLSt, which showed no detectable expression in control animals (Fig. 3). However, increased expression of c-fos mRNA following neuroleptics appeared to precede the increases in NT/N mRNA caused by these drugs. Thus the peak c-fos mRNA increases occurred at 30 min following haloperidol treatment and, by 3 hr after the drug administration, c-fos gene expression in the DLSt was no longer detectable.

In contrast to the DLSt, none of the antipsychotic drugs significantly altered c-fos mRNA expression in the shell of the nucleus accumbens. Thus the average number of labeled cells ± SEM in the various treatment groups was as follows: saline, 2.33 ± 0.66; haloperidol, 4.52 ± 0.74; fluphenazine, 3.63 ± 0.74; clozapine, 2.73 ± 0.66; remoxipride, 3.34 ± 0.41; and thioridazine, 2.33 ± 0.33. The number of autoradiographic grains over these cells also did not differ between

**Table 1.** Effects of muscarinic cholinergic receptor blockade or dopamine D1 and D2 receptor-selective ligands on expression of NT/N mRNA in the nucleus accumbens shell

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, mg/kg</th>
<th>No. of cells</th>
<th>No. of grains per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>—</td>
<td>16.28 ± 2.27</td>
<td>17.14 ± 1.15</td>
</tr>
<tr>
<td>Atropine</td>
<td>2</td>
<td>16.73 ± 2.29</td>
<td>20.56 ± 2.32</td>
</tr>
<tr>
<td>SCH 23390</td>
<td>0.5</td>
<td>18.28 ± 2.21</td>
<td>18.87 ± 1.58</td>
</tr>
<tr>
<td>SKF 38393</td>
<td>9</td>
<td>14.74 ± 3.50</td>
<td>19.79 ± 2.87</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>5</td>
<td>19.83 ± 2.73</td>
<td>20.66 ± 2.33</td>
</tr>
</tbody>
</table>

Animals were treated i.p. with a single dose of saline or drug as indicated. In situ hybridization for NT/N mRNA was carried out as described in the legend to Fig. 1. Hybridization signal in the accumbal shell was quantified as described in the legend to Fig. 2.
**DISCUSSION**

The present study clearly demonstrates that NT-ergic neurons located in distinct regions of the forebrain respond differentially to several typical versus atypical antipsychotic drugs. That is, NT neurons in the shell of the nucleus accumbens appear to be sensitive to all antipsychotics tested, whereas those in the dorsolateral striatum respond only to those drugs that cause acute EPS such as dystonias.

Fig. 3. Detection and quantification of c-fos mRNA in the striatum by *in situ* hybridization following acute antipsychotic drug treatment. Animals were treated as described in the legend to Fig. 1. (Upper) Representative film autoradiograms from animals sacrificed 1 h after saline or drug treatment. Note an increase in the c-fos hybridization signal in the DLSt (indicated by arrowheads) following treatment with haloperidol (H) and fluphenazine (Fl) but not clozapine (Cl), remoxipride (R), or thioridazine (T). (Lower) Densitometric analysis of c-fos mRNA hybridization signal in the DLSt of each treatment group (n = 6). *, P < 0.01 (ANOVA followed by Scheffe’s test) compared to control (C).

**Effects of Muscarinic or Dopamine D1 Receptor Blockade on NT/N and c-fos mRNA Expression in the DLSt.** The second set of studies investigated potential pharmacological mechanisms that may have accounted for the differential responses of NT/N and c-fos gene expression in the DLSt to typical versus atypical antipsychotics. Clozapine and thioridazine possess significant antimuscarinic activity. In addition, clozapine also displays potent dopamine D1 receptor blocking activity. Hence, the effects of muscarinic or D1 blockade alone and in combination with haloperidol were studied. Blockade of either dopamine D1 receptors (with SCH 23390) or muscarinic receptors (with atropine) by itself did not affect the basal expression of either NT/N mRNA or c-fos mRNA in the DLSt neurons. Additionally, administration of neither SCH 23390 nor atropine prior to haloperidol treatment modified the increases in NT/N and c-fos gene expression in the DLSt caused by haloperidol alone (Fig. 4).

Fig. 4. Effects of atropine and SCH 23390 on basal and haloperidol-induced expression of NT/N mRNA (A) and c-fos mRNA (B) in the DLSt. Thirty minutes prior to saline or haloperidol (1 mg/kg) administration, rats were given a single dose of atropine (2 mg/kg) or SCH 23390 (0.5 mg/kg). Separate groups of rats also received either saline (control) or haloperidol alone. In *in situ* hybridization for NT/N and c-fos mRNA and quantification of data were carried out as described in the legends to Figs. 1–3. Note that blockade of muscarinic or dopamine D1 receptors (with atropine and SCH 23390, respectively) neither affected the basal expression of c-fos or NT/N mRNA in the DLSt nor modified the increases in NT/N or c-fos gene expression in the DLSt caused by haloperidol. *, P < 0.001 (ANOVA followed by Scheffe’s test, n = 5 per group) versus the control group.

Interestingly, NT immunoreactivity in the dorsal striatum also increases selectively following chronic treatment (21 days) with haloperidol but not clozapine (31). Although other peptidergic systems such as cholecystokinin, enkephalins, or tachykinins are affected by antipsychotics, they do not show this pattern of region-specific drug selectivity (32–34). The data presented here support the hypothesis that differential sensitivity of NT neurons to typical versus atypical antipsychotics may have a functional significance.

The selective enhancement in NT/N gene expression in the shell of the nucleus accumbens by all antipsychotic drugs suggests the possibility that increased activity of NT neurons may be functionally related to the clinical efficacy of these drugs. Indeed, the accumbal shell with its characteristic limbic connections is a likely target of antipsychotic effects. Additionally, direct injections of NT into the nucleus accumbens antagonize dopamine agonist-induced hyperlocomotion in rats, a preclinical test indicative of antipsychotic efficacy (35). Finally, this effect of NT appears to be mediated via postsynaptic mechanisms (36) and not by effects on presynaptic dopamine transmission. Taken together, these data suggest that NT neurons in the accumbal shell may serve as neuronal targets of antipsychotic drugs such that an increase in the activity of these peptidergic neurons may contribute to the therapeutic effects of these drugs.

In contrast, NT neurons in the DLSt appeared to be sensitive only to the typical antipsychotics. Although thioridazine has the potential to induce motoric effects in humans, its propensity to induce acute dystonias is much lower compared to the classical neuroleptics such as haloperidol. Additionally, it also appears to have a lower potency to induce catalepsy in rodents compared to other neuroleptics (28). Thus, a correlation appears to exist between the propensity of the antipsychotic drugs to cause certain acute EPS in humans (or catalepsy in rodents) and their ability to induce NT/N mRNA expression in the DLSt of the rat. Because this is a predominantly motor region of the brain, our findings suggest a role of NT neurons within the DLSt in the acute EPS induced by these drugs. Involvement of central NT systems in motor control is also evident in the observation that central administration of NT induces catalepsy in mice (37), a behavior that correlates with acute EPS in humans.
These findings suggest that an evaluation of NT/N mRNA expression in humans is warranted to determine if subpopulations of NT neurons in distinct striatal regions may be involved in the therapeutic versus adverse effects of antipsychotic drugs.

Pharmacological mechanisms underlying the differential responses of NT neurons in the DLSt to typical versus atypical antipsychotics remain unclear. However, our data do suggest that blockade of muscarinic or D1 receptors concomitantly with blockade of D2 receptors by antipsychotics is probably not a mechanism resulting in the insensitivity of NT neurons in the DLSt to drugs such as clozapine or thioridazine. That preferential blockade of other subtypes of dopamine receptors (such as D2 versus D4 receptor) or serotonin receptors may lead to the selective effects of typical versus atypical antipsychotics on NT/N mRNA expression is a possibility that requires further investigation.

Our previous studies (22) indicate that haloperidol may increase transcription of the NT/N gene in the DLSt neurons. Present results show rapid and transient increases in c-fos mRNA expression in the DLSt preceding the increases in NT/N mRNA levels following neuroleptic drugs. This indicates a possible role of the transcription regulator fos in neuroleptic-induced increases in NT/N gene transcription in DLSt neurons. Indirect evidence for this possibility is apparent in the present studies since c-fos mRNA expression exhibited the same anatomical specificity and discriminatory selectivity for typical versus atypical antipsychotics as that displayed by NT neurons in the DLSt (only those drugs that increased NT/N gene expression also enhanced c-fos mRNA levels). However, further studies are required to determine the functional relationship (if any) between enhanced expression of c-fos and NT/N mRNA in DLSt caused by neuroleptic drugs. The present data do demonstrate that c-fos and NT/N mRNA expression in DLSt neurons exhibit differential sensitivity to typical versus atypical antipsychotic drugs.

The increased expression of c-fos gene observed here is concordant with previous reports showing increases in c-fos mRNA levels in the striatum by Northern blot analysis (38) and also induction of fos-like immunoreactivity in the DLSt following administration of typical but not atypical antipsychotics (39, 40). On the other hand, there appears to be a discrepancy between expression of fos immunoreactivity and c-fos mRNA in the nucleus accumbens after antipsychotic treatment of the rat. In the present study we did not detect a similar discrepancy in c-fos mRNA expression in the shell of the nucleus accumbens, although previous studies have demonstrated differential increases in fos immunoreactivity in the shell and core following clozapine and haloperidol treatment, respectively (39, 40). It is possible that the in situ hybridization technique used here was not sensitive enough to detect small alterations in c-fos mRNA transcripts in the nucleus accumbens. Another likely explanation for this apparent discrepancy is that antipsychotic drug-induced increases in fos immunoreactivity in the accumbal shell may involve post-transcriptional mechanisms. Nonetheless, the data presented here clearly demonstrate yet another distinction between the responses of nucleus accumbens and DLSt neurons to antipsychotic drugs.

As mentioned earlier, it appears imperative to study the effects of antipsychotic drugs on NT/N mRNA expression in the human striatum as well as to correlate any changes with drug-induced motor dysfunctions in patients to understand clearly the role of striatal NT neurons. However, our data indicate that an assessment of the responses of NT/N and/or c-fos genes in striatal neurons may offer a rapid, sensitive, and reproducible index for predicting acute EPS potential of antipsychotic drugs.

We are grateful for the technical assistance provided by Anne Kalliomaki. This work was supported by the Department of Veterans Affairs, National Institutes of Health Grant NS 20311, and The Scottish Rite Schizophrenia Research Program.