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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.

Jérôme Maheux, Isabelle Éthier, Claude Rouillard and Daniel Lévesque

Neuroscience Unit, Laval University Hospital Research Centre, CRCHUL (CHUQ), Quebec, Qc, Canada G1V 4G2.

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* Address correspondence to: Daniel Lévesque, Ph.D., Centre de recherche en Neuroscience
CHUL, RC-9800, 2705 Boul. Laurier, Sainte-Foy (Québec), Canada
G1V 4G2, Phone: (418) 654-2152, FAX: (418) 654-2753
E-mail: daniel.levesque@crchul.ulaval.ca

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ABBREVIATIONS: NGFI-B, Nerve-Growth Factor Inducible B; Nor-1, Neuron-derived receptor-1; cRNA, complementary RNA; AcC, nucleus accumbens core; AcSh, nucleus accumbens shell; CC, cingulate cortex; DG, dentate gyrus; mPFC, medial prefrontal cortex; StDL, dorsolateral striatum; StDM, dorsomedial striatum; StVL, ventrolateral striatum; StVM, ventromedial striatum; SN, substantia nigra; VTA, ventral tegmental area; RXR, retinoid X receptor; EPS, extrapyramidal symptoms; VEH, vehicle; CLOZ, clozapine; FLU, fluphenazine; CHLOR, chlorpromazine; HAL, haloperidol; OLAN, olanzapine; RIS, risperidone; QUET, quetiapine; RAC, raclopride; NS, non significant

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ABSTRACT

Monitoring gene expression has been intensively used in order 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 dopaminoceptive areas such as the striatum, nucleus accumbens and prefrontal cortex. In order to better understand the relationship between *Nurs* and antipsychotic drug effects, we conducted a comprehensive evaluation of the effect of various typical and atypical antipsychotic drugs on the modulation of *Nurs* mRNA levels. We show that differential patterns of *Nurs* 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 *Nurs* by antipsychotic drugs are correlated with dopamine D_2 receptor in the striatum and, D_2 and D_3 receptor subtypes in the nucleus accumbens. The 5-HT_{2A}/D_2 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 *Nurr1* 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.

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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 D_2 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 D_2 receptors and higher affinity for serotonin 5-HT_{2A} receptors, compared to typical drugs. In fact, a high 5-HT_{2A}/D_2 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. D_3, D_4 or 5-HT_{1A} receptors) remains a matter of debate.

Monitoring gene expression in the central nervous system (CNS) has been intensively used in order 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 (Robertson et al., 1994). Acute administration of antipsychotic drugs likely to produce EPS dramatically elevates the levels of both *c-fos* mRNA and Fos-like immunoreactivity in the dorsolateral striatum. In contrast, atypical antipsychotics which are unlikely to generate EPS either fail to increase or produce minor

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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 following repeated exposure to various treatments. Indeed, chronic administration of typical and atypical antipsychotics produce similar contrasting patterns on ΔFosB immunoreactivity, as previously observed on Fos-like immunoreactivity following 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. *Nurr1* (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 (NGFI-B)) (NR4A1) and *Nor*-1 (Neuron-derived receptor-1, NR4A3) are found in dopaminoceptive 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* (NGFI-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 following acute administration of haloperidol and clozapine (Beaudry et al., 2000; Langlois et al., 2001). Interestingly, induction of *Nur77* in the striatum did

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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 appear after prolonged exposure to antipsychotic drugs in rodents, are profoundly altered by genetic ablation of the *Nur77* gene (knockout) (Ethier et al., 2004a;b). In addition, haloperidol-induced neurotensin and enkephalin expression are reduced in *Nur77* deficient mice (Ethier et al., 2004a). We have also shown that those responses are dependent on the interaction of haloperidol with dopamine D_2 receptors (Ethier et al., 2004a). Antipsychotic drugs also modulate *Nor*-1 in the striatal complex, but its role remains mostly unexplored (Werme et al., 2000; Ethier et al., 2004a).

To better understand the relationship between *Nurs* and antipsychotic drug effects in the CNS, we conducted a comprehensive evaluation of effects of various typical and atypical antipsychotic drugs on the modulation of *Nurs* mRNA levels. We show that differential patterns of *Nurs* expression can be obtained with typical and atypical antipsychotic drugs. Inductions of *Nurs* are correlated with dopamine D_2 and D_3 receptor subtypes and the 5-HT_{2A}/D_2 affinity ratio can be used to predict these patterns of induction. Modulation of *Nurs* by antipsychotics can also be used to calculate an index that is also predictive on the typical *vs* 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 (Charles River, Canada, weighing 20-25 g) were used in the present study. They were

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acutely treated with the different antipsychotic drugs (0.5 ml, i.p.). Typical antipsychotic drugs include haloperidol (0.5 mg/kg), fluphenazine (3 mg/kg), chlorpromazine (20 mg/kg) and raclopride (2 mg/kg). Although raclopride is not used as an antipsychotic drug, it was included in the study because of its highly selective dopamine D_2/D_3 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 clozapine (20 mg/kg), olanzapine (0.5 mg/kg) and quetiapine (10 mg/kg). The dosages were chosen on the basis of clinical equivalency (Deutch and Duman, 1996). Chlorpromazine, risperidone, raclopride and fluphenazine were purchased form Sigma-Aldrich Company (St-Louis, MO). Haloperidol and clozapine were purchased from RBI (Oakville, ON, Canada), olanzapine from Lilly (Zyprexa^R) and quetiapine from AstraZeneka (Seroquel^R). The animals were sacrificed by decapitation under CO_2 anesthesia 1 hour 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^{TM} 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 [^{35}S]UTP-radiolabeled complementary RNA (cRNA) probes were used. The *Nur77* probe preparation and radiolabeling have been described in details elsewhere (Beaudry et al., 2000; Ethier et al., 2004a). The mouse *Nor*-1 probe was generated from a PCR fragment of 393 bp (from nucleotide 572 to 964) subcloned into pBluscript SK$^+$ linearized with *Hind*III to generate to antisense complementary RNA (cRNA). The cRNA probe for *Nurr1* stems from a 403 bp *Eco*RI-BamHI fragment of the full length mouse *Nurr1* cDNA subcloned into pBluescript SK+ and linearized

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with *Xba* I. Single-stranded riboprobes were synthesized and labeled using Promega riboprobe kit (Promega, Madison, WI), [^{35}S]-UTP (Perkin Elmer Inc., Canada) and the RNA polymerase T_7. *In situ* hybridization of riboprobes with tissue sections were done at 56-58°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 (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 CCD camera model XC-77 (Sony) equipped with a 60 mm f/2.8D (Nikon) magnification lens. Images were analyzed using the ImageJ software (Wayne Rasband, NIH). Optical densities of autoradiograms were transformed to µCi/g of tissue using [^{14}C] radioactivity standards (ARC 146A-^{14}C standards, American Radiolabeled Chemicals Inc., St-Louis). 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 (AcC) of the nucleus accumbens, medial prefrontal (mPFC) and cingulate (CC) cortices. *Nurs* mRNA levels were also evaluated in CA1, CA3 and dentate gyrus (DG) regions of the hippocampus. *Nurr1* 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 following 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 4 sections. Average signals from both brain hemispheres were made. All data were then expressed as group mean ± SEM from 5 animals per group (except for the vehicle

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group, which included 8 animals). Statistical comparisons were performed with "StatView" (SAS Institute) program. Statistical analyses of mRNA level variances were performed using a one-way ANOVA. When a significant variance analysis was reported, a Fisher's LSD test was performed as *post hoc* analysis. Correlation analysis between antipsychotic drug inhibitory constants (Ki) 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).

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 and Fig. 2). A preferential expression of *Nur77* is observed in the CA1, compared to CA3 and dentate gyrus (DG) regions of the hippocampus (Table 1). Basal expression of *Nur77* mRNA is barely detectable in the SN and VTA (Table 1 and 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 (Fig. 2 and 4 left panels). However, effects of antipsychotics are more pronounced in the ventrolateral and ventromedial areas (Fig. 4 left panels). For example, haloperidol, raclopride and the highest dose of risperidone (15 mg/kg)

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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 to 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. 4). Huge increases of *Nur77* have been observed in the SN and VTA (2000- to 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. 5). Althought *Nur77* levels in CA3 area was also elevated by some antipsychotics (fluphenazine, chlorpromazine, risperidone and clozapine), the effects are of lower magnitude compared to the CA1 region (Fig. 5). 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 mice forebrain is similar to *Nur77* (Fig. 3 and Table 1). However, the basal levels of *Nor*-1 are 5- to

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10-fold lower than *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, *Nor*-1 being predominantly expresses in the CA3 region, whereas *Nur77* display a higher expression in the CA1 areas, compared to CA3 and DG of the hippocampus (Table 1, Figs 2D and 3D). 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. 5).

The effects of typical antipsychotic drugs on the expression of *Nor*-1 were similar to the effect observed with *Nur77* (Figs 3 and 4, right panels). However, the magnitude of these effects was higher, with an average of 500- to 1500-fold increase of *Nor*-1 in the striatum following acute typical antipsychotic drug administration (Fig. 4). The effect of the typical antipsychotic chlorpromazine in the striatum was also somewhat lower compared to other typical antipsychotic drugs. The higher dose of risperidone (15 mg/kg) was also less effective to induce *Nor*-1 compared to the induction observed with *Nur77* (Fig. 4). Typical antipsychotics also induced strong *Nor*-1 mRNA levels in the nucleus accumbens shell and core and moderate increases in the cortex (Fig. 4, right panels). 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 mRNA levels were strongly upregulated 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

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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. 4).

Effect of antipsychotic drugs on *Nurr1* expression. Basal *Nurr1* expression is strong in the SN and VTA, as previously reported (Zetterström et al., 1996b) (Fig. 6A). Similarly to *Nur77*, *Nurr1* is also preferentially expressed in the CA1 region of the hippocampus, compared to 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. 5). However, some atypical antipsychotics such as risperidone, clozapine and quetiapine significantly increased *Nurr1* levels in the DG region (see Fig. 5).

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 nucleus accumbens shell (AcSh) and ventrolateral striatum (StVL) in order to determine if modulations of *Nurs* 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 percent of inductions of *Nurs* by antipsychotics in the StVL and AcSh (Fig. 7). This allowed us to classify antipsychotics according to the preferential induction of *Nurs* in the striatum. Therefore, our analysis represents a typical index, as opposed

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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; typical antipsychotics having a much stronger StVL minus AcSh difference of induction compared to 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 in between typical and atypical antipsychotics (Fig. 7A). However, the lower dose of risperidone (3 mg/kg) was closer to 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 *Nurs* mRNA levels by antipsychotic drugs and their affinity for aminergic neurotransmitter receptors. In order to identify receptor subtypes involved in the modulation of *Nurs* by antipsychotic drugs, we have performed correlation analysis between modulation of *Nurs* in the StVL and AcSh and selected aminergic neurotransmitter receptors (see Table 2). Induction of *Nur77* in the striatum (StVL) closely correlated with the affinity of dopamine D_2 receptor and histamine H_1 receptor for antipsychotic drugs, whereas the correlation did not reach significance with the D_3 receptor and is inexistent with the serotonin 5-HT_{2A} receptors (Fig. 8, left panels) or 5-HT_{1A} receptor subtypes (not shown). Note that we used the Log (1/Ki) affinity values 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

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dopamine receptors is involved in the induction of higher levels of *Nur77* mRNA, whereas a negative correlation, as seen with histamine H_1 receptor subtype should be interpreted in the opposite direction. In the AcSh, inductions of *Nur77* were correlated with both D_2 and D_3 receptor subtypes, whereas non significant correlations were observed for 5-HT_{2A} and H_1 receptor subtypes (Fig. 8, right panels). It is generally accepted that the affinity ratio of dopamine and serotonin receptors displayed by antipsychotic drugs is a good predictor of their typical *vs* atypical clinical profile (Meltzer, 1999). Indeed, affinity ratios for 5-HT_{2A}/D_2 and 5-HT_{1A}/D_2 correlated strongly with induction of *Nur77* in the StVL (Fig. 9, left panels). The H_1/D_2 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-HT_{2A}/D_2 ratio (Fig. 9, right panels). 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 D_2 and H_1 receptors in the StVL and with D_2 and D_3 receptor subtypes in the AcSh (Fig. 10). The 5-HT_{2A}/D_2, 5-HT_{1A}/D_2 and H_1/D_2 ratios in the StVL also correlated strongly with levels of *Nor*-1 inductions (Fig. 11). Induction of *Nurr1* in the SN and VTA also correlated well with dopamine D_2 receptor affinity for antipsychotic drugs (SN: $r^2 = 0.5275$, p value = 0.0267*; VTA: $r^2 = 0.6450$, p value = 0.0091*).

Discussion

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The present results indicate that typical and atypical antipsychotic drug administration produce 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 *Nurs* expression in those areas. This extends and confirms our previous study comparing haloperidol and clozapine (Beaudry et al., 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 have similar effects in those limbic areas. Indeed, by subtracting *Nurs* 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 D_2 receptor affinities in the striatum and with dopamine D_2 and D_3 receptor subtypes in the nucleus accumbens. In addition, $5\text{-}HT_{2A}/D_2$ and $5\text{-}HT_{1A}/D_2$ affinity ratios can predict levels of inductions of *Nur77* and *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 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 to their pharmacological properties.

These observations on the modulation of *Nurs* expression 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

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significant differences can be noticed. First, induction of *c-fos* is more homogenously distributed amongst striatal areas compared to induction of *Nurs*. The basal expression of *Nurs* is higher then *c-fos* expression and is not evenly distributed in the striatal complex. Indeed, the most dramatic increase of *Nurs* by typical antipsychotic drugs was observed in the ventrolateral portion of the striatum. In the nucleus accumbens, typical antipsychotics induced *Nurs* 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 to 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, while 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)). Co-localization studies with tyrosine hydroxylase and *Nur77* mRNA in animal treated with haloperidol indicate that *Nur77* is indeed induced in TH-containing cell of mesencephalic dopamine neurons (D. Lévesque, unpublished data). Another important difference resides in the fact that the *c-fos* response rapidly desensitized and is progressively replaced by an increased in ΔFosB following 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

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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 D_2 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* a 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., 2004a). 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 in order 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 D_2/D_3 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, while 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

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associated with retinoid receptors (RXR) (Ethier et al., 2004a;b). In fact, RXR are putative heterodimerization partners for *Nur77* and *Nurr1*, but not for *Nor*-1, which cannot form heterodimers with RXR (Zetterström 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 D_2/D_3 receptor signaling.

By calculating a typical index (*Nur* induction in the StVL *minus* AcSh), we have classified antipsychotic 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, chlorpromazine felt in between typical and atypical categories with the atypical index calculated by the group of Robertson and colleagues (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 to 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, while 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 oro-facial dyskinesias (tardive dyskinesias), two very important symptoms induced by typical antipsychotics (Ethier et al., 2004a;b). In addition, we showed that *Nur77* gene ablation (knockout) produced deficits associated with dopamine D_2 receptor signaling (Ethier et al., 2004a). We confirm here that inductions of *Nur77* by typical

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antipsychotic drugs in the striatum are strongly correlated with D_2 receptor affinities. Induction of *Nur77* and *Nor*-1 in the nucleus accumbens was also correlated with D_3 receptor affinities, suggesting that interaction with D_3 receptors might participate in effects of antipsychotic drugs in this brain area.

We are able to predict the clinical profile of antipsychotic drugs using affinities ratios for 5-HT_{2A}/D_2 and 5-HT_{1A}/D_2 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-HT drugs (Tremblay et al., 1998). Similarly, 5-HT drugs can also modulate *Nur77* (Gervais et al., 1999). These similarities between the patterns of *c-fos* induction and *Nurs* (*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 (Bäckman 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 immunoreactivity 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 effect of quetiapine in the prefrontal cortex and CA1 area of the hippocampus is suggested by the modulation of *Nurs* expression in the present study. Interestingly, connections between the medial prefrontal cortex and CA1 region of the hippocampus form the hippocampo-prefrontal cortex pathway which is involved in the dopamine-mediated limbic circuitry (Thierry et al., 2000).

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In summary, our results show that antipsychotic 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 D_2/D_3 receptor subtypes and the 5-HT_{2A}/D_2 affinity ratios can predict the patterns of *Nurs* inductions. In addition, the strong and important modulations of *Nurs* 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 factors specific functions represents an interesting challenge for future research related to the mechanism of action of antipsychotic drugs.

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This article has not been copyedited and formatted. The final version may differ from this version.

References

Atkins JB, Chlan-Fourney J, Nye HE, Hiroi N, Carlezon WAJ and Nestler EJ (1999) Region-specific induction of ΔFosB by repeated administration of typical versus atypical antipsychotic drugs. *Synapse* **33:**118-128.

Bäckman C and Morales M (2002) Acute methamphetamine administration up-regulates NGFI-B mRNA expression in the striatum: Co-localization with C-FOS immunoreactivity. *Synapse* **44:**158-165.

Beaudry G, Langlois M-C, Weppe I, Rouillard C and Lévesque D (2000) Contrasting patterns and cellular specificity of transcriptional regulation of the nuclear receptor nerve growth factor-inducible B by haloperidol and clozapine in the rat forebrain. *J Neurochem* **75:**1694-1702.

Casey DE (1991) Neuroleptic drug-induced extrapyramidal syndromes and tardive dyskinesia. *Schizophr Res* **4:**109-120.

Chen J, Kelz MB, Hope BT, Nakabeppu Y and Nestler EJ (1997) Chronic Fos-related antigens: stable variants of ΔFosB induced in brain by chronic treatments. *J Neurosci* **17:**4933-4941.

Cochran SM, McKerchar CE, Morris BJ and Pratt JA (2002) Induction of differential patterns of local cerebral glucose metabolism and immediate-early genes by acute clozapine and haloperidol. *Neuropharmacology* **43:**394-407.

Deutch AY and Duman RS (1996) The effects of antipsychotic drugs on Fos protein expression in the prefrontal cortex cellular localization and pharmacological characterization. *Neuroscience* **70:**377-389.

Doucet JP, Nakabeppu Y, Bédard PJ, Hope BT, Nestler EJ, Jasmin BJ, Chen JS, Iadarola MJ, St-Jean M, Wigle N, Blanchet P, Grondin R and Robertson GS (1996) Chronic alterations in

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 20, 2024

dopaminergic neurotransmission produce a persistent elevation of ΔFosB-like protein(s) in both the rodent and primate striatum. *Eur J Neurosci* **8:**365-381.

Ethier I, Beaudry G, St-Hilaire M, Milbrandt J, Rouillard C and Lévesque D (2004a) The transcription factor NGFI-B (*Nur77*) and retinoids play a critical role in acute neuroleptic-induced extrapyramidal effect and striatal neuropeptide gene expression. *Neuropsychopharmacology* **29:**335-346.

Ethier I, Kagechika H, Shudo K, Rouillard C and Lévesque D (2004b) Docosahexaenoic acid reduces haloperidol-induced dyskinesias in mice: Involvement of *Nur77* and retinoid receptors. *Biol Psychiatry* **56:**522-526.

Gervais J, Soghomonian J-J, Richard D and Rouillard C (1999) Dopamine and serotonin interactions in the modulation of the expression of the immediate-early transcription factor, nerve growth factor-inducible B, in the striatum. *Neuroscience* **91:**1045-1054.

Giguère V (1999) Orphan nuclear receptors: from gene to function. *Endocr Rev* **20:**689-725.

Guo NN, Vincent SR and Fibiger HC (1998) Phenotypic characterization of neuroleptic-sensitive neurons in the forebrain: Contrasting targets of haloperidol and clozapine. *Neuropsychopharmacology* **19:**133-145.

Herdegen T and Leah JD (1998) Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Res Brain Res Rev* **28:**370-490.

Herrera DG and Robertson HA (1996) Activation of *c-fos* in the brain. *Prog Neurobiol* **50:**83-107.

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS and Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* **13:**1235-1244.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 20, 2024

JPET Fast Forward. Published on December 22, 2004 as DOI: 10.1124/jpet.104.080184
This article has not been copyedited and formatted. The final version may differ from this version.

Kislauskis E and Dobner PR (1990) Mutually dependent response elements in the *cis*-regulatory region of the neurotensin/neuromedin N gene integrate environmental stimuli in PC12 cells. *Neuron* **4:**783-795.

Konradi C, Kobierski LA, Nguyen TV, Heckers S and Hyman SE (1993) The cAMP-response-element-binding protein interacts, but Fos protein does not interact, with the proenkephalin enhancer in rat striatum. *Proc Natl Acad Sci USA* **90:**7005-7009.

Langlois M-C, Beaudry G, Zekki H, Rouillard C and Lévesque D (2001) Impact of antipsychotic drug administration on the expression of nuclear receptors in the neocortex and striatum of the rat brain. *Neuroscience* **106:**117-128.

Marchese G, Bartholini F, Casu MA, Ruiu S, Casti P, Congeddu E, Tambaro S and Pani L (2004) Haloperidol versus risperidone on rat "early onset" vacuous chewing. *Behav Brain Res* **149:**9-16.

Meltzer HY (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* **21 (Suppl.):**S106-S115.

Miwa H, Nishi K, Fuwa T and Mizuno Y (1998) Globus pallidus lesions inhibit the induction of *c-fos* by haloperidol in the basal ganglia output nuclei in rats. *Neurosci Lett* **250:**29-32.

Robertson GS, Matsumura H and Fibiger HC (1994) Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. *J Pharmacol Exp Ther* **271:**1058-1066.

Roth BL, Sheffler D and Potkin SG (2003) Atypical antipsychotic drug actions: unitary or multiple mechanisms for 'atypicality"? *Clin Neurosci Res* **3:**108-117.

Schotte A, Janssen PFM, Gommeren W, Luyten WHML, Van Gompel P, Lesage AS, De Loore K and Leysen JE (1996) Risperidone compared with new and reference antipsychotic drugs: *in vitro* and *in vivo* receptor binding. *Psychopharmacology* **124:**57-73.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 20, 2024

JPET Fast Forward. Published on December 22, 2004 as DOI: 10.1124/jpet.104.080184
This article has not been copyedited and formatted. The final version may differ from this version.

Schwartz J-C, Diaz J, Lévesque D, Pilon C, Dimitriadou V, Griffon N, Lammers CH, Martres M-P and Sokoloff P (1995) The D_2-like subfamily of dopamine receptors, in *Schizophrenia: An Integrated View* (Fog R, Gerlach J, Hemmingsen R, Krogsgaard-Larsen P and Thaysen JH eds) pp 251-262, Copenhagen.

Seeman P (2001) Antipsychotic drugs, dopamine receptors, and schizophrenia. *Clin Neurosci Res* **1:**53-60.

Serretti A, De Ronchi D, Lorenzi C and Berardi D (2004) New antipsychotics and schizophrenia: A review on efficacy and side effects. *Curr Med Chem* **11:**343-358.

Shearman LP and Weaver DR (1997) Haloperidol regulates neurotensin gene expression in striatum of *c-fos*-deficient mice. *Mol Brain Res* **47:**275-285.

St-Hilaire M, Landry É, Lévesque D and Rouillard C (2003) Denervation and repeated L-DOPA induce a coordinate expression of the transcription factor NGFI-B in striatal projection pathways in hemi-parkinsonian rats. *Neurobiol Dis* **14:**98-109.

Suzuki M, Sun YJ, Murata M and Kurachi M (1998) Widespread expression of Fos protein induced by acute haloperidol administration in the rat brain. *Psychiatry Clin Neurosci* **52:**353-359.

Thierry A-M, Gioanni Y, Dégénétais E and Glowinski J (2000) Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus* **10:**411-419.

Tremblay PO, Gervais J and Rouillard C (1998) Modification of haloperidol-induced pattern of *c-fos* expression by serotonin agonists. *Eur J Neurosci* **10:**3546-3555.

Vahid-Ansari F, Nakabeppu Y and Robertson GS (1996) Contrasting effects of chronic clozapine, Seroquel(TM) (ICI 204,636) and haloperidol administration of ΔFosB-like immunoreactivity in the rodent forebrain. *Eur J Neurosci* **8:**927-936.

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JPET Fast Forward. Published on December 22, 2004 as DOI: 10.1124/jpet.104.080184
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Wadenberg MLG, Soliman A, VanderSpek SC and Kapur S (2001) Dopamine D_2 receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. *Neuropsychopharmacology* **25:**633-641.

Werme M, Ringholm A, Olson L and Brené S (2000) Differential patterns of induction of NGFI-B, *Nor*-1 and *c-fos* mRNAs in striatal subregions by haloperidol and clozapine. *Brain Res* **863:**112-119.

Wirtshafter D and Asin KE (1995) Dopamine antagonists induce Fos-like-immunoreactivity in the substantia nigra and entopeduncular nucleus of the rat. *Brain Res* **670:**205-214.

Zetterström RH, Solomin L, Jansson L, Hoffer BJ, Olson L and Perlmann T (1997) Dopamine Neuron Agenesis in *Nurr1*-Deficient Mice. *Science* **276:**248-250.

Zetterström RH, Solomin L, Mitsiadis T, Olson L and Perlmann T (1996a) Retinoid X receptor heterodimerization and developmental expression distinguish the orphan nuclear receptors NGFI-B, *Nurr1*, and *Nor1*. *Mol Endocrinol* **10:**1656-1666.

Zetterström RH, Williams R, Perlmann T and Olson L (1996b) Cellular expression of the immediate-early transcription factors *Nurr1* and NGFI-B suggests a gene regulatory role in several brain regions including the nigrostriatal dopamine system. *Mol Brain Res* **41:**111-120.

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JPET Fast Forward. Published on December 22, 2004 as DOI: 10.1124/jpet.104.080184
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Footnotes

There is no conflict of interest related to the data presented in this work.

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Figure legends

Figure 1. Schematic illustration of the mice brain areas selected for quantitative analysis of changes in *Nurs* expression. Hatched boxes indicate the sampled areas in the medial prefrontal cortex (mPFC) (A), nucleus accumbens shell (AcSh) and Core (AcC) and cingulate cortex (CC) (B), in dorsomedial (StDM), dorsolateral (StDL), ventromedial (StVM) and ventrolateral (StVL) quadrants of the striatum (C) and in substantia nigra (SN), ventral tegmental area (VTA) and CA1, CA3 and dentate girus (DG) regions of the hippocampus (D).

Figure 2. Representative examples of autoradiograms generated with a specific [^{35}S]UTP-labeled *Nur77* mRNA probe after *in situ* hybridization in mice 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). Messsenger RNA levels of *Nur77* were measured in the medial prefrontal cortex (A), nucleus accumbens (B), caudal striatum (C) and substantia nigra and ventral tegmental area (D).

Figure 3. Representative examples of autoradiograms generated with a specific [^{35}S]UTP-labeled *Nor-1* mRNA probe after *in situ* hybridization in mice 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). Messsenger RNA levels of *Nor*-1 were measured in the medial prefrontal cortex (A), nucleus accumbens (B), caudal striatum (C) and substantia nigra and ventral tegmental area (D).

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Figure 4. Histograms illustrating the effect of various typical and atypical antipsychotic drugs administration on *Nur77* (left panels) and *Nor*-1 (right panels) mRNA levels in mice brain. Values are expressed in percent change compared to vehicle-treated (VEH) animals and represent mean ± SEM from 5 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 and *** p< 0.001 *vs* respective vehicle-treated group (VEH); # p<0.05 vs risperidone (15 mg/kg) (RIS15) respective group). Abbreviations: HAL, haloperidol; RAC, raclopride; FLU, fluphenazine; CHLOR, chlorpromazine; RIS3, risperidone (3 mg/kg); CLOZ, clozapine; OLAN, olanzapine; QUET, quetiapine; NS, non significant. See Fig. 1 for abbreviations and specific brain areas used for quantification. See Material and Methods section for doses of antipsychotics used.

Figure 5. Histograms illustrating the effect of various typical and atypical antipsychotic drugs administration on *Nur77* (top panel), *Nor*-1 (middle panel) and *Nurr1* (lower panel) mRNA levels in mice hippocampal areas (CA1, CA3 and dentate gyrus, DG). Values are expressed in percent change compared to vehicle-treated (VEH) animals and represent mean ± SEM from 5 animals. Mean absolute values of control animals (VEH) of *Nur77*, *Nor*-1 and *Nurr1* can be found in Table 1 (* p<0.05 *vs* respective vehicle-treated group (VEH); # p<0.05 vs risperidone (15 mg/kg) (RIS15) respective group)). Abbreviations: HAL, haloperidol; RAC, raclopride; FLU, fluphenazine; CHLOR, chlorpromazine; RIS3, risperidone (3 mg/kg); CLOZ, clozapine; OLAN, olanzapine; QUET, quetiapine. See Fig. 1 for abbreviations and specific brain areas used for quantification. See Material and Methods section for doses of antipsychotics used.

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Figure 6. Effect of various atypical and atypical antipsychotic drugs administration on *Nurr1* mRNA levels. (A) Representative examples of autoradiograms generated with a specific [^{35}S]UTP-labeled *Nurr1* mRNA probe after *in situ* hybridization in mice 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 mice substantia nigra (SN) and ventral tegmental area (VTA). Values are expressed in percent change compared to vehicle-treated (VEH) animals and represent mean ± SEM from 5 animals. Mean absolute values of control animals (vehicle) of *Nurr1* can be found in Table 1 (* $p<0.05$, ** $p<0.01$ and *** $p< 0.001$ vs respective vehicle-treated group (VEH)). Abbreviations: HAL, haloperidol; FLU, fluphenazine; CHLOR, chlorpromazine; RIS3, risperidone (3 mg/kg); RIS15, risperidone (15 mg/kg); CLOZ, clozapine; QUET, quetiapine; NS, non significant. See Material and Methods section for doses of antipsychotics used.

Figure 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 shell of nucleus accumbens (AcSh) from the modulation observed in the ventrolateral portion of the striatum (StVL) induced by each antipsychotic drugs. Values are expressed in residual percent of variation from means of StVL *minus* AcSh *Nurs* modulations for each drug.

Figure 8. Correlation analysis of the modulation of *Nur77* in the ventrolateral (StVL) portion of the striatum (left panels) and the shell of nucleus accumbens (AcSh) (right panels) with affinities at various amiergic neurotransmitter receptors, including dopamine D_2 and D_3 receptor subtypes, serotonin 5-HT_{2A} and histamine H_1 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

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presentation. Linear regression analysis on transformed Ki values of antipsychotic drugs at respective receptor subtypes *vs* percentage of variation of *Nur77* mRNA levels were performed. The linear regression was performed with 95% confidence intervals. Goodness of fits is illustrated with the calculated r squared (r^2) and the P value statistical analysis indicates if the slope is significantly different from zero (indicated by an asterisk, $p < 0.05$).

Figure 9. Correlation analysis of the modulation of *Nur77* in the ventrolateral (StVL) portion of the striatum (left panels) and the shell of nucleus accumbens (AcSh) (right panels) with affinity ratios of antipsychotics for $5\text{-HT}_{2A}/D_2$, $5\text{-HT}_{1A}/D_2$ and H_1/D_2 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 *vs* percentage of variation of *Nur77* mRNA levels were performed. The linear regression was performed with 95% confidence intervals. Goodness of fits is illustrated with the calculated r squared (r^2) and the P value statistical analysis indicates if the slope is significantly different from zero (indicated by an asterisk, $p< 0.05$).

Figure 10. Correlation analysis of the modulation of *Nor*-1 in the ventrolateral (StVL) portion of the striatum (left panels) and the shell of nucleus accumbens (AcSh) (right panels) with affinities at various amiergic neurotransmitter receptors, including dopamine D_2 and D_3 receptor subtypes, serotonin 5-HT_{2A} and histamine H_1 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 *vs* percentage of variation of *Nor*-1 mRNA levels were performed. The linear regression was performed with 95% confidence intervals. Goodness of fits is illustrated with the calculated r

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squared (r^2) and the P value statistical analysis indicates if the slope is significantly different from zero (indicated by an asterisk, $p<0.05$).

Figure 11. Correlation analysis of the modulation of *Nor*-1 in the ventrolateral (StVL) portion of the striatum (left panels) and the shell of nucleus accumbens (AcSh) (right panels) with affinity ratios of antipsychotics for $5\text{-HT}_{2A}/D_2$, $5\text{-HT}_{1A}/D_2$ and H_1/D_2 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 *vs* percentage of variation of *Nor*-1 mRNA levels were performed. The linear regression was performed with 95% confidence intervals. Goodness of fits is illustrated with the calculated r squared (r^2) and the P value statistical analysis indicates if the slope is significantly different from zero (indicated by an asterisk, $p<0.05$).

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Table 1. Means of absolute values for *Nurs* mRNA levels in vehicle-treated animals in brain areas analyzed.

Brain areas	mRNA levels (µCi/g tissue)		
	Nur77	*Nor*-1	*Nurr1*
StDL	1.325 ± 0.172	0.087 ± 0.021	-
StDM	2.095 ± 0.301	0.250 ± 0.047	-
StVL	0.420 ± 0.038	0.019 ± 0.007	-
StVM	0.456 ± 0.059	0.068 ± 0.018	-
AcSh	1.337 ± 0.126	0.312 ± 0.035	-
AcC	0.882 ± 0.157	0.108 ± 0.016	-
CC	5.035 ± 0.659	0.438 ± 0.054	-
mPFC	3.994 ± 0.717	0.458 ± 0.033	-
SN	0.067 ± 0.007	0.015 ± 0.003	0.859 ± 0.040
VTA	0.033 ± 0.005	0.009 ± 0.002	0.687 ± 0.012
CA1	4.827 ± 1.008	0.898 ± 0.037	0.782 ± 0.017
CA3	1.281 ± 0.093	1.455 ± 0.020	0.348 ± 0.016
DG	0.448 ± 0.077	0.435 ± 0.028	0.151 ± 0.021

Values represent mean ± SEM from 8 vehicle-treated animals.

Abbreviations are: AcC, nucleus accumbens core; AcSh, nucleus accumbens shell; CA1, field CA1 of hippocampus; CA3, field CA3 of hippocampus; CC, cingulate cortex; DG, dentate gyrus; mPFC, medial prefrontal cortex; SN, substantia nigra; StDM, dorsomedial striatum; StDL, dorsolateral striatum; StVM, ventromedial striatum; StVL, ventrolateral striatum; VTA, ventral tegmental area.

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Table 2. Selected aminergic neurotransmitter receptor affinities for antipsychotic drug used in the present study.

	Inhibitory constants (Ki) of animergic receptor subtypes (nM)					
Antipsychotics	D_1[c]	D_2[a]	D_3[b,d]	5-HT_{2A}[b]	5-HT_{1A}[c]	H_1[a]
Typical						
Haloperidol	83	2.4	15	60	1202	4160
Fluphenazine	24	0.6	0.2	3.8	145	67
Chlorpromazine	112	6.7	6	2	3115	0.18
Raclopride	>10000	1.15	4	4400	>10000	8430
Atypical						
Risperidone	267	1.65	3.6	0.2	427	27
Clozapine	189	187	270	4	105	0.23
Olanzapine	58	31	23	3.4	2063	0.65
Quetiapine	712	700	520	135	431	2.2

Data represent mean Ki values obtained from:

[a] Roth et al., 2003 (Roth et al., 2003)

[b] Seeman et al., 2001 (Seeman, 2001)

[c] NIMH Psychoactive Drug Screening Program Ki database: http://pdsp.cwru.edu/pdsp.asp

[d] Schwartz et al., 1995 (Schwartz et al., 1995)

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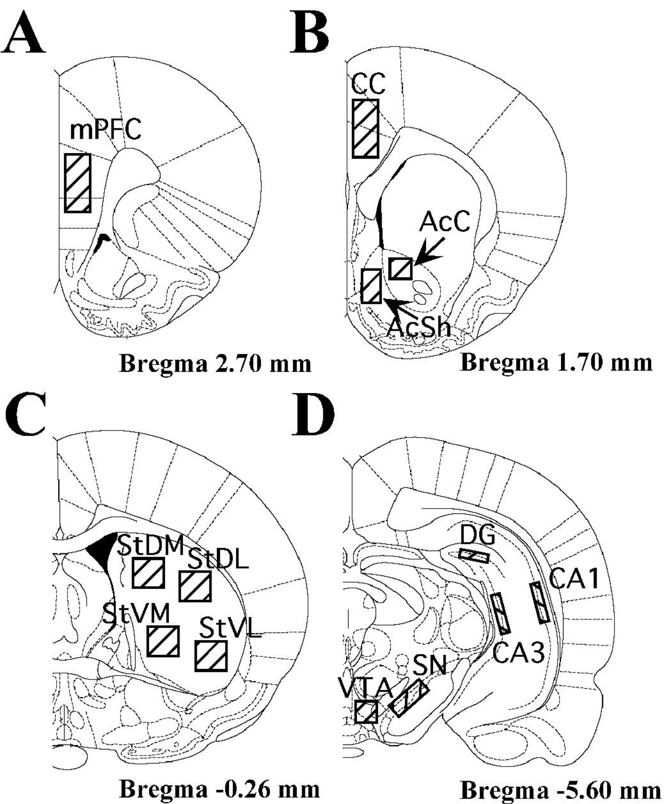


Figure 1

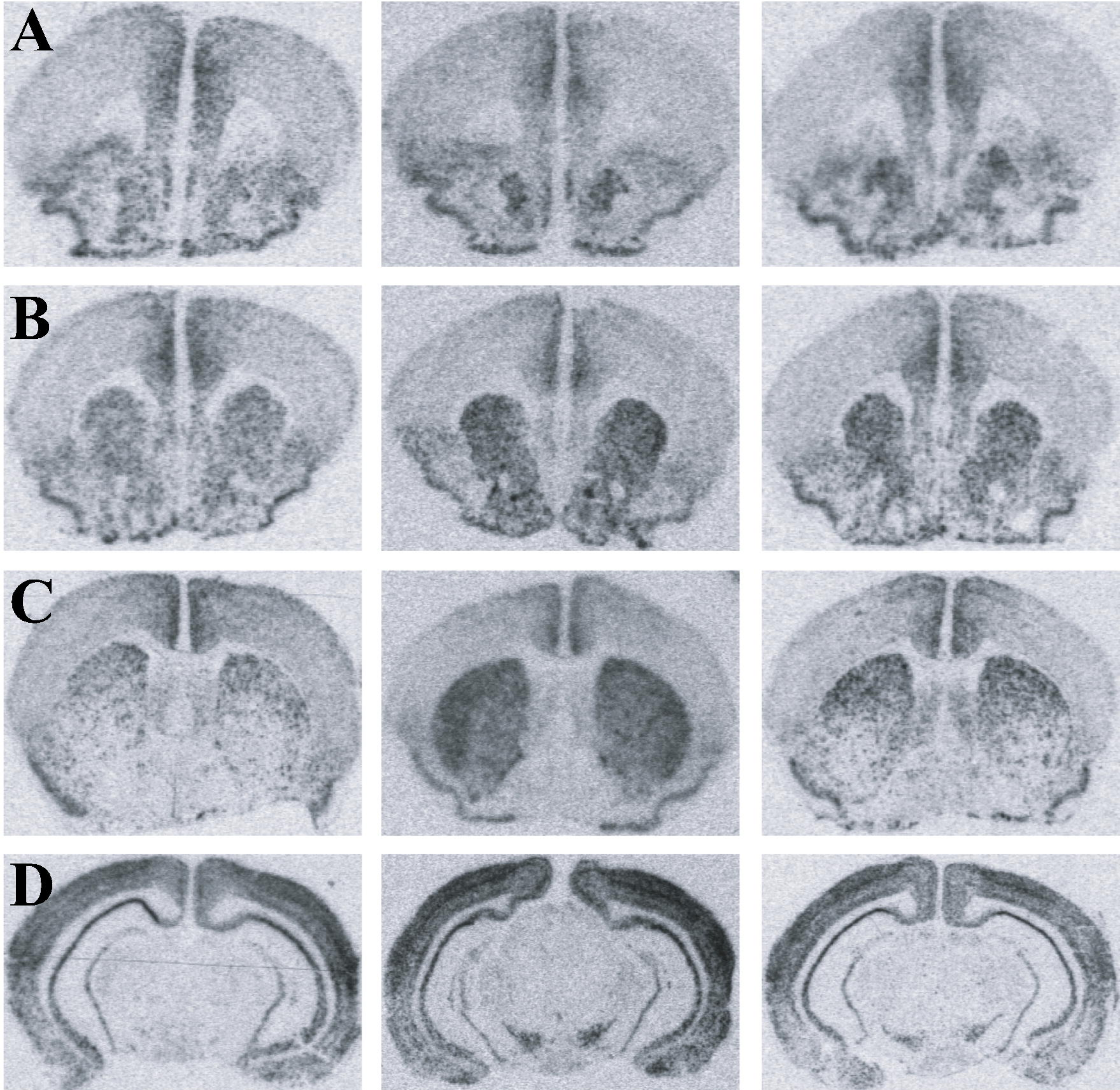
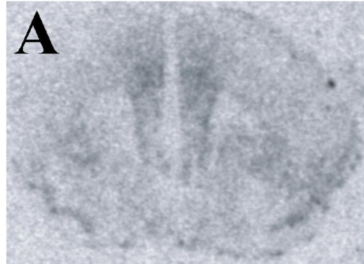
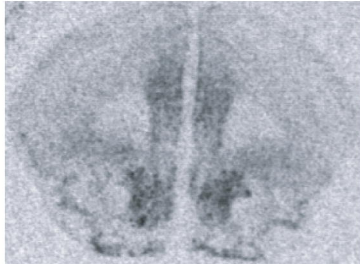
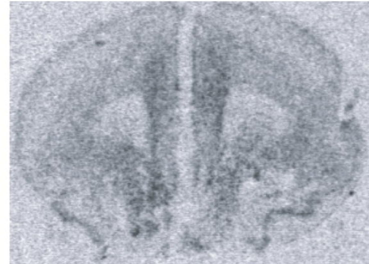
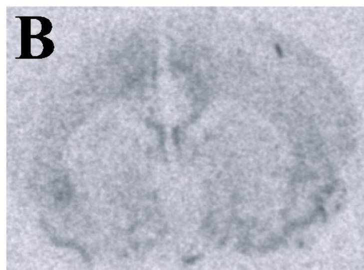


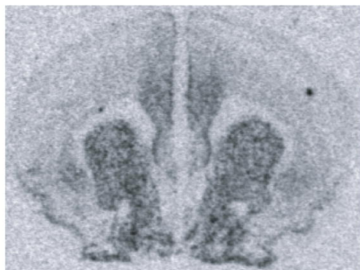
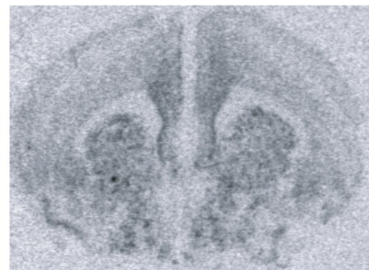
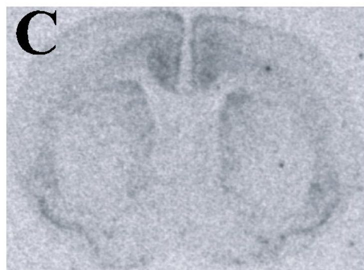
Figure 2

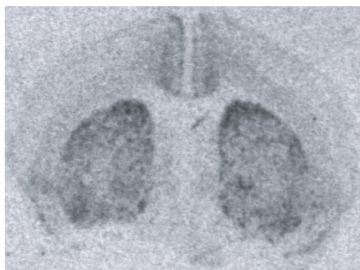
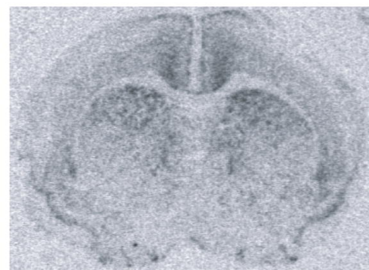
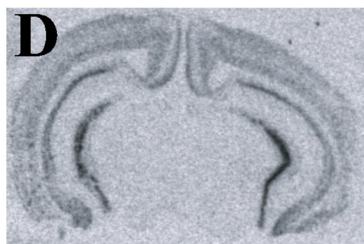
Nor-1

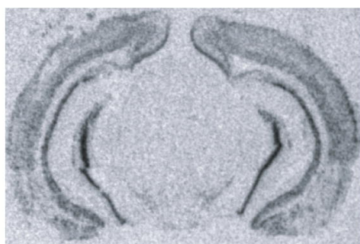
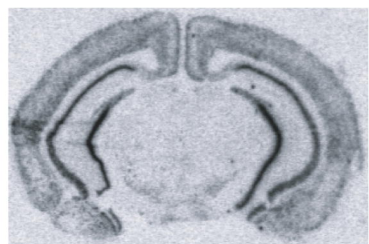
VEH **RAC** **OLAN**



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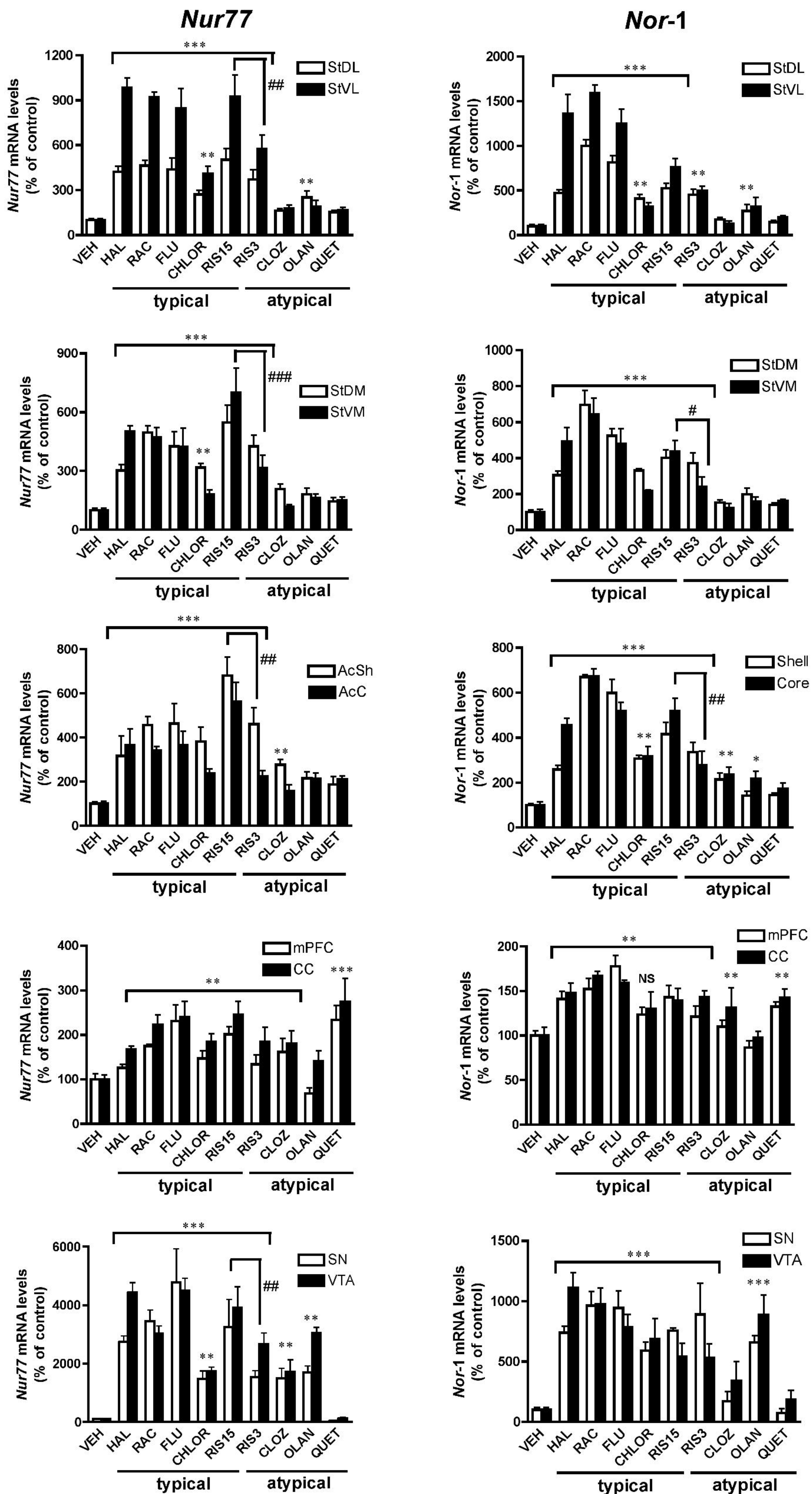

Figure 4

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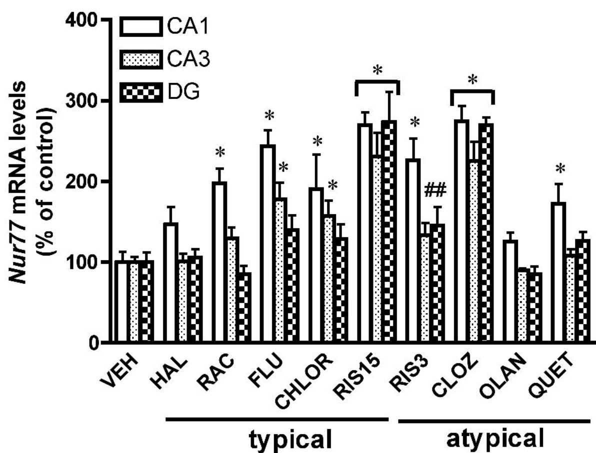
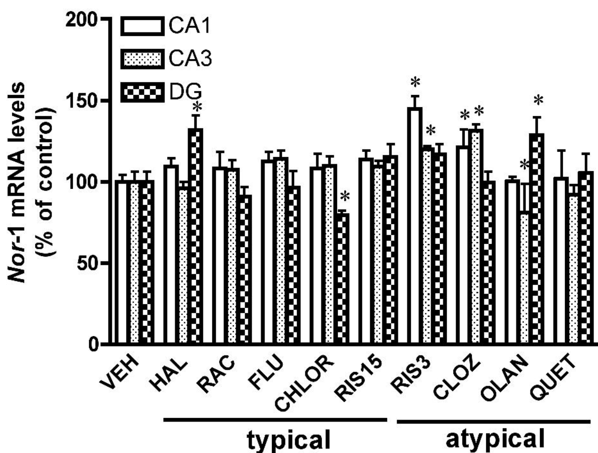




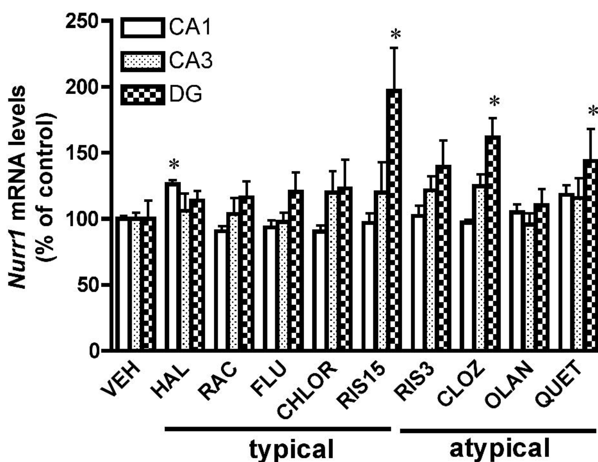


Figure 5

A

VEH RAC OLAN

B

SN
VTA

***

Nurr1 mRNA levels (% of control)

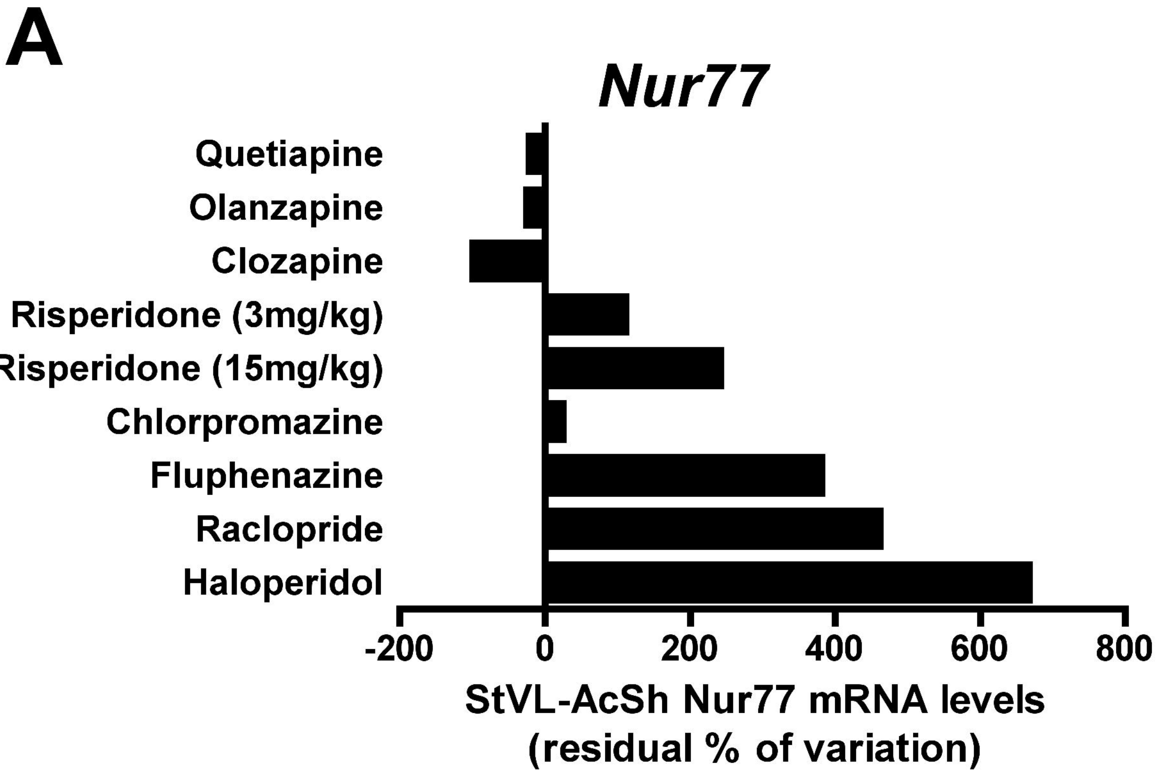
200
150
100
50
0

NS
*
*

VEH HAL RAC FLU CHLOR RIS15 RIS3 CLOZ OLAN QUET

typical atypical

Figure 6



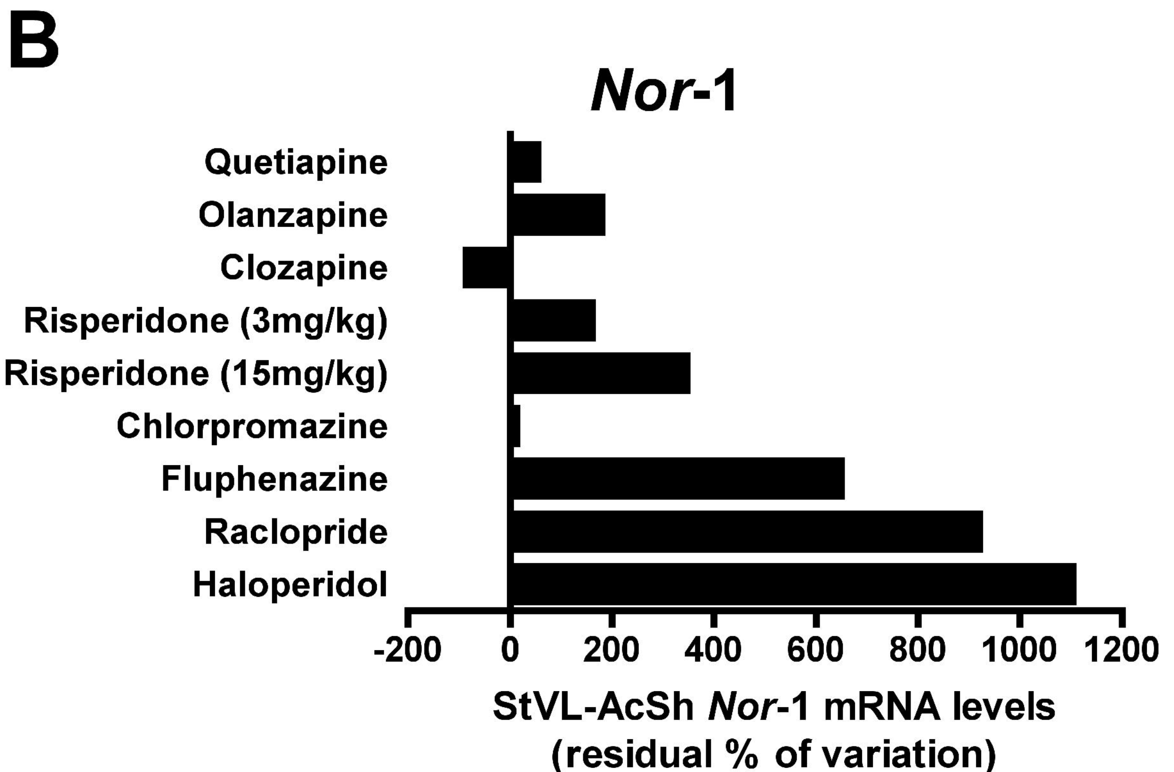
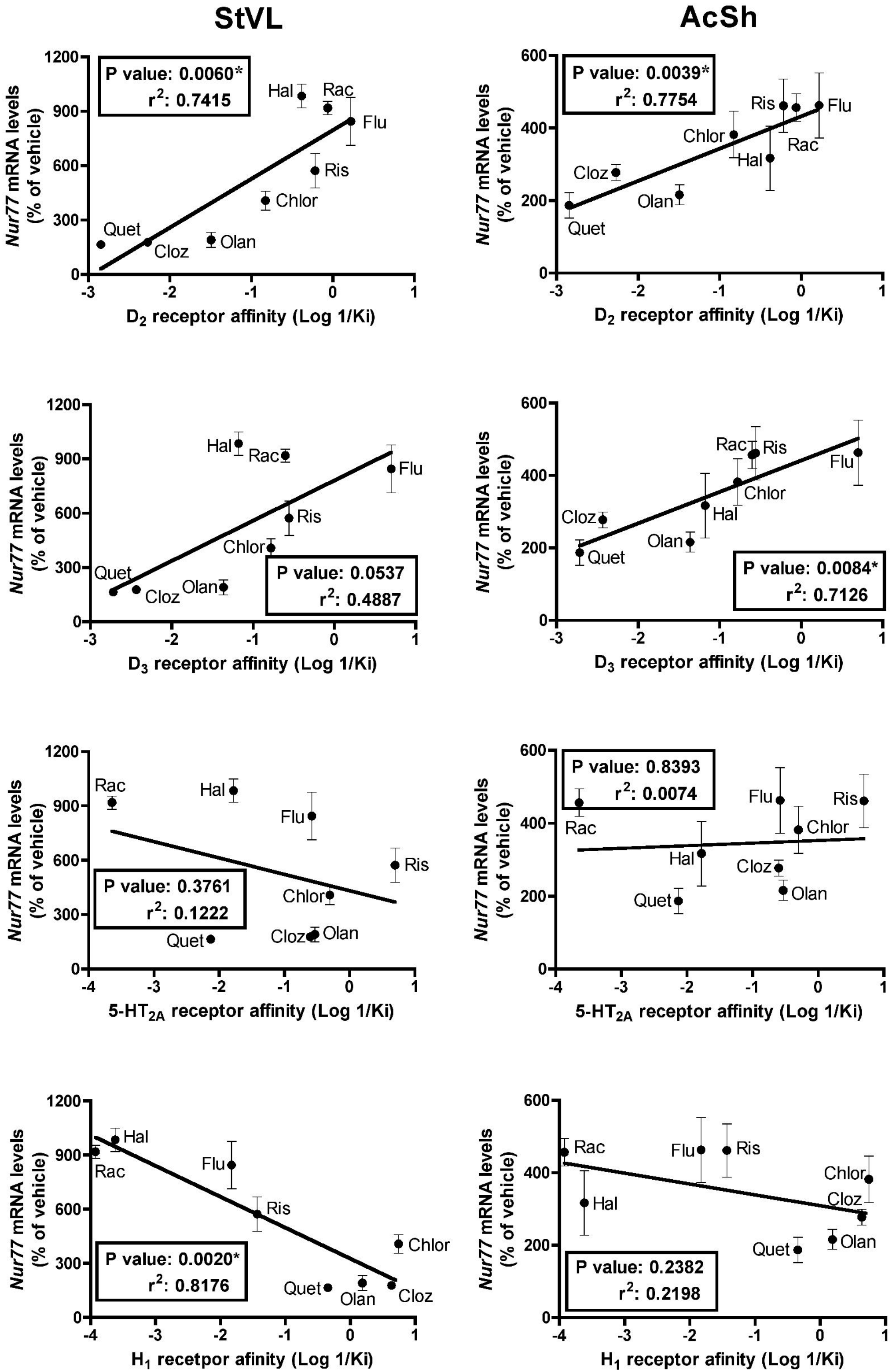


Figure 7

Nur77



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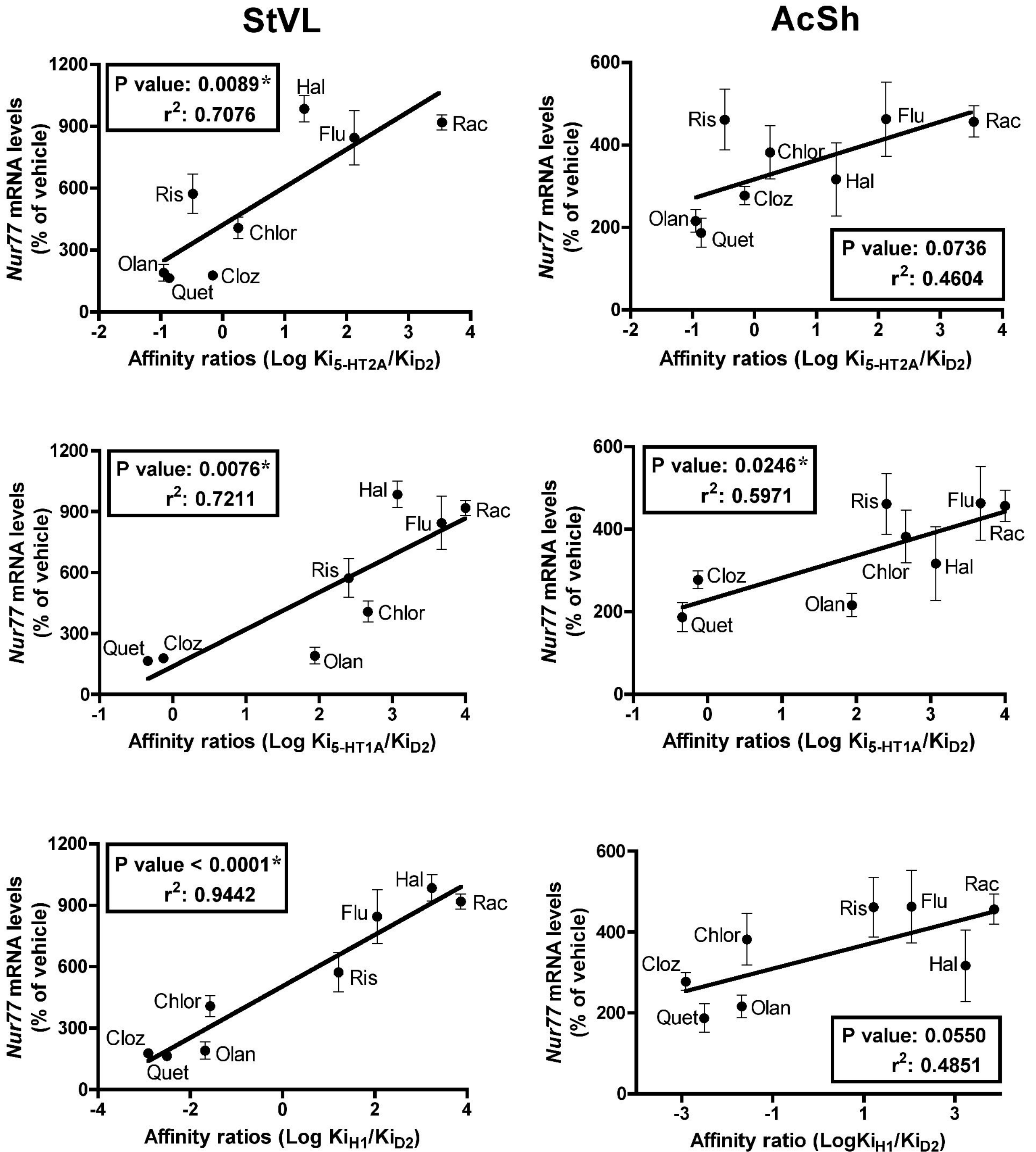

Figure 9

Maheux et al., JPET

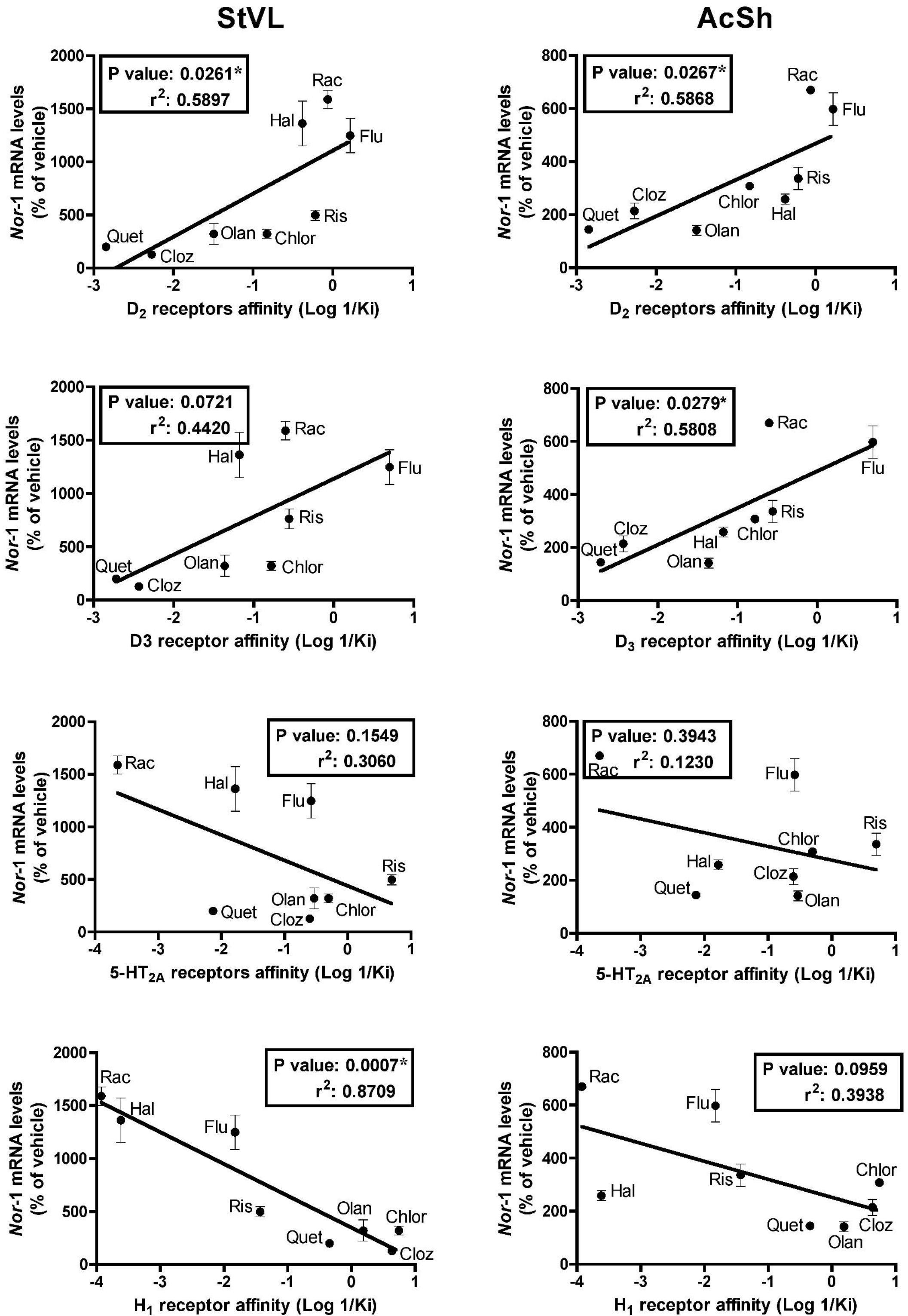

Figure 10

Nor-1

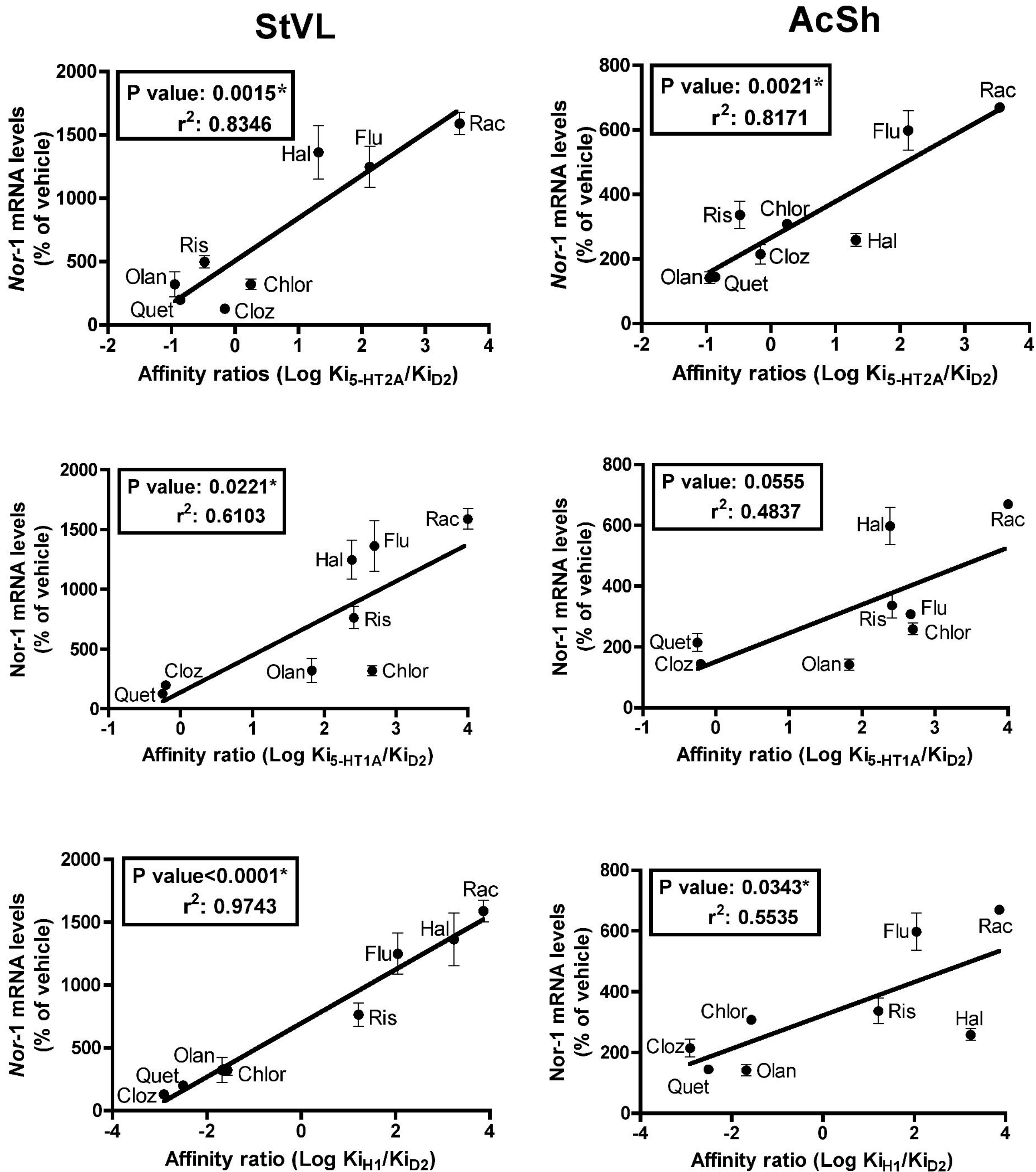

Figure 11