Induction Patterns of Transcription Factors of the Nur Family (Nurr1, Nur77, and Nor-1) by Typical and Atypical Antipsychotics in the Mouse Brain: Implication for Their Mechanism of Action

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ABSTRACT

Monitoring gene expression has been intensively used to identify neurobiological and neuroanatomical substrates associated with administration of antipsychotic drugs. Transcription factors of the Nur family (Nurr1, Nur77, and Nor-1) are orphan nuclear receptors that have been recently associated with dopamine neurotransmission. Nurr1 is involved in midbrain dopamine neuron development. Nur77 and Nor-1 are expressed in dopaminergic areas such as the substantia nigra, nucleus accumbens, and prefrontal cortex. To better understand the relationship between Nur and antipsychotic drug effects, we conducted a comprehensive evaluation of the effect of various typical and atypical antipsychotic drugs on the modulation of Nur mRNA levels. We show that differential patterns of Nur expression can be obtained with typical and atypical antipsychotic drugs. Modulation of Nur77 and Nor-1 mRNA expression by antipsychotics can be used to calculate an index that is predictive of the typical or atypical profile of antipsychotic drugs. Inductions of Nur by antipsychotic drugs are correlated with dopamine D2 receptor in the striatum and D2 and D3 receptor subtypes in the nucleus accumbens. The 5-hydroxytryptamine2A/D2 affinity ratio of antipsychotics can also predict these patterns of inductions. In addition to classical gene patterns induced in the striatal complex (striatum, accumbens) and cortex, most antipsychotic drugs tested strongly induced Nur77, Nor-1, and increased Nur1 mRNA levels in the substantia nigra and ventral tegmental area. These data suggest that typical and atypical antipsychotic drugs might induce in multiple brain regions distinct Nur-dependent transcriptional activities, which may contribute to their pharmacological effects.

Antipsychotic drugs currently used in the treatment of schizophrenia can be classified as either typical or atypical neuroleptics. Typical neuroleptics such as haloperidol and chlorpromazine have a high propensity to cause a variety of extrapyramidal symptoms (EPS) (Casey, 1991). New generation of atypical antipsychotics such as clozapine and olanzapine are defined as drugs active in the treatment of schizophrenia but with lesser propensity to induce EPS (Serretti et al., 2004). All typical antipsychotic drugs are potent D2 receptor antagonists. Their effects on the mesolimbic dopamine pathway play a role in their antipsychotic actions, whereas those on the nigrostriatal dopamine pathway are responsible for the generation of EPS (Wadenberg et al., 2001). Receptor interaction analysis revealed that atypical antipsychotics tend to have a lower affinity for D2 receptors and higher affinity for 5-HT2A (serotonin) receptors, compared with typical drugs. In fact, a high 5-HT2A/D2 affinity ratio can be used to predict an atypical profile for most antipsychotic drugs (Meltzer, 1999; Roth et al., 2003). Contribution of other dopamine and serotonin receptor subtypes (e.g., D3, D4, or 5-HT1A receptors) remains a matter of debate.

Monitoring gene expression in the central nervous system has been intensively used to identify neurobiological and neuroanatomical substrates associated with administration of antipsychotic drugs. Indeed, induction patterns of fos-like immunoreactivity, the protein products of the immediate-early gene c-fos, in the rat forebrain can be used as a predictor for the typical or atypical profile of antipsychotics (Roberton et al., 1994). Acute administration of antipsychotic drugs likely to produce EPS dramatically elevates the levels of Nur77 and Nor-1 mRNA expression by antipsychotics can be used to calculate an index that is predictive of the typical or atypical profile of antipsychotic drugs. Inductions of Nur by antipsychotic drugs are correlated with dopamine D2 receptor in the striatum and D2 and D3 receptor subtypes in the nucleus accumbens. The 5-hydroxytryptamine2A/D2 affinity ratio of antipsychotics can also predict these patterns of inductions. In addition to classical gene patterns induced in the striatal complex (striatum, accumbens) and cortex, most antipsychotic drugs tested strongly induced Nur77, Nor-1, and increased Nur1 mRNA levels in the substantia nigra and ventral tegmental area. These data suggest that typical and atypical antipsychotic drugs might induce in multiple brain regions distinct Nur-dependent transcriptional activities, which may contribute to their pharmacological effects.

ABBREVIATIONS: EPS, extrapyramidal symptom; 5-HT, 5-hydroxytryptamine (serotonin); cRNA, complementary RNA; SIDL, dorsolateral striatum; StVL, ventrolateral striatum; AcSh, nucleus accumbens shell; AcC, nucleus accumbens core; StDM, dorsomedial striatum; SVM, ventromedial striatum; mPFC, medial prefrontal cortex; CA1, field CA1 of hippocampus; CA3, field CA3 of hippocampus; DG, dentate gyrus; SN, substantia nigra; VTA, ventral tegmental area; AP-1, activator protein-1; RXR, retinoid X receptor.
of both c-fos mRNA and Fos-like immunoreactivity in the dorsal lateral striatum. In contrast, atypical antipsychotics that are unlikely to generate EPS either fail to increase or produce minor elevation of c-fos or Fos-like immunoreactivity in this brain area (for review, see Herrera and Robertson, 1996; Herdegen and Leah, 1998). However, down-regulation of c-fos response is a general phenomenon that has been reported to occur with repeated exposure to a variety of treatments (Hope et al., 1994). The Fos-like family also includes two other gene products, FosB and ΔFosB (Chen et al., 1997). The ΔFosB product is remarkably resistant to degradation, and it accumulates after repeated exposure to various treatments. Indeed, chronic administration of typical and atypical antipsychotics produce similar contrasting patterns on ΔFosB immunoreactivity, as observed previously on Fos-like immunoreactivity after acute treatment (Doucet et al., 1996; Vahid-Ansari et al., 1996; Atkins et al., 1999).

Other transcription factors are closely associated with dopamine and serotonin neurotransmission. Members of the Nur family are of particular interest. They are orphan members of the nuclear receptor family of steroid/thyroid-like receptors (Giguère, 1999). Three members of the Nur family have been identified. Nur1 (NR4A2) is expressed in dopaminergic neurons of the substantia nigra and ventral tegmental area. This nuclear receptor is essential for the development and maintenance of mesencephalic dopamine neurons (Zetterström et al., 1997). However, its role in fully mature dopamine neurons remains uncertain. Nur77 (also known as nerve-growth factor inducible B) (NR4A1) and Nur-1 (neuron-derived receptor-1, NR4A3) are found in dopaminergic areas, including the striatum, nucleus accumbens, olfactory tubercle, and prefrontal and cingulate cortices (Zetterström et al., 1996b; Beaudry et al., 2000; Werme et al., 2000). We have previously shown that Nur77 (nerve-growth factor inducible B) mRNA levels can be modulated by dopamine and serotonin agonists (Gervais et al., 1999), as well as by dopamine antagonists (antipsychotic drugs) (Beaudry et al., 2000). Contrasting patterns of Nur77 expression were demonstrated after acute administration of haloperidol and clozapine (Beaudry et al., 2000; Langlois et al., 2001). Interestingly, induction of Nur77 in the striatum did not desensitize and remain present after chronic administration (Beaudry et al., 2000). Neuroleptic-induced acute parkinsonism (catalepsy) and vacuous chewing movements (similar to tardive dyskinesias) responses, which occur after prolonged exposure to antipsychotic drugs in rodents, are profoundly altered by genetic ablation of the Nur77 gene (knockout) (Éthier et al., 2004a,b). In addition, haloperidol-induced neurotensin and enkephalin expression are reduced in Nur77-deficient mice (Éthier et al., 2004a). We have also shown that those responses are dependent on the interaction of haloperidol with dopamine D2 receptors (Éthier et al., 2004a). Anti-psychotic drugs also modulate Nor-1 in the striatal complex, but its role remains mostly unexplored (Werme et al., 2000; Éthier et al., 2004a).

To better understand the relationship between Nur and antipsychotic drug effects in the central nervous system, we conducted a comprehensive evaluation of effects of various typical and atypical antipsychotics on the modulation of Nur mRNA levels. We show that differential patterns of Nur expression can be obtained with typical and atypical antipsychotics. Inductions of Nur are correlated with dopamine D2 and D3 receptor subtypes, and the 5-HT2/D2 affinity ratio can be used to predict these patterns of induction. Modulation of Nur by antipsychotics can also be used to calculate an index that is also predictive on the typical versus atypical profile of antipsychotic drugs.

Materials and Methods

Animal Care and Treatments. All procedures, including means to minimize discomfort, were reviewed and approved by the Laval University Animal Care Committee. Wild-type C57BL/6 mice (weighing 20–25 g; Charles River Canada, Montreal, PQ, Canada) were used in the present study. They were acutely treated with the different antipsychotic drugs (0.5 ml i.p.). Typical antipsychotic drugs include 0.5 mg/kg haloperidol, 3 mg/kg fluphenazine, 20 mg/kg chlorpromazine, and 2 mg/kg raclopride. Although raclopride is not used as an antipsychotic drug, it was included in the study because of its highly selective dopamine D2/D3 pharmacological profile. We also used two doses of risperidone (3 and 15 mg/kg), which have been associated with an atypical and typical profile in animal models, respectively (Marchese et al., 2004). The other atypical antipsychotic drugs investigated were 20 mg/kg clozapine, 0.5 mg/kg olanzapine, and 10 mg/kg quetiapine. The dosages were chosen on the basis of clinical equivalency (Deutch and Duman, 1996). Chlorpromazine, risperidone, raclopride, and fluphenazine were purchased form Sigma-Aldrich (St. Louis, MO). Haloperidol and clozapine were purchased from Sigma/RBI (Oakville, ON, Canada), olanzapine (Zyprexa) was from Eli Lilly & Co. (Indianapolis, IN), and quetiapine (Seroquel) was from AstraZeneca Pharmaceuticals LP (Wilmington, DE). The animals were sacrificed by decapitation under CO2 anesthesia 1 h after drug administration. After decapitation, brains were
rapidly removed and immediately immersed into cold isopentane (−40°C) for a few seconds and kept at −80°C until used.

In Situ Hybridization Procedure. Cryostat coronal brain sections (12 μm) were mounted onto Snowcoat X-tra slides (Surgipath, Winnipeg, MA, Canada) and stored at −80°C until used. Brain sections were fixed in 4% paraformaldehyde at 4°C for 20 min. Specific 35S-UTP-radiolabeled complementary RNA (cRNA) probes were used. The Nur77 probe preparation and radiolabeling have been described in detail previously (Beaudry et al., 2000; Ethier et al., 2004a). The mouse Nor-1 probe was generated from a polymerase chain reaction fragment of 393 base pairs (from nucleotide 572 to 964) subcloned into pBluescript SK+ linearized with HindIII to generate to antisense cRNA. The cRNA probe for Nurr1 stems from a 403-base pair EcoRI-BamHI fragment of the full-length mouse Nurr1 cDNA subcloned into pBluescript SK+ and linearized with XbaI. Single-stranded riboprobes were synthesized and labeled using Promega riboprobe kit (Promega, Madison, WI), 35S-UTP (PerkinElmer Life and Analytical Sciences, Woodbridge, ON, Canada) and the RNA polymerase T7. In situ hybridization of riboprobes with tissue sections were done at 56 to 58°C, overnight, in a standard hybridization buffer containing 50% formamide (Beaudry et al., 2000; Langlois et al., 2001; Ethier et al., 2004a). Tissue sections were then apposed against BiomaxMR (Eastman Kodak, New Haven, CT) radioactive sensitive films for 2 to 5 days.

Levels of radioautographic labeling on films were quantified by computerized densitometry. Digitized brain images were obtained by a charge-coupled device camera model XC-77 (Sony, Tokyo, Japan) equipped with a 60-mm f/2.8D (Nikon, Tokyo, Japan) magnification lens. Images were analyzed using the ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD). Optical densities of autoradiograms were transformed to microcurie per gram of

![Fig. 2. Representative examples of autoradiograms generated with a specific 35S-UTP-labeled Nur77 mRNA probe after in situ hybridization in mouse brain sections from vehicle- (VEH), raclopride- (RAC), typical antipsychotic, and olanzapine (OLAN, atypical)-treated animals. We performed the analysis at four different levels (see Fig. 1 for corresponding bregma levels). Messenger RNA levels of Nur77 were measured in the medial prefrontal cortex (A), nucleus accumbens (B), caudal striatum (C), and substantia nigra, ventral tegmental area, and hippocampus (D).](image-url)
Fig. 3. Histograms illustrating the effect of various typical and atypical antipsychotic drugs administration on Nur77 (left) and Nor-1 (right) mRNA levels in mouse brain. Values are expressed in percentage of change compared with vehicle-treated (VEH) animals and represent mean ± S.E.M. from five animals. Mean absolute values of control animals (VEH) of Nur77 and Nor-1 can be found in Table 1 (*, p < 0.05; **, p < 0.01; ***, p < 0.001 versus respective VEH group (#, p < 0.05 versus respective risperidone (15 mg/kg) (RIS15) group). CHLOR, chlorpromazine; CLOZ, clozapine; FLU, fluphenazine; HAL, haloperidol; OLAN, olanzapine; QUET, quetiapine; RAC, raclopride; RIS3, risperidone (3 mg/kg). See Fig. 1 for abbreviations and specific brain areas used for quantification. See Materials and Methods for doses of antipsychotics used.
tissue using $^{14}$C radioactivity standards (ARC 146A-$^{14}$C standards; American Radiolabeled Chemicals, St. Louis, MO). For Nur77 and Nor-1, brain areas investigated included the dorsolateral (StDL), dorsomedial (StDM), ventrolateral (StVL), and ventromedial (StVM) portions of the striatum, the shell (AcSh), and core of the nucleus accumbens (AcC), medial prefrontal (mPFC), and cingulate cortices (CC). Nur mRNA levels were also evaluated in CA1, CA3, and den- tate gyrus (DG) regions of the hippocampus. Nur77 mRNA levels were measured in both the substantia nigra (SN) and the ventral tegmental area (VTA). We show for the first time that that Nur77 and Nor-1 are induced in the SN and VTA after antipsychotic drug treatments. Therefore, their mRNAs were also measured in these brain areas. Figure 1 illustrates the exact coordinates and brain areas used for quantification of mRNA levels.

**Statistical Analysis.** For each animal and for all brain regions investigated, we measured Nur mRNA levels in four sections. Average signals from both brain hemispheres were made. All data were then expressed as group mean ± S.E.M. from five animals per group (except for the vehicle group, which included eight animals). Statistical comparisons were performed with StatView (SAS Institute, Cary, NC) program. Statistical analyses of mRNA level variances were performed using a one-way analysis of variance. When a significant variance analysis was reported, a Fisher’s least significant difference test was performed as post hoc analysis. Correlation analysis between antipsychotic drug inhibitory constants ($K_i$) at various neurotransmitter receptors, as well as affinity ratios, and Nur (Nur77 and Nor-1) mRNA levels were performed. Correlation coefficients were determined by least-squares linear regression, and significance was tested using the null hypothesis with GraphPad Prism version 4.0 software (GraphPad Software Inc., San Diego, CA).

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**Fig. 4.** Histograms illustrating the effect of various typical and atypical antipsychotic drugs administration on Nur77 (top), Nor-1 (middle), and Nurr1 (bottom) mRNA levels in mice hippocampal areas (CA1, CA3, and DG). Values are expressed in percentage of change compared with vehicle-treated (VEH) animals and represent mean ± S.E.M. from five animals. Mean absolute values of control animals (VEH) of Nur77, Nor-1, and Nurr1 can be found in Table 1 [* , p < 0.05 versus respective VEH group; #, p < 0.05 versus respective risperidone (15 mg/kg) (RIS15) group]. CHLOR, chlorpromazine; CLOZ, clozapine; FLU, fluphenazine; HAL, haloperidol; OLAN, olanzapine; QUET, quetiapine; RAC, raclopride; RIS3, risperidone (3 mg/kg). See Fig. 1 for abbreviations and specific brain areas used for quantification. See Materials and Methods for doses of antipsychotics used.

**Fig. 5.** Representative examples of autoradiograms generated with a specific $^{35}$S-UTP-labeled Nor-1 mRNA probe after in situ hybridization in mouse brain sections from vehicle- (VEH), raclopride- (RAC, typical antipsychotic), and olanzapine (OLAN, atypical)-treated animals. We performed the analysis at four different levels (see Fig. 1 for corresponding bregma levels). Messenger RNA levels of Nor-1 were measured in the medial prefrontal cortex (A), nucleus accumbens (B), caudal striatum (C), and substantia nigra, ventral tegmental area, and hippocampus (D).
Results

Effect of Antipsychotic Drugs on Nur77 Expression.
The basal pattern of distribution of Nur77 mRNA observed here is similar to the one previously reported in rats (Zetterström et al., 1996b; Beaudry et al., 2000; Werme et al., 2000) and mice (Ethier et al., 2004a). Significant basal expression is observed in the dorsomedial and dorsolateral portions of the striatum, the medial prefrontal and cingulate cortices, and the nucleus accumbens (Table 1; Fig. 2). A preferential expression of Nur77 is observed in the CA1, compared with CA3 and DG regions of the hippocampus (Table 1). Basal expression of Nur77 mRNA is barely detectable in the SN and VTA (Table 1; Fig. 2). Typical antipsychotic drugs induced strong Nur77 mRNA levels in the motor areas of the striatal complex, including dorsolateral, dorsomedial, ventrolateral, and ventromedial portions of the striatum (Figs. 2 and 3, left). However, effects of antipsychotics are more pronounced in the ventrolateral and ventromedial areas (Fig. 3, left). For example, haloperidol, raclopride, and the highest dose of risperidone (15 mg/kg) induced up to 9-, 5-, and 3- to 4-fold increases of Nur77 mRNA levels in the ventrolateral, ventromedial, and dorsolateral portions of the striatum, respectively. Note that the effects of chlorpromazine are always lower compared with the other typical antipsychotics. The effects of typical antipsychotics are more modest in the nucleus accumbens shell and core, as well as in the medial prefrontal and cingulate cortices; however, effects remain highly significant (Fig. 3). Huge increases of Nur77 have been observed in the SN and VTA (2000–5000-fold increases).

In general, atypical antipsychotic drugs did not significantly induce Nur77 expression in the striatum (with the exception of olanzapine, which induced a moderate increase in the STDL). The lower dose of risperidone (3 mg/kg), which can be associated with an atypical profile in animal models (Marchese et al., 2004), induced Nur77 in the striatum. However, the effects of this dose were always lower than those of the highest dose (15 mg/kg). Atypical antipsychotics induced a strong significant increase of Nur77 in the cortex and the VTA, with the exception of quetiapine. Olanzapine failed to induce Nur77 in the shell of the nucleus accumbens and cortex. It is noteworthy that a higher dose of olanzapine (5 mg/kg) produced a pattern of Nur77 expression similar to typical antipsychotic drugs (data not shown). Interestingly, the only effect of quetiapine was observed in the cortex.

With the exception of haloperidol and olanzapine, all antipsychotic drugs increased Nur77 mRNA levels in CA1 region of the hippocampus (Fig. 4). Although Nur77 levels in CA3 area were also elevated by some antipsychotics (fluphenazine, chlorpromazine, risperidone, and clozapine), the effects are of lower magnitude compared with that of the CA1 region (Fig. 4). Only risperidone and clozapine modified levels of Nur77 mRNA in the DG.

Effect of Antipsychotic Drugs on Nor-1 Expression.
Basal expression of Nor-1 mRNA in mouse forebrain is similar to that of Nur77 (Fig. 5; Table 1). However, the basal levels of Nor-1 are 5- to 10-fold lower than that of Nur77, suggesting that cells expressing Nor-1 represent a smaller subpopulation or that the number of mRNA copies per cell is lower with Nor-1. Interestingly, Nor-1 and Nur77 seem to have a complementary expression in the hippocampus, with Nor-1 being predominantly expressed in the CA3 region, whereas Nur77 displays a higher expression in the CA1 areas, compared with CA3 and DG of the hippocampus (Table 1; Figs. 2D and 5D). In contrast to Nur77, antipsychotic drugs did not alter the expression of Nor-1 in the CA1 region. Only risperidone (3 mg/kg) and clozapine increased Nor-1 levels in CA3 (Fig. 4).

The effects of typical antipsychotic drugs on the expression of Nor-1 were similar to the effect observed with Nur77 (Fig. 3, right, and Fig. 5). However, the magnitude of these effects was higher, with an average of 500- to 1500-fold increase of Nor-1 in the striatum after acute typical antipsychotic drug administration (Fig. 3). The effect of the typical antipsychotic chlorpromazine in the striatum was also somewhat lower compared with that of other typical antipsychotic drugs. The higher dose of risperidone (15 mg/kg) was also less effective to induce Nor-1 compared with the induction observed with Nur77 (Fig. 3). Typical antipsychotics also induced strong Nor-1 mRNA levels in the nucleus accumbens shell and core and moderate increases in the cortex (Fig. 3, right). The difference between the low and high dose of risperidone was less marked than for Nur77, but significant differences can be observed as well, especially in the dorsoventral area of the striatum and the core of the nucleus accumbens. Nor-1

Fig. 6. Effect of various typical and atypical antipsychotic drugs administration on Nurr1 mRNA levels. A, representative examples of autoradiograms generated with a specific 35S-UTP-labeled Nurr1 mRNA probe after in situ hybridization in mouse brain sections from vehicle (VEH), raclopride (RAC, typical antipsychotic), and olanzapine (OLAN, atypical)-treated animals. B, histograms illustrating the effect of antipsychotic drugs on Nurr1 mRNA levels in mouse SN and VTA. Values are expressed in percentage of change compared with VEH-treated animals and represent mean ± S.E.M. from five animals. Mean absolute values of control animals (vehicle) of Nurr1 can be found in Table 1 (⁎, p < 0.05; **, p < 0.01; ***p < 0.001 versus respective VEH-treated group). CHLOR, chlorpromazine; CLOZ, clozapine; FLU, fluphenazine; HAL, haloperidol; QUET, quetiapine; RIS3, risperidone (3 mg/kg); RIS15, risperidone (15 mg/kg). See Materials and Methods section for doses of antipsychotics used.
mRNA levels were strongly up-regulated in the SN and VTA with all the typical antipsychotic drugs (about 500- to 1000-fold increase). Atypical antipsychotic drugs did not induce Nor-1 mRNA levels in the striatum with the exception of a mild effect of olanzapine in the dorsolateral portion of the striatum (similarly to the effect of olanzapine on Nur77 expression in this region). Olanzapine did not induce Nor-1 (as well as Nur77) in the cortex. However, it induced a strong Nor-1 mRNA signal in the SN and VTA. Quetiapine modulated Nor-1 only in the cortex, an effect that is similar to the effect of this drug on Nur77 (Fig. 3).

**Effect of Antipsychotic Drugs on Nurr1 Expression.**
Basal Nurr1 expression is strong in the SN and VTA, as reported previously (Zetterström et al., 1996b) (Fig. 6A). Similarly to Nur77, Nurr1 is also preferentially expressed in the CA1 region of the hippocampus, compared with CA3 and DG areas (Fig. 6A). With the exception of quetiapine and chlorpromazine, all antipsychotic drugs induced Nurr1 mRNA in the VTA, whereas only typical antipsychotics induced Nurr1 in the SN (Fig. 6B). It is interesting to note that contrary to Nur77 and Nor-1 that are barely detectable in basal conditions in the SN/VTA complex but strongly induced by most of the antipsychotic drugs used here, Nurr1 mRNA expression was not induced in new brain areas after antipsychotic administration. Very few antipsychotic drugs altered Nurr1 expression in the hippocampus (Fig. 4). However, some atypical antipsychotics such as risperidone, clozapine, and quetiapine significantly increased Nurr1 levels in the DG region (Fig. 4).

**Fig. 7.** Typical index of the modulation of Nur77 (A) and Nor-1 (B) by typical and atypical antipsychotic drugs. The index is obtained by subtracting the variation of Nur levels in the AcSh from the modulation observed in the StVL induced by each antipsychotic drugs. Values are expressed in residual percentage of variation from means of StVL – AcSh Nur modulations for each drug.
Classification of Typical and Atypical Antipsychotics on the Basis of the Difference between Nur mRNA Expression in the Striatum and Nucleus Accumbens.

We compared neuroleptic-induced Nur mRNA expression in the AcSh and StVL to determine whether modulations of Nur mRNA by antipsychotics may serve as a biochemical index for their typical or atypical profile. These evaluations were based on a similar analysis performed by George Robertson and colleagues (Robertson et al., 1994), with minor modifications. We have subtracted percentages of induction of Nur by antipsychotics in the StVL and AcSh (Fig. 7). This allowed us to classify antipsychotics according to the preferential induction of Nur in the striatum. Therefore, our analysis represents a typical index, as opposed to the index calculated using Fos-like immunoreactivity, which represented an atypical index (Robertson et al., 1994). According to this analysis, we have been able to clearly delineate typical and atypical antipsychotics, with typical antipsychotics having a much stronger StVL minus AcSh difference of induction compared with atypical antipsychotics. The only exception was chlorpromazine, which reacted like an atypical antipsychotic in this analysis. The atypical antipsychotics quetiapine, olanzapine, and clozapine showed negative StVL-AcSh nur77 levels, which clearly distinguished them from typical antipsychotics. Risperidone was found to be in between typical and atypical antipsychotics (Fig. 7A). However, the lower dose of risperidone (3 mg/kg) was closer to that of atypical antipsychotics. A similar typical index was obtained with induction patterns of nor-1 expression (Fig. 7B). We have chosen the StVL area for the analysis because it represented the area where we observed the strongest effects of antipsychotics. However, similar classifications were obtained using other portions of the striatum (data not shown).

Correlations between the Modulation of Nur mRNA Levels by Antipsychotic Drugs and Their Affinity for Aminergic Neurotransmitter Receptors. To identify receptor subtypes involved in the modulation of Nur by antipsychotic drugs, we have performed correlation analysis between modulation of Nur in the StVL and AcSh and selected aminergic neurotransmitter receptors (Table 2). Induction of Nur77 in the striatum (StVL) closely correlated with the affinity of dopamine D2 receptor and histamine H1 receptor subtypes for antipsychotic drugs, whereas the correlation did not reach significance with the D3 receptor and it does not exist with the serotonin 5-HT2A receptors (Fig. 8, left) or 5-HT1A receptor subtypes (data not shown). Note that we used the log (1/Ki) affinity value transformation for performing the correlations (from Ki values presented in Table 2) for a more convenient presentation. Thus, the positive correlations observed with dopamine receptors indicate that the high affinity of antipsychotics for dopamine receptors is involved in the induction of higher levels of Nur77 mRNA, whereas a negative correlation, as seen with histamine H1 receptor subtype, should be interpreted in the opposite direction. In the AcSh, inductions of Nur77 were correlated with both D2 and D3 receptor subtypes, whereas nonsignificant correlations were observed for 5-HT2A and H1 receptor subtypes (Fig. 8, right). It is generally accepted that the affinity ratio of dopamine and serotonin receptors displayed by antipsychotic drugs is a good predictor of their typical versus atypical clinical profile (Meltzer, 1999). Indeed, affinity ratios for 5-HT2A/D2 and 5-HT1A/D2 correlated strongly with induction of Nur77 in the StVL (Fig. 9, left). The H1/D2 ratio also predicted the pattern of modulation of Nur77 by antipsychotic drugs. Correlations of affinity ratios and Nur77 induction were less important in the AcSh, and significant correlations where not reached with 5-HT2A/D2 ratio (Fig. 9, right). Similar results were obtained in ventromedial, dorsomedial, and dorsolateral parts of the striatum (data not shown).

Correlations of the induction of nor-1 expression with aminergic neurotransmitter receptor affinity of antipsychotic drugs or affinity ratios were also highly significant with the dopamine D2 and H1 receptors in the StVL and with D2 and D3 receptor subtypes in the AcSh (Fig. 10). The 5-HT2A/D2, 5-HT1A/D2, and H1/D2 ratios in the StVL also correlated strongly with levels of nor-1 inductions (Fig. 11). Induction of nur1 in the SN and VTA also correlated well with dopamine D2 receptor affinity for antipsychotic drugs (SN: r2 = 0.5275, *p = 0.0267; VTA: r2 = 0.6450, *p = 0.0091).

Discussion

The present results indicate that typical and atypical antipsychotic drug administration produces distinct patterns of induction of transcription factors of the Nur family. Typical antipsychotic drugs strongly induced Nur77 and nor-1 in striatal areas associated with the control of locomotor functions, whereas atypical drugs induced only mild Nur expression in these areas. This extends and confirms our previous study comparing haloperidol and clozapine (Beaudry et al.,

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<th>Antipsychotic</th>
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Data represent mean K values obtained from National Institute of Mental Health Psychoactive Drug Screening Program Ki database at http://pdsp.cwru.edu/pdsp.asp, Roth et al. (2003), Seeman et al. (2001), and Schwartz et al. (1995).
2000) and indicates that other antipsychotic drugs share similar properties. Although induction of Nur77 and Nor-1 in the nucleus accumbens shell and core is of lower magnitude, both typical and atypical drugs have similar effects in those limbic areas. Indeed, by subtracting Nur inductions in the striatum and nucleus accumbens, we have calculated a typical index that corresponds to the typical or atypical profile of antipsychotic drugs used in the present study. Thus, measurement of Nur levels in the striatal complex (striatum and nucleus accumbens) may have a predictive value regarding the typical or atypical profile of an antipsychotic drug. Induction of Nur77 and Nor-1 by antipsychotic drugs correlated very well with dopamine D₂ receptor affinities in the striatum and with dopamine D₂ and D₃ receptor subtypes in the nucleus accumbens. In addition, 5-HT₂A/D₂ and 5-HT₁A/D₂ affinity ratios can predict levels of inductions of Nur77 and

**Nur77**

![Fig. 8. Correlation analysis of the modulation of Nur77 in the StVL portion of the striatum (left) and the AcSh (right) with affinities at various aminergic neurotransmitter receptors, including dopamine D₂ and D₃ receptor subtypes, 5-HT₂A, and histamine H₁ receptor subtypes. Inhibitory constant (Kᵢ) values used for the analysis can be found in Table 2. They were transformed into log (1/Kᵢ) for a more convenient presentation. Linear regression analysis on transformed Kᵢ values of antipsychotic drugs at respective receptor subtypes versus percentage of variation of Nur77 mRNA levels were performed. The linear regression was performed with 95% confidence intervals. Goodness of fit is illustrated with the calculated r², and the p value statistical analysis indicates whether the slope is significantly different from zero (*, p < 0.05).](image-url)
Nor-1 by typical or atypical antipsychotics used in the present study. Finally, we showed for the first time that antipsychotic drugs might alter the transcriptional activity of dopamine neurons by inducing huge increases of Nur77 and Nor-1 and also by up-regulating Nurr1 in the SN and VTA. This suggests that the effect of both typical and atypical antipsychotic drugs at dopamine neurons in the SN/VTA complex might participate in their pharmacological properties.

These observations on the modulation of Nur by antipsychotic drugs are somewhat similar to modulations of Fos-like members that have been well documented (for review, see Herrera and Robertson, 1996; Herdegen and Leah, 1998). However, some significant differences can be noticed. First, induction of c-fos is more homogenously distributed among striatal areas compared with induction of Nur. The basal expression of Nur is higher than c-fos expression and is not evenly distributed in the striatal complex. Indeed, the most dramatic increase of Nur by typical antipsychotic drugs was observed in the ventrolateral portion of the striatum. In the nucleus accumbens, typical antipsychotics induced Nur similarly in the shell and core of the nucleus accumbens (Ethier et al., 2004a), whereas typical antipsychotics induced stronger c-fos response in the core (Robertson et al., 1994). In the cortex, antipsychotics induced higher c-fos expression in the prefrontal cortex compared with cingulate cortex, whereas both typical and atypical antipsychotics tend to induce stronger Nur mRNA levels in the cingulate cortex. Most importantly, antipsychotic drugs did not induce c-fos in dopamine neurons, whereas all antipsychotics, except quetiapine, induced very strong Nur77, Nor-1 as well as Nurr1 mRNA levels in both SN and VTA. In fact, previous reports showed that some antipsychotic drugs induced c-fos expression in the substantia nigra pars reticulata, but not in dopamine neuron (pars compacta) in vivo (Wirtshafter and Asin, 1995; Miwa et al., 1998; Cochran et al., 2002) (but see Suzuki et al., 1998). Colocalization studies with tyrosine hydroxylase and Nur77 mRNA in animal treated with haloperidol indicate that Nur77 is indeed induced in tyrosine hydroxylase-containing cell of mesencephalic dopamine neurons (D. Lévesque, unpublished data). Another important difference resides in the fact that the c-fos response rapidly desensitized

![Nur77](image-url)
and is progressively replaced by an increased in ΔFosB after chronic treatment. In the case of Nur77, we have shown that even after prolonged treatment dopamine drugs the response of Nur77 remains unchanged (Beaudry et al., 2000; St-Hilaire et al., 2003). However, similar studies need to be performed for Nurr1 and Nor-1.

We have previously shown that haloperidol induces Nur77 specifically in enkephalin-positive cells in the striatum (Beaudry et al., 2000). This is similar to what have been observed with induction of c-fos by this typical antipsychotic (Guo et al., 1998). This raises the possibility that typical antipsychotics can induce a concerted c-fos- and Nur77-dependent transcriptional activity in a subset of cells expressing enkephalin and D2 receptors, i.e., in neurons belonging to the indirect striatal output pathway. For example, it has been shown that neurotensin expression can be dependent on Fos/Jun transcriptional activity via an AP-1-responsive element present in its promoter (Kislauskis and Dobner, 1990). However, neurotensin is still up-regulated by haloperidol in c-fos knockout mice (Shearman and Weaver, 1997). On the other hand, neurotensin regulation by haloperidol is reduced by about 50% in Nur77 knockout mice (Ethier et al.,

![Graphs showing the modulation of Nor-1 in the StVL and AcSh regions of the striatum.](https://example.com/graphs.png)

**Fig. 10.** Correlation analysis of the modulation of Nor-1 in the StVL portion of the striatum (left) and the AcSh (right) with affinities at various aminergic neurotransmitter receptors, including dopamine D2 and D3 receptor subtypes, 5-HT2A, and histamine H1 receptor subtypes. Inhibitory constant (Ki) values used for the analysis can be found in Table 2. They were transformed into log (1/Ki). Linear regression analysis on transformed Ki values of antipsychotic drugs at respective receptor subtypes versus percentage of variation of Nor-1 mRNA levels were performed. The linear regression was performed with 95% confidence intervals. Goodness of fit is illustrated with the calculated r², and the p value statistical analysis indicates whether the slope is significantly different from zero (+, p < 0.05).
Then, it is conceivable that the neurotensin promoter is sensitive to both AP-1 and Nur77 transcriptional activities, which may act in synergy to fully activate the neurotensin gene promoter. Enkephalin is another putative target for such a Nur77-dependent activity. We reported recently that haloperidol-induced enkephalin expression is almost abolished in Nur77 deficient mice (Ethier et al., 2004a). Interestingly, the induction of enkephalin by haloperidol is not dependent on c-fos, despite the presence of an AP-1 regulatory element in the preproenkephalin promoter (Konradi et al., 1993). Additional experiments are needed to directly assess Nur77-dependent transcriptional activity of neurotensin and enkephalin gene promoters.

In general, the patterns of induction of Nor-1 are similar to the patterns of induction of Nur77, suggesting that Nor-1 also represents a target for antipsychotic drugs actions. Correlation of the induction of Nor-1 with D2/D3 receptors also indicates that Nor-1 is a target of intracellular signaling events associated with these dopamine receptor subtypes. However, the transcriptional activity associated with Nor-1 may be distinct from Nur77. Indeed, we have shown that Nor-1 knockout mice displayed normal cataleptic response to haloperidol, whereas this behavior is strongly altered in Nur77-deficient mice (Ethier et al., 2004a). One possible reason for this difference may reside in the fact that Nur77-dependent activity in the striatum seems to be associated with retinoid receptors (RXRs) (Ethier et al., 2004a,b). In fact, RXRs are putative heterodimerization partners for Nur77 and Nurr1, but not for Nor-1, which cannot form heterodimers with RXRs (Zetterstro¨m et al., 1996a). Thus, this suggests that Nur77 and Nor-1, although they are both induced by antipsychotic drugs, may have distinct functions or belong to different biochemical pathways associated with dopamine D2/D3 receptor signaling.

By calculating a typical index (Nur induction in the StVL – AcSh), we have classified antipsychotics drugs used in the present study into two categories that fit very well with their typical or atypical profile in clinics. One noticeable exception is chlorpromazine, which displayed an atypical profile with Nur biochemical markers. Interestingly, chlor-
prominent in both typical and atypical categories with the atypical index calculated by Robertson et al. (1994) using c-fos expression. However, based on its clinical profile, this drug should display a typical profile. The reasons for this discrepancy are unclear. It possibly reflects the peculiar pharmacological profile of this conventional neuroleptic. Although the two doses of risperidone used in the present study are somewhat high compared with clinical dosages actually used in humans (Schotte et al., 1996), they generated distinct patterns of Nur expression. The lower dose was acting more like an atypical antipsychotic, whereas the higher dose produced an effect similar to that of typical antipsychotics. In animal models, a similar high dose was associated with generation of EPS (Marchese et al., 2004). This is reminiscent to the effect of this antipsychotics reported in clinics, where EPS can be significantly induced with high dose of risperidone. We recently observed that Nur77 is associated with the production of acute parkinsonism (catalepsy) and orofacial dyskinesias (tardive dyskinesias), two very important symptoms induced by typical antipsychotics (Éthier et al., 2004a,b). In addition, we showed that Nur77 gene ablation (knockout) produced deficits associated with dopamine D2 receptor signaling (Éthier et al., 2004a). We confirm here that inductions of Nur77 by typical antipsychotic drugs in the striatum are strongly correlated with D2 receptor affinities. Induction of Nur77 and Nor-1 in the nucleus accumbens was also correlated with D3 receptor affinities, suggesting that interaction with D3 receptors might participate in effects of antipsychotic drugs in this brain area.

We are able to predict the clinical profile of antipsychotic drugs using affinity ratios for 5-HT2A/D2 and 5-HT2C/D2 and Nur mRNA levels. Indeed, we have previously shown that the pattern of c-fos expression induced by haloperidol (typical) can be transformed into a pattern resembling the one induced by clozapine (atypical) with addition of 5-HT2A-blockers (Treblay et al., 1998). Similarly, 5-HT2A drugs can also modulate Nur77 (Gervais et al., 1999). These similarities between the patterns of c-fos induction and Nur (Nur77 and Nor-1) in some brain areas suggest that antipsychotic drugs can trigger Fos- and Nur-related transcriptional activities in the same cell population. Indeed, it has been recently observed that amphetamine induced both c-fos and Nur77 in the same cells in the striatum (Backman and Morales, 2002).

The pattern of Nur induction by quetiapine is very peculiar. The only significant effects of quetiapine were observed in the cortex and the hippocampus, whereas the striatum, nucleus accumbens, SN, and VTA were not affected. This pattern of induction is in contrast to quetiapine-induced c-fos expression and ΔFosB immuneactivity patterns (Robertson et al., 1994; Vahid-Ansari et al., 1996). However, the dose of quetiapine used in the present study is somewhat lower than the doses used in previous reports. Nevertheless, a preferential induction of Nur77 in the prefrontal cortex and CA1 area of the hippocampus is suggested by the modulation of Nur expression in the present study. Interestingly, connections between the medial prefrontal cortex and CA1 region of the hippocampus that form the hippocampo-prefrontal cortex pathway are involved in the dopamine-mediated limbic circuitry (Thierry et al., 2000).

In summary, our results show that antipsychotics drugs profoundly modulate transcriptional activities of various nuclear receptors belonging to the Nur family in many brain areas associated with their therapeutic activity. This suggests that Nur-dependent transcriptional activity may play a role in antipsychotic drug-induced gene expression. These effects are related to their actions at dopamine D2/D2 receptor subtypes, and the 5-HT2A/D2 affinity ratios can predict the patterns of Nur inductions. In addition, the strong and important modulations of Nur expression in the SN and VTA suggest a direct activity of antipsychotics at mesencephalic dopamine neurons, which may participate to their pharmacological profile. Dissection of Nur transcription factor-specific functions presents an interesting challenge for future research related to the mechanism of action of antipsychotic drugs.

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