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Modulation of haloperidol-induced patterns of the transcription factor *Nur77* and *Nor-1* expression by serotonergic and adrenergic drugs in the mouse brain

Jérôme Maheux[1], **Laura Vuillier**[1], **Mylène Mahfouz**[1], **Claude Rouillard**[2,3], and **Daniel Lévesque**[1]

[1]Faculté de Pharmacie, Université de Montréal, Montréal, Qc H3C 3J7, Canada

[2]Département de Psychiatrie et Neuroscience, Faculté de Médecine, Université Laval, Canada

[3]Neuroscience, Centre de Recherche du CHUQ (CRCHUQ), Québec, Qc, Canada

Abstract

Different patterns of expression of the transcription factors of *Nur77* and *Nor-1* are induced following acute administration of typical and atypical antipsychotic drugs. The pharmacological profile of atypical antipsychotics suggests that serotonergic and/or adrenergic receptors might contribute to these reported differences. In order to test this possibility, we examined the abilities of serotonin 5-HT_{1A} and 5-$HT_{2A/2C}$, and α_1- and α_2-adrenergic receptor drugs to modify the pattern of *Nur77* (NR4A1) and *Nor*-1 (NR4A3) mRNA expression induced by haloperidol. Various groups of mice were treated with either saline, DOI, a 5-$HT_{2A/2C}$ agonist, MDL11939, a 5-HT_{2A} antagonist, 8-OH-DPAT, a 5-HT_{1A} agonist, prazosin, an α_1-adrenergic antagonist and idazoxan, an α_2-adrenergic antagonist, alone or in combination with haloperidol. The 5-$HT_{2A/2C}$ agonist DOI alone significantly increased *Nur77* expression in the medial striatum and nucleus accumbens. DOI reduced *Nor-1* expression, while MDL11939 increased the expression of this transcript in the cortex. Prazosin reduced *Nur77* expression in the dorsal striatum and nucleus accumbens. Interestingly, 8-OH-DPAT and MDL11939 partially prevented haloperidol-induced *Nur77* up-regulation, while MDL11939 completely abolished *Nor*-1 expression in the striatum. In addition, MDL11939 decreased haloperidol-induced *Nur77* and *Nor-1* mRNA levels in the ventral tegmental area. On the contrary, idazoxan (α_2 antagonist) consistently potentiated haloperidol-induced *Nur77*, but not *Nor-1* mRNA levels in the striatum, whereas prazosin (α_1 antagonist) remained without effect. Taken together, these results show the ability of a 5-HT_{1A} agonist or a 5-HT_{2A} antagonist to reduce haloperidol-induced *Nur77 and Nor-1* striatal expression, suggesting that these serotonin receptor subtypes participate in the differential pattern of gene expression induced by typical and atypical antipsychotic drugs.

Address for correspondence: Dr D. Levesque, Faculty of Pharmacy, University of Montreal, Pavillon Jean Coutu, C.P. 6128 Succursale Centre-Ville, Montreal, Qc H3C 3J7, Canada. Tel. : 1-514-343-6339 Fax : 1-514-343-7073, daniel.levesque.1@umontreal.ca.

Statement of Interest

None.

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Introduction

Nuclear receptors are a vast family of proteins that regulate gene transcription. These receptors provide multicellular organisms with a means to directly control gene expression in response to a wide range of developmental and physiological cues, as well as to internal and environmental stimuli. The NR4A subgroup is constituted of three closely related receptors (collectively called *Nurs*) ; i.e. *Nur77* [NR4A1; also known as nerve-growth-factor inducible gene B (NGFI-B) and TR3], *Nurr1* (NR4A2) and *Nor-1* (NR4A3) (for review, see Maxwell & Muscat, 2005). *Nurs* are classified as early response genes and are induced by a wide range of stimuli, including growth factors, cytokines, peptide hormones, stress and neurotransmitters. Their ability to sense and rapidly respond to changes in the cellular environment appears to be a hallmark of this subgroup. The NR4A subgroup members are expressed in various part of the brain, but a close neuroanatomical association can be observed with the dopamine system. Basal expression of *Nurr1* is found in the hippocampus, hypothalamus, cortex and most notably in midbrain areas containing dopamine producing neurons, i.e. the substantia nigra (SN) and ventral tegmental area (VTA) (Gofflot *et al.* 2007; Zetterström *et al.* 1996). Contrary to *Nurr1*, which is enriched in the mesencephalon, *Nur77* and *Nor-1* are mainly expressed in the forebrain, i.e. the olfactory bulb and tubercle, cortex, striatum, nucleus accumbens, hippocampal formation, hypothalamus and amygdala (Beaudry *et al.* 2000; Gofflot *et al.* 2007; Ponnio & Conneely, 2004; Werme *et al.* 2000; Zetterström *et al.* 1996). Their expression patterns suggest an involvement in various brain functions including cognition, emotion, reward, motivated behaviour, learning, memory and locomotion. Unfortunately, only a few of these functions have been thoroughly investigated so far.

Although gene targets for *Nur77* and *Nor-1* were not systematically characterized in the central nervous system, some evidence strongly suggest that neuropeptides known to be related to the dopamine system such as enkephalin and neurotensin might represent transcriptional targets for *Nur77* (Ethier *et al.* 2004*a*; St-Hilaire *et al.* 2006). In addition, we have shown that catechol-*O*-methyltransferase (COMT) mRNA levels and activity are reduced in *Nur77*-deficient mice, suggesting that *Nur77* could modulate COMT expression and participates in the control of enzymatic degradation of dopamine (Gilbert *et al.* 2006).

Antipsychotic drugs currently used in the treatment of schizophrenia can be classified as either typical or atypical antipsychotic drugs or neuroleptics. Typical neuroleptics such as haloperidol have a high propensity to cause a variety of extrapyramidal motor symptoms (Casey, 1991). New-generation atypical antipsychotics such as clozapine and olanzapine are defined as drugs active in the treatment of schizophrenia but with a lesser propensity to induce motor symptoms (Serretti *et al.* 2004). Haloperidol, a typical neuroleptic, increased *Nur77* and *Nor-1* mRNA levels in the striatum, a brain region involves in the control of locomotion (Beaudry *et al.* 2000; Maheux *et al.* 2005; Werme *et al.* 2000). Using *Nur77* and

Nor-1 induction patterns by various typical and atypical antipsychotic drugs, we have shown that modulation of *Nur77* and *Nor*-1 mRNA levels can be used to calculate an index predictive of the typical *vs.* atypical profile of antipsychotic drugs (Maheux *et al.* 2005). Inductions of *Nurs* (*Nur77* and *Nor-1*) can be correlated with dopamine D_2 and D_3 receptor affinities and serotonin (5-HT) 5-HT_{2A}/D_2 affinity ratios could also be used to predict *Nur77* and *Nor-1* patterns of expression. Interestingly, *Nur77* mRNA up-regulation is maintained upon chronic typical antipsychotic drug treatments without any apparent desensitization, suggesting that *Nur77* not only participates in the initiation of a neuroadaptive signalling cascade, but also in more prolonged effects (Beaudry *et al.* 2000; Langlois *et al.* 2001). As opposed to *Nurr1*, mRNA levels of *Nur77* and *Nor-1* are extremely low in the SN and VTA in basal conditions in the adult brain (Maheux *et al.* 2005). However, their expression can be significantly increased in these brain areas by administration of both typical and atypical antipsychotic drugs (Maheux *et al.* 2005). These data strongly suggest that *Nur77* and *Nor-1* expression is tightly regulated in central dopamine systems (for a review, see Lévesque & Rouillard, 2007).

It is generally recognized that the atypical profile of antipsychotic drugs is associated with their additional interaction with 5-HT receptor subtypes, i.e. blockade of 5-HT_{2A} and agonism at the 5-HT_{1A} receptors (Ichikawa & Meltzer, 2000; Ichikawa *et al.* 2001; Meltzer & Huang, 2008; Meltzer *et al.* 2003). These pharmacological properties are thought to be responsible for their lower propensity to generate extrapyramidal motor side-effects. Indeed, blockade of 5-$HT_{2A/2C}$ or activation of 5-HT_{1A} in combination with administration of a typical neuroleptic was shown to prevent haloperidol-induced catalepsy (Ohno *et al.* 2008, 2009). Interaction of antipsychotic drugs with 5-HT_{2A} and/or 5-HT_{1A} receptors has also been shown to increase cortical dopamine release (Ichikawa & Meltzer, 2000; Ichikawa *et al.* 2001; Meltzer & Huang, 2008; Meltzer *et al.* 2003), suggesting that the effects on 5-HT receptor subtypes might also contribute to their antipsychotic clinical profile in addition to reducing motor side-effects.

Most antipsychotic drugs also interact with α_1- and α_2-adrenergic receptor subtypes. Although recent reports indicates that *Nur77* and *Nor-1* can be modulated by β-adrenergic receptor activity in the periphery (Maxwell & Muscat, 2005; Myers *et al.* 2009; Pearen *et al.* 2008) and by adrenergic drugs in the pineal gland (Humphries *et al.* 2004), no data is available on the modulation of *Nur77* and *Nor-1* by selective α-adrenergic receptors in the central nervous system. While most antipsychotic drugs display a strong α_1-adrenergic receptor interaction, some atypical antipsychotic drugs such as clozapine and risperidone also display high affinity for the α_2-adrenergic receptor subtype (Bymaster *et al.* 1996; Schotte *et al.* 1996). Interestingly, α_2-adrenergic receptor blockade enhanced cortical dopamine release, reduced dopamine D_2 receptor antagonist-induced conditioned avoidance response, in addition to reversing haloperidol-induced catalepsy (Hertel *et al.* 1999; Invernizzi *et al.* 2003; Wadenberg *et al.* 2007). Thus, α_2-adrenergic receptors might also contribute to the clinical profile of these atypical antipsychotic drugs.

Distinct patterns of immediate-early gene (IEG) modulation have also been associated with the respective clinical profiles of typical and atypical antipsychotic drugs (Beaudry *et al.* 2000; Maheux *et al.* 2005; Robertson *et al.* 1994; Werme *et al.* 2000). An important number

of studies on the effects of typical and atypical antipsychotics on the region-specific expression of IEG, such as Fos and *Nur* families, have been performed. Specifically, cortical IEG expression by atypical antipsychotics, such as clozapine, has been speculated to be indicative of beneficial effects against negative symptoms of schizophrenia, whereas typical antipsychotics, such as haloperidol, induce striatal IEG expression, an effect that is thought to be related to the extrapyramidal side-effects liability of dopamine D_2 receptor antagonists (Bruins Slot *et al.* 2009; Maheux *et al.* 2005; Merchant & Dorsa, 1993; Robertson *et al.* 1994).

Since *Nur77* and *Nor-1* are distinctly modulated by typical and atypical antipsychotic drugs (Maheux *et al.* 2005) and *Nur77* is closely related to antipsychotic drug motor effects such as catalepsy (acute Parkinsonism) and tardive dyskinesia (Ethier *et al.* 2004*a*, *b*), we conducted a series of pharmacological investigations aimed at exploring the contribution of serotonergic and adrenergic receptor subtypes in the pattern of expression *Nur77* and *Nor-1* induced by the conventional antipsychotic drug haloperidol. We report that 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors contribute significantly to the gene expression patterns induced by this antipsychotic drug.

Materials and methods

Animals

The experiments were performed on male wild-type C57BL/6 mice (Charles River, Canada) weighing 20–25 g. Animals were housed in groups of five per cage. They were maintained on a 12-h light/dark cycle (lights on 07:00 hours) under controlled temperature (24 °C) and humidity (40–50%). Food and drinking water were available *ad libitum*. Handling of mice was performed in accordance with the Canadian Guide for the Care and Use of Laboratory Animals and all procedures, including means to minimize discomfort, were approved by the institutional Animal Care Committee of University of Montreal. In order to minimize the possibility of stress-induced *Nur* expression, mice were handled for about 3 d prior to the day of experiment and we initiated strict environmental controls on all experimental procedures, including the use of one animal handler and injecting animals within the same room. After drug administration, the animals were kept in their home cage until anaesthetized and sacrificed.

Drugs

A 5 mg/ml solution of haloperidol was obtained commercially from Sabex Inc. (Canada) and diluted in saline to its final concentration. (±)DOI-HCl [(±) [1-(2,5-dimethoxy-4-iodophenyl)-aminopropane]-hydrochloride], (±)-8-OH-DPAT-HBr [8-hydroxy-2-(di-*n*-propylamino)-tetralin]-hydrobromide], prazosin and idazoxan were obtained from Sigma-Aldrich Canada Inc. (Canada). MDL11939 (*α*-phenyl-1-(2-phenylethyl)-4-piperidine-methanol), was purchased from Tocris Bioscience (USA).

Experimental protocols

Mice were acutely treated intraperitoneally (i.p.) with the different drugs in a final volume of 0.5 ml. Two series of experiments were performed in this study. Each group of animals

consisted of five mice. For serotonin agents, treatments were: saline, DOI, a 5-$HT_{2A/C}$ agonist (2.5 mg/kg), 8-OH-DPAT, a selective 5-HT_{1A} agonist (2.5 mg/kg), MDL11939, a 5-HT_{2A} antagonist (2 mg/kg), haloperidol, a typical antipsychotic drug (0.5 mg/kg), DOI +haloperidol, 8-OH-DPAT+ haloperidol and MDL11939+haloperidol. For adrenergic agents, treatments were: saline, prazosin, a selective α_1-adrenergic antagonist (1 mg/kg), idazoxan, a selective α_2-adrenergic antagonist (1 mg/ kg), haloperidol (0.5 mg/kg), prazosin +haloperidol and idazoxan+haloperidol. All serotonergic or adrenergic drugs and saline were administered 30 min before saline (control groups) or haloperidol, so that all animals had received two injections. The animals were sacrificed by decapitation under CO_2 anaesthesia 1 h after saline (controls) or haloperidol drug administration. All drug dosages and the time of sacrifice were based on data in the literature showing modulations of *Nur77*, *Nor-1* or other IEGs, such as c-*fos*, and preliminary work from our laboratory (Beaudry *et al.* 2000; Gervais *et al.* 1999; Maheux *et al.* 2005; Marcus *et al.* 2005; Tremblay *et al.* 1998; Wadenberg *et al.* 2000). 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 (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-radiolabelled complementary RNA (cRNA) probes were used. The *Nur77* probe preparation and radiolabelling have been described in detail elsewhere (Beaudry *et al.* 2000; Ethier *et al.* 2004*a*). The mouse *Nor-1* probe was generated from a PCR fragment of 393 bp (from nucleotides 572 to 964) subcloned into pBluscript SK $^{+}$ linearized with *Hin*dIII to generate to antisense cRNA (Maheux *et al.* 2005). Single-stranded riboprobes were synthesized and labelled using Promega riboprobe kit (Promega, USA), [^{35}S]UTP (PerkinElmer Inc., Canada) and the RNA polymerase T_7. *In-situ* hybridization of riboprobes with tissue sections was performed at 56–58 °C, overnight, in a standard hybridization buffer containing 50% formamide (Beaudry *et al.* 2000; Ethier *et al.* 2004*a*; Langlois *et al.* 2001; Maheux *et al.* 2005). Tissue sections were then apposed against BiomaxMR (Kodak, USA) radioactive sensitive films for 2–5 d.

Quantification analysis

Levels of radioautographic labelling 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 analysed using the Image J 1.43u software (Wayne Rasband, NIH). Optical densities of autoradiograms were transformed to nCi/g of tissue using [^{14}C] radioactivity standards (ARC 146A-^{14}C standards, American Radiolabelled Chemicals Inc., USA). Brain areas investigated included the dorsolateral (StDL), dorsomedial (StDM), ventrolateral (StVL) and ventromedial (StVM) portions of the striatum, the shell (NAcS) and core (NAcC) of the nucleus accumbens, medial prefrontal cortex (mPFC), cingulate cortex (CC), substantia nigra pars compacta (SNc) and VTA. 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 on four different 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. Homogeneity of variances was first determined with Bartlett's χ^2 test, and square root or log data transformation was performed to increase homogeneity when necessary. Statistical analyses of mRNA level variances were performed using a one-way ANOVA. When a significant variance analysis was observed, Tukey's test was performed as *post-hoc* analysis. Statistical analyses and graphs were performed with GraphPad Prism version 4.0 software (GraphPad Software Inc., USA).

Results

As previously reported, there is significant basal expression of *Nur77* and *Nor-1* mRNA in the mouse forebrain including the StDM, StDL, mPFC, CC and NAc. Interestingly, *Nur77* and *Nor-1* mRNA are barely detectable in the SN/VTA complex in untreated animals (Maheux *et al.* 2005; Zetterström *et al.* 1996). As previously observed, haloperidol induced strong upregulations of *Nur77* and *Nor-1* mRNA levels in the StVL and StVM and in the SN/VTA complex (Maheux *et al.* 2005). In this study, haloperidol only upregulated *Nor*-1 in the PFC and CC. The absolute *Nur77* and *Nor-1* mRNA values in the vehicle-treated animals expressed in nCi/g of tissue are presented in Table 1. These values have been used to determine % of control levels presented in the following figures and tables.

Effects of serotonergic drugs on haloperidol-induced *Nur* expression

Treatment of animals with the 5-$HT_{2A/2C}$ agonist DOI alone increased *Nur77* mRNA levels in the NAc (Table 2) and medial striatum (Fig. 2, left panels). However, DOI remained without effect on haloperidol-induced *Nur77* mRNA levels in all brain areas investigated (Fig. 2, Table 2). Administration of the 5-HT_{1A} agonist 8-OH-DPAT alone did not alter *Nur77* expression, but significantly reduced haloperidol-induced *Nur77* mRNA levels in the striatum (except in the StVM portion) and NAcS (Fig. 2, Table 2). The 5-HT_{2A} antagonist MDL11939 also remained inactive when administered alone, except for a small increase in the CC (Table 2). But, it significantly reduced haloperidol-induced *Nur77* expression in the striatum, except for the StDM subterritory (Fig. 2). MDL11939 also decreased haloperidol-induced *Nur77* expression in the VTA, but remained without effect in the SNc (Table 2). We did not observe any significant modulation of *Nur77* in the PFC or CC (Table 2).

DOI alone significantly reduced *Nor-1* mRNA levels in the PFC and CC, whereas MDL11939 significantly up-regulated *Nor-1* expression in the cortex (Table 3). However, DOI did not alter haloperidol-induced *Nor-1* expression in any of the brain areas investigated (Fig. 3, Table 3). The 5-HT_{1A} agonist 8-OH-DPAT alone did not alter the expression of *Nor-1* in the brain area investigated (Fig. 3, Table 3). However, pre-treatment with the 5-HT_{1A} agonist was able to reduce haloperidol-induced *Nor*-1 mRNA levels in many brain areas, including the cortex, VTA and striatum (Fig. 3, Table 3). The most striking effects were observed with pre-treatment with the 5-HT_{2A} antagonist MDL11939, which strongly

reduced or totally prevented haloperidol-induced *Nor-1* in all the brain areas investigated, except for the SNc (Fig. 3, Table 3).

Effects of adrenergic drugs on haloperidol-induced *Nur* expression

Treatment with prazosin (an α_1-adrenergic antagonist) alone significantly reduced basal *Nur77* mRNA levels in the NAc (Table 4) and dorsal striatum (Fig. 4, top panels), while idazoxan (an α_2-adrenergic antagonist) had no effect in all brain regions investigated (Fig. 4, Table 4). On the other hand, pre-treatment with idazoxan potentiated haloperidol-induced *Nur77* expression in the SNc, NAcS and all striatal subterritories (Fig. 4, Table 4). Pre-treatment with prazosin also induced a potentiation of haloperidol-induced *Nur77* mRNA levels in the VTA (Table 4) and a small but significant increase in the NAcC (Table 4). But, prazosin had no effect on haloperidol-induced *Nur77* expression in all striatal subterritories (Fig. 4).

Contrary to *Nur77* expression, adrenergic drugs had no effect on basal or haloperidol-induced *Nor-1* expression (Fig. 5), except for the α_2-adrenergic antagonist idazoxan, which potentiated haloperidol-induced *Nor-1* expression in the NAcS (Table 5). Idazoxan also selectively reduced haloperidol-induced *Nor-1* in the VTA, whereas prazosin remained without effect (Table 5).

Discussion

We and others have previously shown that typical and atypical antipsychotic drugs induced distinct patterns of expression of *Nur77* and *Nor-1*mRNA levels in brain areas related to their clinical efficacy, i.e. the PFC, CC, NAc, striatum and SN/VTA complex (Beaudry *et al.* 2000; Bruins Slot *et al.* 2009; Maheux *et al.* 2005; Werme *et al.* 2000). These transcription factors are strongly up-regulated in striatal areas associated with locomotor functions by typical antipsychotic drugs, whereas atypical drugs induced only mild effects in these areas. Given the preferentially high affinity of atypical antipsychotics for some 5-HT receptors and most notably for 5-HT_{1A} and 5-HT_{2A} receptors, it has been postulated for decades that 5-HT and its receptors are key mediators for the actions of atypical antipsychotics (Meltzer, 1999). In support of this hypothesis, we show here that a 5-HT_{1A} receptor agonist or a 5-HT_{2A} antagonist can reduce haloperidol-induced *Nur77* and *Nor-1* mRNA up-regulation in the striatum. As previously demonstrated with c-*fos* expression (Bruins Slot *et al.* 2009; Ohno *et al.* 2008; Tremblay *et al.* 1998), the present results demonstrate that it is possible to transform the pattern of expression of *Nur77* and *Nor-1* induced by haloperidol into a pattern resembling that induced by atypical antipsychotics simply with the addition of drugs targeting these 5-HT receptor subtypes. We also demonstrate that adrenergic receptor blockade is not involved. On the contrary, α_2-adrenergic receptor blockade potentiates the effect of haloperidol on *Nur77* mRNA levels in the striatum.

Interestingly, our results suggest that both 5-HT_{2A} and α_1-adrenergic receptors, but not 5-HT_{1A} receptors, are involved in *Nur* expression in basal conditions. Briefly, basal expression of *Nur77* in the striatum is modulated by 5-HT_{2A} and α_1-adrenergic drugs, whereas basal expression of *Nor-1* in the cortex is modulated only by 5-HT_{2A} receptors. Concomitant activation of serotonin 5-HT_{2A} receptors has no effect, but blockade of 5-HT_2 receptors

strongly reduced haloperidol-induced *Nur77* expression in the ventral striatum, VTA, and almost totally abolished haloperidol-induced expression of *Nor-1* in all the brain areas investigated, except within the SN. The regionally selective effect of MDL11939 (5-HT_{2A} antagonist) might be explained in part by the medioventral to dorsolateral gradients of expression of this 5-HT_2 receptor subtype (Appel *et al.* 1990; Laprade *et al.* 1996). The α_1-adrenergic antagonist prazosin had no effect on haloperidol-induced *Nur77* expression, whereas the α_2-adrenergic receptor antagonist idazoxan potentiated haloperidol-induced *Nur77* expression in the NAcS, the entire striatum and SN. No effect was observed on *Nor-1* expression. Activation of 5-HT_{1A} receptors had no effect on basal expression of *Nur77* and *Nor-1*. However, when these nuclear receptors are up-regulated by haloperidol, both 5-HT_{1A} agonism or 5-HT_{2A} antagonism can be involved, whereas adrenergic receptors are not. On the other hand, haloperidol-induced *Nur77* and *Nor-1* expression are further up-regulated in the NAcS by a 5-HT_{1A} agonist. Taken together these results on gene transcription are consistent with the beneficial effect of 5-HT_{1A} and 5-HT_{2A} receptor subtypes in reducing dopamine nigrostriatal pathway activity, while promoting dopamine mesolimbic pathway activity.

Therefore, our results clearly indicate that 5-HT receptors contribute to the distinct transcriptional patterns induced by typical and atypical antipsychotic drugs. But how? There are at least two possibilities. First, it could be an indirect effect through the modulation of dopamine release. Indeed, there is considerable evidence obtained from microdialysis and electrophysiological studies that 5-HT_{2A} receptor antagonists modulate differentially nigrostriatal, mesolimbic and mesocortical dopamine systems (Meltzer *et al.* 2003). Administration of selective D_2 antagonists to rodents produces large increases in extracellular dopamine concentrations in the striatum and NAc and only a modest one in the PFC (Meltzer *et al.* 2003). Administration of a selective 5-HT_{2A} receptor antagonist alone had little effect on dopamine release in any of these brain regions. However, when the 5-HT_{2A} and the D_2 antagonists are combined, there is an increase in dopamine release in the PFC, but no change in the striatum (Andersson *et al.* 1995; Liégeois *et al.* 2002; Westerink *et al.* 2001). These differential effects on regional dopamine release are thought to be involved in both gene expression patterns and in the differential clinical profile of atypical antipsychotic drugs. A second possibility is a direct effect on gene expression pattern via their effects on various receptor subtypes. We and others have previously demonstrated that *Nur77* and *Nor-1* can be modulated by 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors (present data and Bruins Slot *et al.* 2009; Gervais *et al.* 1999). We show here that DOI, a 5-$HT_{2A/2C}$ agonist, administered alone increased *Nur77* in NAc and medial striatum (limbic portions of the striatal complex), while it reduced *Nor-1* in the PFC and CC. On the other hand, MDL11939, a preferential antagonist of the 5-HT_{2A} receptor, increased *Nor-1* in cortical areas. This strongly suggests that *Nur77* and *Nor-1* are differentially modulated by 5-HT neurotransmission in the mouse forebrain. However, DOI did not alter haloperidol-induced *Nur77* and *Nor-1* expression patterns. This indicates that activation of 5-HT_2 receptors participates in the tonic basal expression of *Nur77*, but it is the blockade of 5-HT_2 that is responsible for the reduction of haloperidol-induced *Nur77* expression. In addition, haloperidol-induced *Nor-1* mRNA levels are strongly modulated by 5-HT antagonists in striatal and extra-striatal brain areas, suggesting that this transcription factor is particularly

sensitive to manipulation of 5-HT neurotransmission. It is interesting to note that vector delivery of a *Nor-1* short-hairpin RNA (shRNA) in the brain ameliorated depressive-like behaviours in Wistar–Kyoto rats (Schaffer *et al.* 2010).

It is also interesting to observe that in absence of D_2 blockade, the 5-HT_2 antagonist increased *Nor-1* mRNA levels, while it reduced haloperidol-induced *Nor-1* expression in the cortex. At the present time, we have no explanation for this paradoxical effect of MDL11393. The 5-HT_{1A} agonist (8-OH-DPAT) is also able to reduce haloperidol-induced *Nor-1* expression in the cortex. Modulations of the haloperidol-induced pattern of *Nor-1* expression in the cortex are reminiscent of the modulation of dopamine release in the cortex upon combined administration of a 5-HT_{1A} agonist or a 5-HT_{2A} antagonist with a dopamine D_2 receptor antagonist, as previously discussed. Interestingly, these effects were not observed with *Nur77*, suggesting that *Nor-1* might be a better marker of atypicality in the cortex compared to *Nur77*.

It has been shown that striatal 5-HT_2 receptors exert a positive control on basal dopamine release and mediate a tonic inhibitory serotonergic tone on dopamine neurons in the VTA. Consequently, administration of 5-HT_2 receptor antagonists can directly increase dopamine release in the NAc and the PFC, and 5-HT_2 agonists can suppress dialysate levels of dopamine in the frontal cortex (Gobert & Millan, 1999; Millan *et al.* 1998). Interestingly, MDL11939 (5-HT_2 antagonist) displayed a selective effect on *Nur77* and *Nor-1* mRNA levels in the VTA, compared to the SN indicating that a 5-HT_2 antagonist can also exert a selective transcriptional activity within dopamine neurons of the mesolimbic and mesocortical pathways.

8-OH-DPAT, a 5-HT_{1A} agonist, tends to reduce *Nur77* in the striatum, which is consistent with a previous report (Gervais *et al.* 1999). Although we used a higher dose of 8-OH-DPAT, modulations of haloperidol-induced *Nur77* and *Nor-1* expression in the striatum by this 5-HT_{1A} agonist are also consistent with the report of Bruins Slot and colleagues (2009). The partial activity of the 5-HT_{1A} agonist might result from interactions at both pre-synaptic autoreceptor and post-synaptic sites by the present 8-OH-DPAT dose. These effects are also similar to previous data on striatal Fos immunohistochemistry or c-*fos* mRNA levels (Bruins Slot *et al.* 2009; Ohno *et al.* 2008; Tremblay *et al.* 1998). One of the proposed mechanism for these effects is a reduction of the activity of 5-HT projections (through stimulation of pre-synaptic 5-HT_{1A} autoreceptors or blockade of post-synaptic 5-HT_{2A} receptors) that inhibit dopaminergic nigrostriatal neurons, thus increasing striatum dopamine levels, which partially or totally overcome the blockade of D_2 receptors by the antipsychotic drug.

Post-synaptic α_1- and α_2-adrenergic receptors are highly expressed in the cerebral cortex, while presynaptic α_2-adrenergic receptors are also present in noradrenergic terminals and locus coeruleus neurons. Therefore, they are well placed to exert an important modulation on dopamine neurotransmission (Shen & Gundlach, 2000). The present results indicate that *Nur77*, but not *Nor-1* expression, can be modulated by an α_2-adrenergic antagonist in the mouse forebrain in the presence of haloperidol, whereas the α_1-adrenergic drug remained without effect in the striatum and cortex. Similar data were obtained using c-*fos* expression (Fink-Jensen *et al.* 1995) or catalepsy behaviour (Wadenberg *et al.* 2000, 2007).

Interestingly, it has been shown that prazosin can selectively modulate the firing pattern of dopamine neurons in the VTA (Grenhoff & Svensson, 1993). We also observed a selective transcriptional activity on *Nur77* by prazosin in the VTA, while idazoxan had a preferential activity in the SN. Potentiation of the effect of haloperidol by idazoxan was surprising because it has been shown that α_2-adrenergic antagonists are able to reduce haloperidol-induced catalepsy (Invernizzi *et al.* 2003; Wadenberg *et al.* 2007). However, the mechanism of the effect of α_2-adrenergic receptors might be complex, since 5-HT neurotransmission seems to be involved in the effect of idazoxan (Invernizzi *et al.* 2003). In addition, the contribution of other receptor targets, such as histamine H_1, 5-HT_6, 5-HT_7 or muscarinic M_1–M_4, cannot be excluded. Further experiments will be necessary to determine their contributions to *Nur77* and *Nor-1* gene expression patterns. Muscarinic M_1–M_4 receptors are of particular interest (Conn *et al.* 2009), but co-administration of scopolamine, a muscarinic M_1–M_4 antagonist, failed to reduced haloperidol-induced *Nur77* expression in the striatum (J. Maheux & D. Lévesque, unpublished observations).

In summary, our results clearly indicate that 5-HT receptors contribute to the distinct transcriptional patterns induced by typical and atypical antipsychotic drugs. While the exact nature of the influence of antipsychotic drug-induced *Nur77* and *Nor-1* expression on downstream *in-vivo* responses remains to be clarified, the region-specific modulations of these transcription factors may constitute useful markers of antipsychotic drug activity and to help predict the clinical profile of potential antipsychotic drugs in development.

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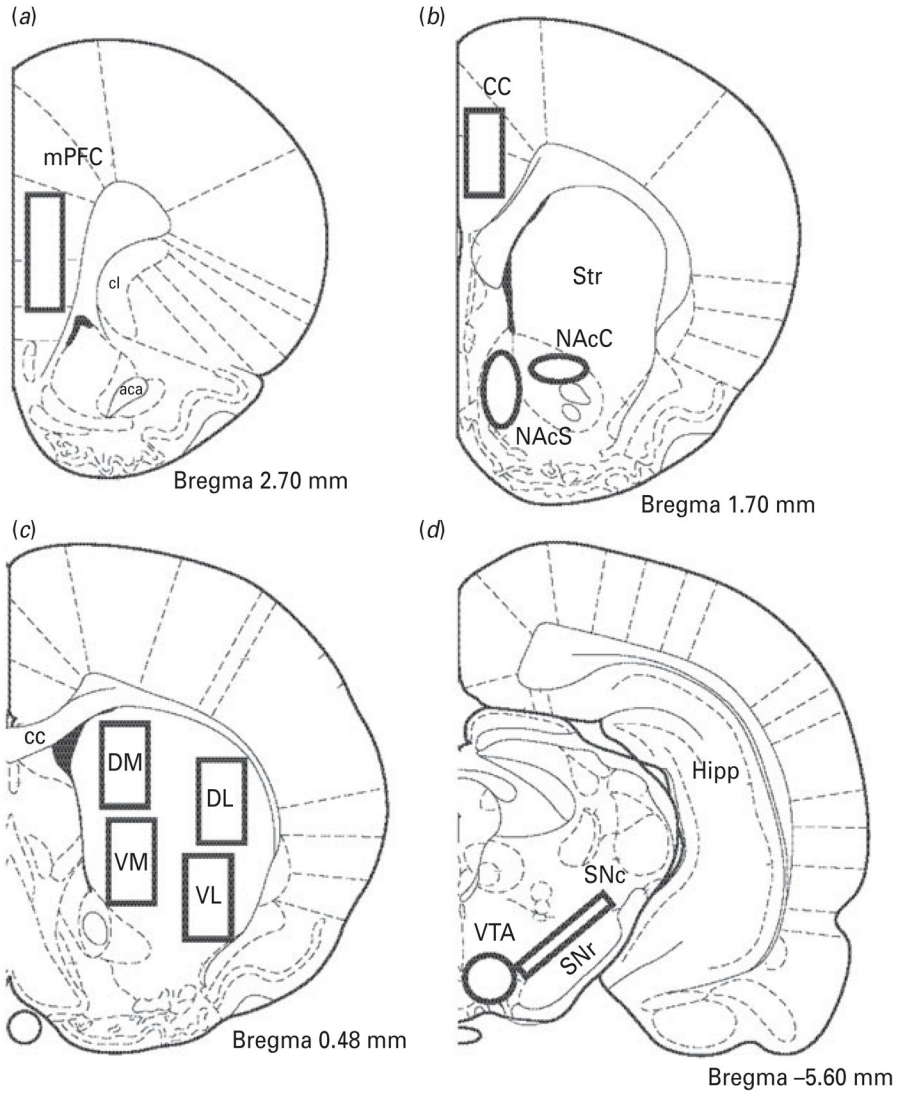

Fig. 1.

Schematic illustration of the mouse brain regions selected for quantitative analysis of *Nur* mRNA levels. Blank boxes indicate the sampled areas in (*a*) the medial prefrontal cortex (mPFC), (*b*) nucleus accumbens core (NAcC), nucleus accumbens shell (NAcS), and cingulate cortex (CC), (*c*) dorsomedial (DM), dorsolateral (DL), ventromedial (VM) and ventrolateral (VL) portions of the striatum, and (*d*) substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Corresponding Bregma levels are indicated in respective diagrams. Other abbreviations shown are : aca, anterior commissure ; cl, clustrum; Str, striatum; cc, corpus callosum; Hipp, hippocampus; SNr, substantia nigra pars reticulata.

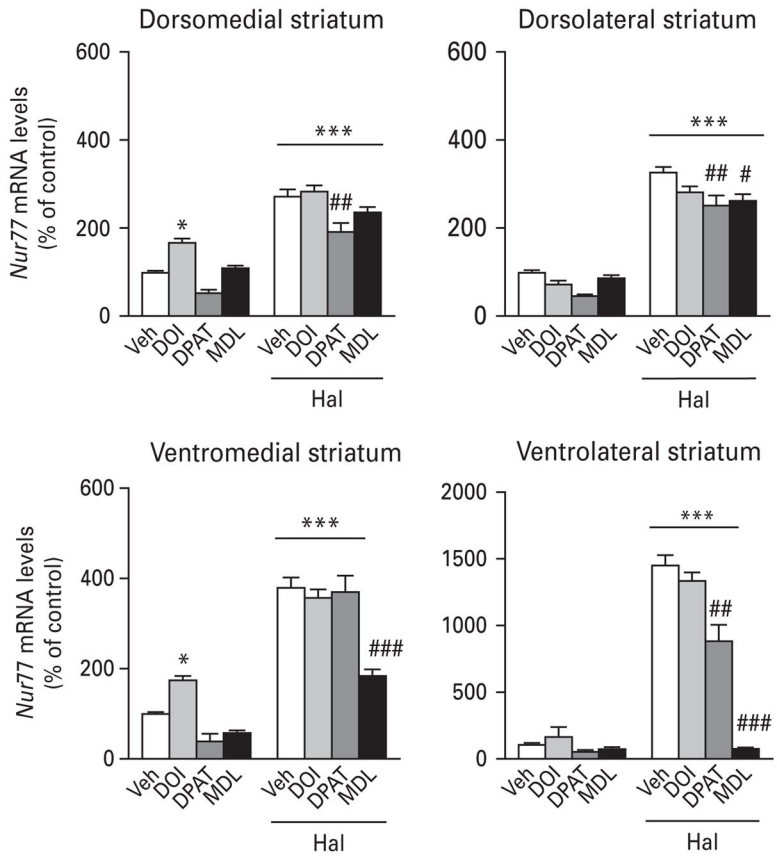

Fig. 2.

Histograms illustrating the effect of 5-HT drugs on haloperidol-induced *Nur77* mRNA levels in the mouse striatum. Values are expressed as percentage of change compared to vehicle-treated animals (control) and represent mean±S.E.M. Each group included five animals. See Materials and methods section for dose regimens. Veh, Vehicle ; DPAT, 8-OH-DPAT; MDL, MDL11939 ; Hal, haloperidol (* $p<0.05$, *** $p<0.001$ *vs.* Veh; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ *vs.* Hal).

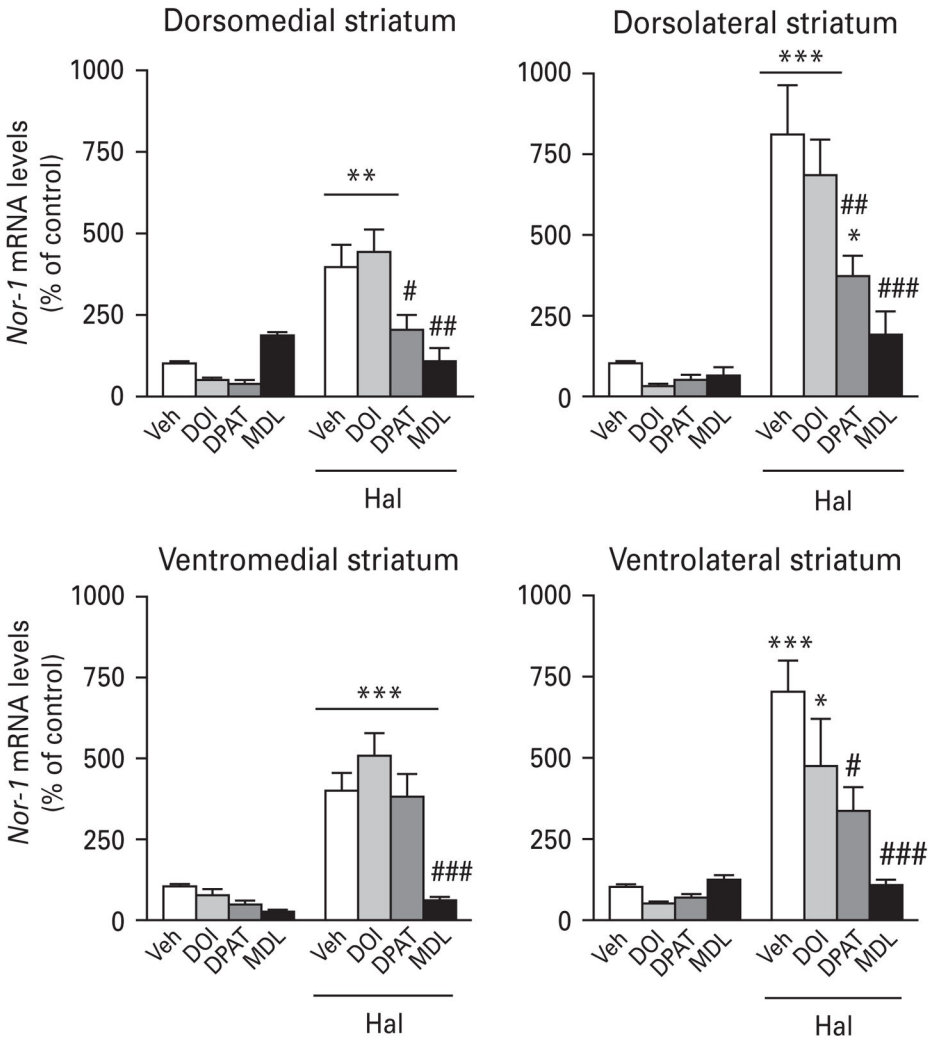

Fig. 3.

Histograms illustrating the effect of 5-HT drugs on haloperidol-induced *Nor-1* mRNA levels in the mouse striatum. Values are expressed as percentage of change compared to vehicle-treated animals (control) and represent mean±S.E.M. Each group included five animals. See Materials and methods for dose regimens. Veh, Vehicle ; DPAT, 8-OH-DPAT; MDL, MDL11939 ; Hal, haloperidol (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* Veh; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ *vs.* Hal).

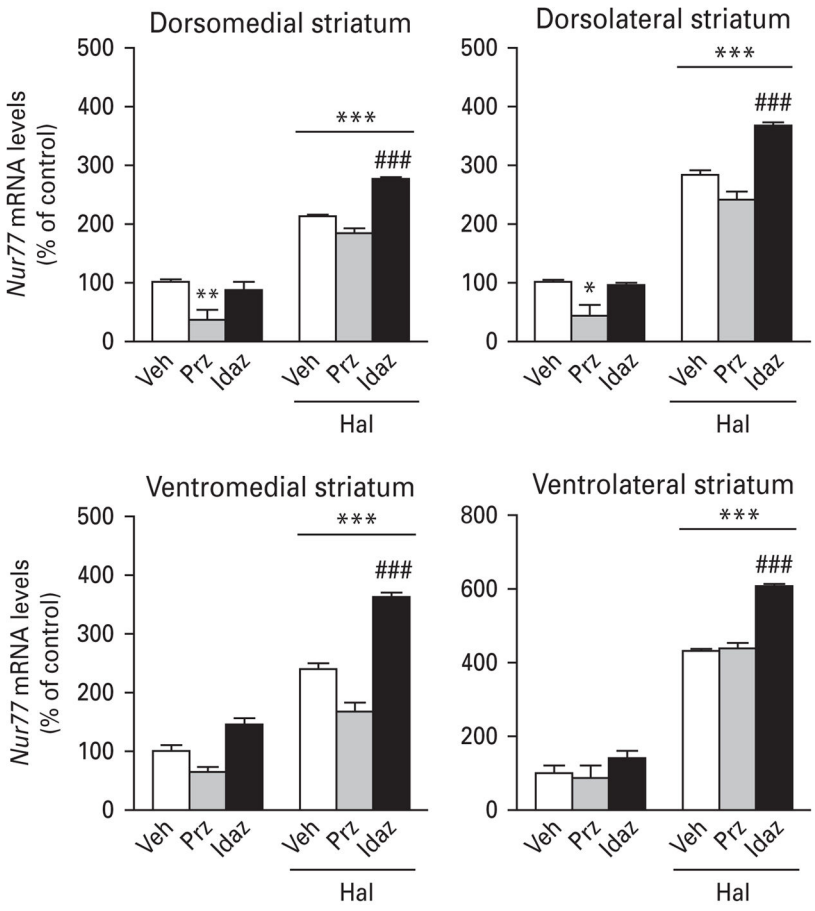

Fig. 4.

Histograms illustrating the effect of adrenergic drugs on haloperidol-induced *Nur77* mRNA levels in the mouse striatum. Values are expressed as percentage of change compared to vehicle-treated animals (control) and represent mean±S.E.M. Each group included five animals. See Materials and methods section for dose regimens. Veh, Vehicle ; Prz, prazosin; Idaz, idazoxan; Hal, haloperidol (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* Veh; ### $p<0.001$ *vs.* Hal).

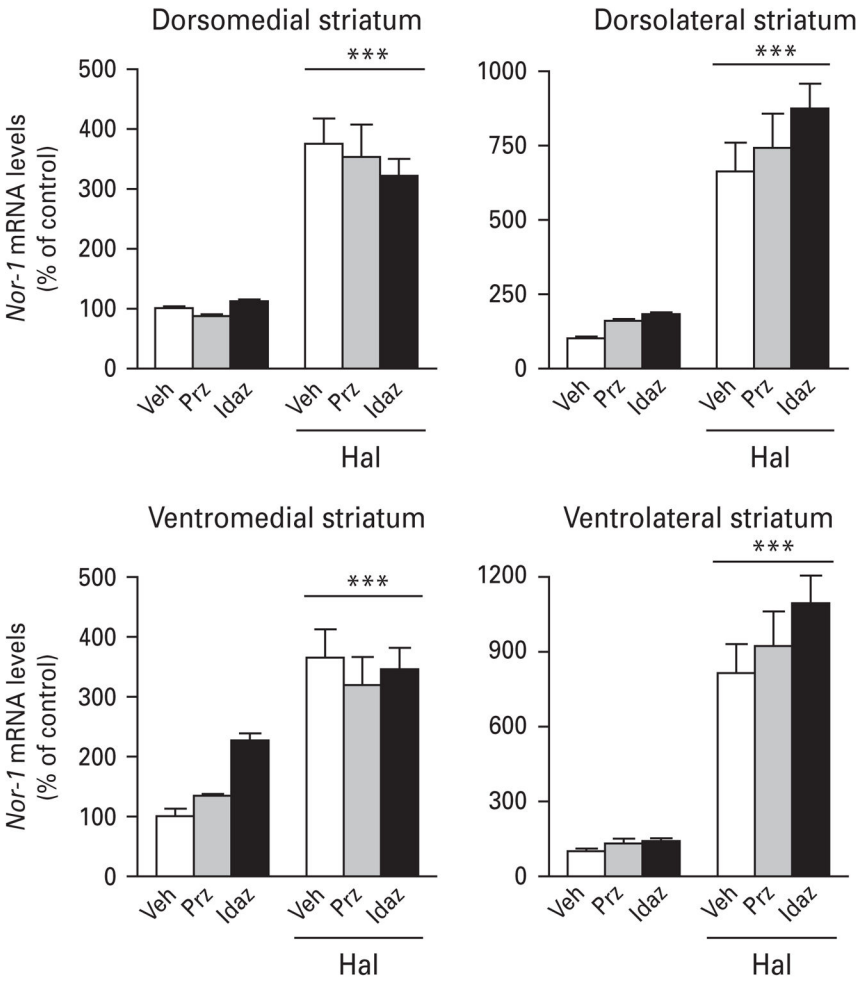

Fig. 5.

Histograms illustrating the effect of adrenergic drugs on haloperidol-induced *Nor-1* mRNA levels in the mouse striatum. Values are expressed as percentage of change compared to vehicle-treated animals (control) and represent mean±S.E.M. Each group included five animals. See Materials and methods section for dose regimens. Veh, Vehicle ; Prz, prazosin ; Idaz, idazoxan; Hal, haloperidol (*** $p<0.001$ *vs.* Veh).

Table 1

Nur77 and *Nor-1* mRNA levels in vehicle-treated animals in the brain areas analysed

	mRNA levels (nCi/g tissue)	
Brain areas	***Nur77***	***Nor-1***
mPFC	389±46	123±12
CC	445±33	106±16
NAcS	103±13	42±4
NAcC	151±21	29±2
StDM	207±11	70±6
StDL	177±14	19±2
StVM	105±8	24±4
StVL	38±7	31±4
SNc	4±2	8±1
VTA	6±2	5±2

CC, Cingulate cortex ; mPFC, medial prefrontal cortex ; NAcC, nucleus accumbens core ; NAcS, nucleus accumbens shell ; SNc, substantia nigra pars compacta ; StDM, dorsomedial striatum; StDL, dorsolateral striatum ; StVM, ventromedial striatum ; StVL, ventrolateral striatum; VTA, ventral tegmental area.

Values represent mean±S.E.M. from vehicle-treated animals (n=5).

Table 2

Effects of serotonin drugs on *Nur77* expression in extra-striatal areas

	Veh	DOI	8-OH-DPAT	MDL11939	Hal	DOI+Hal	8-OH-DPAT+Hal	MDL11939+Hal
***Nur77* mRNA levels (% of vehicle)**								
mPFC	100±12	133±14	125±11	149±11	148±12	122±19	115±7	143±15
CC	100±7	133±8	100±12	143±11 *	116±7	100±11	140±13	135±6
NAcS	100±12	275±36 *	233±32	153±18	338±21 **	434±39 ***	562±75 ***##	442±38 ***
NAcC	100±14	218±26 *	149±39	209±31	267±42 **	347±27 ***	305±31 ***	323±23 ***
SNc	100±58	264±103	201±129	63±36	2146±342 ***	1777±235 ***	1996±303 ***	1726±337 ***
VTA	100±40	144±43	93±17	111±31	1418±155 ***	1186±180 ***	1024±37 ***	848±115 ***##

CC, Cingulate cortex ; Hal, haloperidol; mPFC, medial prefrontal cortex ; NAcC, nucleus accumbens core ; NAcS, nucleus accumbens shell ; SNc, substantia nigra pars compacta ; VTA, ventral tegmental area ; Veh, vehicle.

Values represent mean±S.E.M. from five animals per group

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$ *vs.* Veh group;

\#\# $p<0.01$ *vs.* haloperidol group.

Table 3

Effects of serotonin drugs on *Nor-1* expression in extra-striatal areas

	Veh	DOI	8-OH-DPAT	MDL11939	Hal	DOI+Hal	8-OH-DPAT+Hal	MDL11939+Hal
***Nor-1* mRNA levels (% of vehicle)**								
mPFC	100±10	35±3*	71±18	194±27*	241±38*	236±50*	115±16$^{\#}$	68±20$^{\#\#}$
CC	100±15	34±9*	58±15	220±45*	228±34*	211±56*	119±20$^{\#}$	41±9$^{\#\#}$
NAcS	100±9	46±16	180±58	257±58	589±60**	526±131**	773±80***	101±19$^{\#\#\#}$
NAcC	100±8	80±25	124±40	314±76*	654±94***	642±148**	407±66**	108±22$^{\#\#\#}$
SNc	100±18	118±46	47±7	42±17	975±162***	682±88**	889±135***	743±125***
VTA	100±36	159±47	103±19	123±34	1608±188***	1307±199***	1128±41$^{***\#}$	911±114$^{***\#\#}$

CC, Cingulate cortex ; Hal, haloperidol; mPFC, medial prefrontal cortex ; NAcC, nucleus accumbens core ; NAcS, nucleus accumbens shell ; SNc, substantia nigra pars compacta ; VTA, ventral tegmental area ; Veh, vehicle.

Values represent mean±S.E.M. from five animals per group

$^{*}p<0.05$,

$^{**}p<0.01$,

$^{***}p<0.001$ *vs.* Veh group;

$^{\#}p<0.05$,

$^{\#\#}p<0.01$,

$^{\#\#\#}p<0.001$ *vs.* haloperidol group.

Table 4

Effects of adrenergic drugs on *Nur77* expression in extra-striatal areas

	Veh	Prazosin	Idazoxan	Haloperidol	Prazosin+Hal	Idazoxan+Hal
***Nur77* mRNA levels (% of vehicle)**						
mPFC	100±14	73±4	151±10	116±9	131±9	150±18
CC	100±16	62±16	125±16	109±11	124±8	148±9
NAcS	100±12	41±20 *	77±14	221±11 ***	216±4 ***	304±10 $^{***\#\#}$
NAcC	100±1	38±4 **	96±5	250±9 ***	197±6 $^{***\#\#}$	263±6 ***
SNc	100±23	132±35	141±20	772±80 ***	827±93 ***	1094±119 $^{***\#}$
VTA	100±18	113±18	104±11	475±60 ***	707±69 $^{***\#}$	618±55 ***

CC, Cingulate cortex ; Hal, haloperidol; mPFC, medial prefrontal cortex ; NAcC, nucleus accumbens core ; NAcS, nucleus accumbens shell ; SNc, substantia nigra pars compacta; VTA, ventral tegmental area ; Veh, vehicle.

Values represent mean±S.E.M. from five animals per group

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$ *vs.* Veh group;

$^{\#}$ $p<0.05$,

$^{\#\#}$ $p<0.01$ *vs.* haloperidol group.

Table 5

Effects of adrenergic agents on *Nor-1* expression in extra-striatal areas

	Veh	Prazosin	Idazoxan	Hal	Prazosin+Hal	Idazoxan+Hal
***Nor-1* mRNA levels (% of vehicle)**						
mPFC	100±11	114±7	110±5	72±3	110±14	110±17
CC	100±8	110±11	109±9	81±7	129±10$^{\#}$	88±9
AcSh	100±4	113±11	109±16	312±29***	384±21***	452±36$^{***\#\#}$
AcC	100±10	127±14	117±7	333±29***	366±53***	304±21***
SNc	100±17	89±29	85±6	208±29*	216±24*	195±19*
VTA	100±14	77±22	72±19	177±15*	175±8*	95±8$^{\#}$

CC, Cingulate cortex ; Hal, haloperidol; mPFC, medial prefrontal cortex ; NAcC, nucleus accumbens core ; NAcS, nucleus accumbens shell ; SNc, substantia nigra pars compacta; VTA, ventral tegmental area ; Veh, vehicle.

Values represent mean±S.E.M. from five animals per group

* $p<0.05$,

*** $p<0.001$ *vs.* Veh group;

$^{\#}$ $p<0.05$,

$^{\#\#}$ $p<0.01$ *vs.* haloperidol group.