Haloperidol-induced striatal *Nur77* expression in a non-human primate model of tardive dyskinesia

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Abstract

Tardive dyskinesia (TD) is a delayed and potentially irreversible motor complication arising in patients chronically exposed to antipsychotic drugs. As several modern (so-called atypical) antipsychotic drugs are common offenders, the widening clinical indications for prescription as well as exposure of vulnerable individuals, TD will remain a significant drug-induced unwanted side effect. In addition, the pathophysiology of TD remains elusive and therapeutics difficult. Based on rodent experiments, we have previously shown that the transcriptional factor *Nur77* (also known as NGFI-B or Nr4a1) is induced in the striatum following antipsychotic drug exposure as part of a long-term neuroadaptive process. To confirm this, we exposed adult capuchin (*Cebus apella*) monkeys to prolonged treatments with haloperidol (median 18.5 months, N=11) or clozapine (median 6 months, N=6). Six untreated animals were used as controls. Six haloperidol-treated animals developed mild TD movements similar to those found in humans. No TD was observed in the clozapine group. Postmortem analysis of *Nur77* expression measured by *in situ* hybridization revealed a stark contrast between the two drugs, as *Nur77* mRNA levels in the caudate-putamen were strongly upregulated in animals exposed to haloperidol while spared following clozapine treatment. Interestingly, within the haloperidol-treated group, TD-free animals showed higher *Nur77* expression in putamen subterritories compared to dyskinetic animals. This suggests that *Nur77* expression might be associated with a reduced risk to TD in this experimental model and could provide a novel target for drug intervention.

Keywords

NGFI-B; Nr4a1; transcription factor; typical antipsychotic drug; clozapine; caudate nucleus; putamen; abnormal involuntary movements; monkey; *in situ* hybridization

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Conflict of interest

The authors have no actual or potential conflict of interest including any financial, personal or other relationship with other people or organizations that could inappropriately influence, or be perceptive to influence the present work.

Introduction

Tardive dyskinesia (TD) is a troublesome and potentially irreversible motor complication encompassing all persistent, abnormal, involuntary movements occurring in the setting of chronic therapy with dopamine receptor-blocking agents, such as antipsychotic drugs and metoclopramide (Kenney *et al.*, 2008; Blanchet *et al.*, 2012). The resulting movement disorder is most often stereotyped in nature and typically involves the orofacial musculature. As TD remains an elusive drug complication over 50 years since its initial description, it is not surprising that the treatment options available are non-specific and produce mixed results (van Harten & Tenback, 2011).

New generations of antipsychotic drugs such as clozapine and olanzapine are defined as drugs active in the treatment of schizophrenia but with a lesser propensity to induce motor symptoms (Miyamoto *et al.*, 2005). Unfortunately, the early hope that second-generation (so-called atypical) antipsychotic drugs would afford a disappearance of TD has been challenged by a recent literature review, which reports that a proportion of one-third of patients chronically exposed to commonly used antipsychotic drugs such as risperidone and olanzapine still displays TD (Woods *et al.*, 2010). In fact, the only atypical antipsychotic drug associated with a non significant induction of TD is clozapine (Tarsy & Baldessarini, 2006). The annual risk remains greater in older adults, particularly in those living with a dementing illness (Lee *et al.*, 2005), and severe forms of TD still develop with the new antipsychotic drugs (de Leon, 2007). As a consequence of the persistence of TD in the community, combined with the aging of the population, and the enlarging spectrum of conditions for which antipsychotic drugs are prescribed (*e.g.*, bipolar disorder, depression), TD will remain a significant problem in human pharmacotherapy. In addition, the results of recent large trials and meta-analyses have shown that there is no effectiveness or tolerability advantage when comparing the first and second generations of antipsychotic drugs (Rosenheck *et al.*, 2003; Jones *et al.*, 2006; Leucht *et al.*, 2009; Peluso *et al.*, 2012). Therefore, a better understanding of the molecular mechanisms leading to tardive motor complications may help to design antipsychotic drugs devoid of these untoward effects or develop more effective adjunct anti-dyskinetic agents.

It is well documented that typical antipsychotic drugs rapidly induce genes like *c-fos*, Zif268 and *Nur77* (Nerve-growth factor Inducible gene B [NGFI-B], Nr4a1) in the rodent striatum, a key structure in the regulation of motor control (Herrera & Robertson, 1996; Herdegen & Leah, 1998; Lévesque & Rouillard, 2007). *Nur77* is a transcription factor of the nuclear receptor family that is rapidly induced after treatment with typical antipsychotic drugs (D₂ antagonists) (Beaudry *et al.*, 2000; Maheux *et al.*, 2005). Previous reports from our laboratory implicated *Nur77* in a neuroadaptive response mounted against abnormal motor behaviors following typical antipsychotic drug exposure in rodents (reviewed in (Lévesque & Rouillard, 2007)). For example, *Nur77* knockout mice not only showed spontaneous vacuous chewing movements (VCMs), a behavior that displays some similarities to human TD (Turrone *et al.*, 2002; Blanchet *et al.*, 2012), but also worsened haloperidol-induced VCMs compared to wild type mice (Ethier *et al.*, 2004b). This suggests that neurochemical changes induced by genetic deletion of *Nur77* might have recapitulated some susceptibility processes observed in TD. *Nur77* can display a functional transcriptional activity through

heterodimerization with Retinoid X Receptor (RXR) (Perlmann & Jansson, 1995). Interestingly, we have shown that retinoid drugs (called rexinoids) can modulate haloperidol-induced VCMs in wild-type mice, whereas rexinoids remained without effect in *Nur77* knockout mice (Ethier *et al.*, 2004b). This suggests that *Nur77*/RXR transcriptional complex might represent an interesting pharmacological target to reduce abnormal involuntary movements induced by antipsychotic drugs (Lévesque & Rouillard, 2007). In addition, *Nur77* mRNA up-regulation is sustained during subchronic typical antipsychotic drug administration in rodents without apparent desensitization, suggesting that *Nur77* not only participate in the initiation of a neuroadaptive signaling cascade, but also to more prolonged effects (Beaudry *et al.*, 2000; Langlois *et al.*, 2001).

We also recently showed that a single nucleotide polymorphism (SNP, rs2603751) located in the 3'-UTR (untranslated regulatory region) of the *Nur77* mRNA displayed a nominal association with the risk of developing TD, as well as with TD intensity based on Abnormal Involuntary Movement Scale (AIMS) scores, in a group of schizophrenia patients (Novak *et al.*, 2010).

In the present study, we chronically exposed *Cebus apella* monkeys to typical and atypical antipsychotic drugs in an attempt to associate TD induction and intensity of *Nur77* mRNA levels in various parts of the monkey basal ganglia. The results indicate that *Nur77* mRNA levels are upregulated within the haloperidol-treated group and TD-free animals showed higher *Nur77* expression compared to dyskinetic animals.

Material and methods

Animals and treatments

Handling of primates proceeded in accordance to the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures, including means to minimize discomfort, were approved by the Institutional Animal Care Committee of University of Montreal. Animals were housed separately in observation cages in a temperature-controlled room and exposed to a 12-hour light/dark cycle. They were fed once daily in the afternoon, and water was provided *ad libitum*. The number of animals was kept to a minimum, and all efforts were made to avoid animal stress and suffering.

Twenty-four adult female capuchin (*Cebus apella*) monkeys were used. All monkeys first underwent bilateral ovariectomy in order to reproduce the hormonal status and TD susceptibility of postmenopausal women (Yassa & Jeste, 1992). Twelve animals were injected on a weekly basis with a depot preparation of haloperidol decanoate (0.1–0.9 mg/kg, i.m.) for up to 36 months. A low dose was given initially and titrated upward, and the maintenance dose individually adjusted to prevent overt sedation, bradykinesia, or acute dystonic reaction. Benzotropine i.m. was occasionally needed during the initial titration phase to stop prolonged dystonic reactions. The drug protocol was briefly suspended a few times to ensure that TD manifestations were not masked by parkinsonian features. During the last 3 weeks of exposure, the haloperidol group (N=11) was switched to an oral preparation (0.125–0.3 mg/kg, twice daily) given in a fruit within a dose range proposed previously (Gunne & Barany, 1979), the last dose being administered 3 hours prior to sacrifice, in order

to lessen the variability in drug absorption and dopamine receptor blockade between animals prior to brain collecting. In our experience, such a brief oral drug switch has no impact on group allocation and TD intensity. A separate drug-exposed group of 6 animals received the atypical antipsychotic clozapine individually adjusted to tolerance, given twice daily (5–8 mg/kg/dose) in a fruit and treated 5 days a week for 6 months, until 3 hours prior to sacrifice (cumulative doses ranging from 600–960 mg/kg). Clozapine was chosen because it represents the best TD-sparing atypical antipsychotic drug (Woods *et al.*, 2010). Six animals were left untreated as controls. One TD-free animal died unexpectedly 22 months after initiation of haloperidol exposure.

TD measure

The orofacial (forehead, lips, tongue, jaw), neck, trunk, and limb movements were scored along a primate equivalent of the Abnormal Involuntary Movements Scale (AIMS), with each body segment scored between 0–4 points from absent to severe TD, for a maximum score of 40 points. During drug exposure, the presence of TD was deemed unequivocal when it was persistent over time and when a minimum of two separate body segments scored at least 1 point, or a minimum of one body part scored at least 2 points. Motor rating took place over 15 min each hour for 4 consecutive hours in the morning in the fasting state, before administration of the antipsychotic drug dose, behind a one-way mirror, with the animals kept in their home cage. The final score used for each animal is the cumulative score obtained for all 4 observations.

Tissue preparations

Three hours after drug dosing, the animals were anesthetized with ketamine (15 mg/kg, i.m.) followed by pentobarbital (35 mg/kg, i.v.). After clamping the abdominal aorta, they were perfused intracardially with 3 liters of physiologic saline solution. The brains were then rapidly removed, immersed in 2-methylbutane at -50°C for 15 sec, and then kept frozen at -80°C . Hemisected brains were cut into coronal sections of 12 μm on a cryostat (-20°C). The slices were thaw-mounted onto SuperFrostPlus (Fisher Scientific Ltd, Nepean, ON, Canada) 75 $\times$ 50 mm slides and stored at -80°C until use.

Complementary RNA probe preparation and synthesis

In order to label *Nur77* mRNA in monkey brain tissues, we have produced a complementary RNA (cRNA) probe from total RNA of human caudate-putamen tissues (Mahmoudi *et al.*, 2009). The cRNA probe for *Nur77* stems from an 814 bp (nucleotides 19 to 832) fragment of the full-length human cDNA (GeneBank accession no: NM002135) subcloned into pCRII/TOPO and linearized with Bam HI. The antisense probe was synthesized with a T₇ RNA polymerase. Synthesis of the specific [³⁵S]UTP-labeled cRNA probe for *Nur77* was performed as previously described (Beaudry *et al.*, 2000; Mahmoudi *et al.*, 2009).

In situ hybridization procedure

In situ hybridization with tissue sections was done at 58°C overnight in a standard hybridization buffer containing 50% formamide (Mahmoudi *et al.*, 2009). Tissue sections were then apposed against BiomaxMR (Kodak, New Haven, CT) radioactive sensitive films

for 5 to 7 days. Quantification of the autoradiograms was performed using computerized analysis (ImageJ 1.45s software, Wayne Rasband, NIH). Digital brain images were taken using a Grayscale Digital Camera (Model CFW-1612M, Scion Corporation, Maryland, USA). Optical gray densities were transformed into nCi/g of tissue equivalent using standard curves generated with ^{14}C -microscales (ARC 146A- ^{14}C standards, American Radiolabelled Chemicals Inc., St-Louis). Brain areas investigated included anterior and posterior parts of the caudate nucleus and putamen corresponding to Bregma 2.70 to 0.45 (anterior caudate-putamen) and Bregma -6.30 to -8.10 (posterior caudate-putamen) in the atlas of Paxinos, respectively (Paxinos, 2009). Anterior and posterior caudate-putamens have been subdivided into medial and lateral caudate (CM, CL), and dorsolateral (PDL), dorsomedial (PDM), ventrolateral (PVL) and ventromedial putamen (PVM). Average levels of labeling for each area were calculated from three adjacent brain sections of the same animals. Background intensities were taken in white matter tracts for each section and were subtracted from every measurement.

Statistical analysis

Statistical analysis was performed with Prism version 5.0 program (Graph Pad Software Inc. San Diego, CA, USA). All data were expressed as group means $\pm$ S.E.M. We performed a Shapiro-Wilk normality test to estimate if the data display a Gaussian distribution (normally distributed population). All data sets passed the normally test (with an α level of 0.05) indicating that no significant departure from normally can be assumed. Then, we proceeded to parametric statistical comparison of mRNA levels in controls, haloperidol- and clozapine-treated groups using a one-way analysis of variance (ANOVA). We first performed a test of homogeneity using Bartlett's test, followed by a logarithmic transformation (when Bartlett's test was significant) and finally applied a one-way ANOVA on transformed data. When the ANOVA revealed significant differences, a Tukey's test was performed as *post hoc* analysis. Comparison between dyskinetic and non-dyskinetic haloperidol-treated animals was performed using an unpaired t-test with a two-tailed p value. Statistical significance was set at $p < 0.05$.

Results

Behavioral observations

The chronic haloperidol regimen proposed was well tolerated. Motor slowing, action tremor, and sedation were generally mild if present. Occasional acute dystonic reactions prior to TD induction occurred in 6 out of 12 animals, rapidly reversed with benztropine, and minimized by a reduction in antipsychotic drug dose. We feel this is advantageous to avoid interference with food intake, optimize general well-being, and facilitate TD rating. A total of 6 (4 with early dystonic reactions on record) capuchins treated with haloperidol developed lasting TD. The latency of onset of TD was variable, with a mean ($\pm$ SD) of 19 ($\pm$ 12) months (median, 18.5 months; range 7–35 months), while the mean duration of drug exposure in the TD-free animals was 19 ($\pm$ 4.5) months (median, 17.5 months; range 16–27 months). The cumulative i.m. drug exposure (mg/kg) up to the onset of TD or the time of the last observation for the TD-free group was 34.3 ($\pm$ 37.9) and 41.5 ($\pm$ 16.4), respectively. The abnormal purposeless movements were similar to those found in humans and typically stereotyped in nature, at

times admixed with mild dystonic features. Variable orofacial dyskinetic movements were seen in all but one animal, including forehead contractions, chewing movements, tongue protrusions, and lip retraction (Fig. 1). Neck rotations, brief back extension, and flexion/extension movements of the toes, were also seen. Upper limb involvement was rarely observed. The mean cumulative motor score of haloperidol-treated animals showing TD was 16.5 (± 8.5) points. The orofacial scores accounted for 39% of the mean TD score for the whole group. In contrast, the chronic clozapine treatment was well tolerated and caused only mild behavioral changes such as a decrease in general activity, sedation, and hypersalivation. No acute dystonic reaction or TD was documented.

Nur77 mRNA levels were selectively induced by haloperidol

Nur77 mRNA levels were evaluated using a specific complementary RNA probe from the human *Nur77* DNA sequence. The hybridization signal was consistent with previous *Nur77* mRNA reports in primates (Mahmoudi *et al.*, 2009). The non-specific signal was very low (not shown). A relatively high basal *Nur77* expression was observed in caudate-putamen nuclei (Fig. 2, Table 1), whereas very low basal levels of *Nur77* were observed in nucleus accumbens (Acc), and external and internal segments of the globus pallidus (GPe and GPi, respectively) (Fig. 2). Haloperidol treatment strongly increased *Nur77* mRNA levels in all caudate and putamen subterritories in the anterior level (Fig. 3), while the typical antipsychotic drug significantly increase *Nur77* mRNA levels in posterior lateral caudate, and posterior ventromedial and ventrolateral putamen (Fig. 4). Clozapine treatment remained without effect in all caudate-putamen areas (Figs. 3 and 4). Haloperidol also induced *Nur77* expression in various cortical areas including frontal, insular, temporal and secondary somatosensory cortices (Fig. 2). In the nucleus accumbens, both haloperidol and clozapine tended to increase *Nur77* (CTL = 0.4 ± 0.2 ; HAL = 9.6 ± 3.6 and CLZ = 11.2 ± 5.4 nCi/g of tissue), but significance was not reached because of the important variability inherent to such low signals.

Nur77 mRNA levels were selectively elevated in some putamen areas in animals not showing TD

We then performed a further analysis in which we separated TD from TD-free haloperidol-treated animals. No significant *Nur77* mRNA levels difference between dyskinetic and non-dyskinetic haloperidol-treated animals was observed in the anterior caudate and putamen (Fig. 3, insets). However, animals not showing TD tended to have higher *Nur77* mRNA levels compared to dyskinetic animals in anterior lateral putamen subterritories, but these statistical comparisons did not reach significance (Fig. 3, insets). Interestingly, the statistical analysis indicated that animals not showing TD had significant higher *Nur77* mRNA levels compared to dyskinetic animals in posterior PDM, PVM and PVL putamen subterritories (Fig. 4, insets). According to the present results, it seems that the relationship between *Nur77* expression and TD scores is all or none in nature instead of a linear relationship. However, the limited TD score range obtained in our sample and the relatively small number of animals in the TD group (5) might be responsible for the lack of linearity between TD scores and *Nur77* expression in the present experiment.

Discussion

The present results confirm the sensitivity of *Cebus apella* monkeys to display TD during long-term antipsychotic drug exposure, half of our animals under chronic depot haloperidol developing TD after a median period of 18 months. The abnormal movements observed were similar to those found in humans and typically stereotyped in nature, without concomitant acute dystonic reaction. Clozapine caused no acute dystonic reaction or TD during the 6-month exposure, reflecting clinical experience (Woods *et al.*, 2010). But, since clozapine exposure was shorter than haloperidol in the present experiment, the two drugs cannot be directly compared in terms of their liability to trigger TD. For the purpose of drug impregnation, we feel the oral clozapine dose selected (between 5–8 mg/kg/dose, twice daily) was sufficient and compared favorably to the chronic oral daily dosing protocol (5.2 mg/kg/day) previously administered to rhesus monkeys (Lidow & Goldman-Rakic, 1994). Single s.c. dosing at 2 mg/kg is not well tolerated in capuchins with TD and triggers an atypical acute dystonic reaction (Peacock & Gerlach, 1999). Further, single oral doses of 10 mg/kg have produced a marked decrease in locomotion in rhesus (Kumar *et al.*, 2003).

Chronic treatments with typical antipsychotic drugs induced immediate-early genes such as stable forms of Fos (FosB/ FosB) or *Nur77* in the striatum of rodents, whereas atypical antipsychotic drugs do not, suggesting that induction of immediate-early genes in the striatum is associated with the sustain extrapyramidal side effects of typical antipsychotic drugs (Beaudry *et al.*, 2000; Rodriguez *et al.*, 2001; Grande *et al.*, 2004). Acute haloperidol as well as antagonist commonly used to treat nausea and associated with a metoclopramide, a 5-HT₃/D₂ high incidence of TD (Pasricha *et al.*, 2006), can induce Fos immunoreactivity in monkey caudate-putamen (Deutch *et al.*, 1996). However, no data is available on the modulation of immediate-early genes after prolonged antipsychotic drug exposure in primates. The possibility of functionally significant species differences is particularly important to consider since differences between rodents and primates in certain anatomical, pharmacological, and molecular features of forebrain dopamine system have been observed (Berger *et al.*, 1991; Joel & Weiner, 2000). The present results show that caudate-putamen upregulation of *Nur77* expression is selectively associated with the chronic administration of a typical antipsychotic drug (haloperidol), whereas the atypical antipsychotic drug clozapine administration spared *Nur77* expression in the caudate-putamen. This nicely supports previous experiments in rodents that have shown a differential regulation of striatal *Nur77* expression between typical and atypical antipsychotic drugs (Beaudry *et al.*, 2000; Werme *et al.*, 2000; Maheux *et al.*, 2005), and indicates that the long-term modulation of *Nur77* expression induced by antipsychotic drugs in rodents can be extended to primates. Although both haloperidol and clozapine tended to increase *Nur77* mRNA levels in the nucleus accumbens, in accordance with rodent data (Beaudry *et al.*, 2000; Werme *et al.*, 2000; Maheux *et al.*, 2005), basal *Nur77* expression in the nucleus accumbens is much lower than in caudate-putamen in primates, whereas it has similar expression in both brain areas in rodents (Beaudry *et al.*, 2000; Werme *et al.*, 2000; Maheux *et al.*, 2005).

In mice, we have shown that gene ablation of *Nur77* (knockout) generated spontaneous vacuous chewing movements (VCMs) reminiscent of TD, and exacerbated haloperidol-induced VCMs (Ethier *et al.*, 2004b). This suggests that *Nur77* upregulation induced by this

typical antipsychotic drug is associated with cellular neuroadaptive events that tend to protect against the development of involuntary abnormal movements. The upregulation of *Nur77* in posterior putamen subterritories specifically observed in our TD-free monkeys is supportive of this view. Conceivably, this system is eventually overwhelmed during chronic drug exposure, leading to the delayed expression of motor complications associated with antipsychotic drugs. Alternatively, the regulation of *Nur77* in central dopaminergic circuits under conditions of dopamine D₂ receptor blockade could be genetically determined. We recently identified a single nucleotide polymorphism (SNP, rs2603751) located in the 3'-untranslated region (3'-UTR) of the *Nur77* mRNA that displayed a nominal association with the risk of TD, as well as with the severity of TD based on AIMS scores in a group of schizophrenia patients (Novak *et al.*, 2010). The non coding 3'-UTR of transcripts has been recognized to be important for the control of gene expression (Shim & Karin, 2002), raising the possibility that the SNP rs2603751 might alter *Nur77* expression. Indeed, using reporter plasmids in HEK293 cells containing the *Nur77* 3'-UTR along with the native allele (T allele), or the mutated allele (C allele) associated with a higher risk of TD, we observed that the expression of the reporter gene is significantly reduced in the presence of the *Nur77* 3'-UTR containing the mutated allele (unpublished observation), suggesting that this SNP might be associated with a reduction of *Nur77* expression. Thus, it is tempting to speculate that the induction of TD by a typical antipsychotic drug is linked with lower *Nur77* expression and that individuals bearing this SNP have a higher susceptibility to develop TD, compared to those bearing the native allele. In order to support this claim, the presence of this *Nur77* SNP correlated with high TD scores and low brain *Nur77* expression levels must be sought in a large number of patients treated with antipsychotic drugs.

The absence of modulation of *Nur77* expression in non-dyskinetic clozapine-treated monkeys may seem contradictory to haloperidol data, where non-dyskinetic animals showed higher *Nur77* mRNA levels. However, the pharmacological profile of clozapine is much more complex than haloperidol. We have previously shown that the differential effect of typical and atypical antipsychotic drugs on *Nur77* expression is at least in part explained by interactions of atypical drugs with serotonin 5-HT_{1A} and 5-HT_{2A/C} receptor subtypes, which prevent the induction of striatal *Nur77* expression (Maheux *et al.*, 2012). In other words, the resulting intracellular signaling pathways induced by certain atypical antipsychotic drugs, such as clozapine, which combines complex effects on dopamine, 5-HT, and many other receptor activities, do not trigger TD, thereby avoiding the need to switch on nuclear *Nur77* transcription.

Interestingly, the relationship between *Nur77* mRNA expression and TD was observed in medio-dorsal portions of the caudal putamen (Fig. 4), which display sensorimotor functionality relative to their cortical inputs in primates (Parent & Hazrati, 1995). In other areas sharing associative functionality, such as the caudate nucleus and anterior putamen (Parent & Hazrati, 1995), *Nur77* mRNA levels were upregulated, but no relationship between *Nur77* expression and TD was apparent. The animals exposed for several months to haloperidol developed TD mainly in the orofacial region, but other body segments, including neck and forelimbs, were also affected. Thus, although somatotopic organization of the primate basal ganglia associates ventral portions of the putamen with orofacial movements

(Nambu, 2011), neuroanatomical alterations in brain dopamine circuits in TD could well vary from one animal to another.

In conclusion, the latency of onset, individual susceptibility, phenomenologic expression of the purposeless movements, and persistence of TD signs, make the non-human primate experimental TD model convincingly similar to the human condition. In this unique model, we confirmed the inverse relationship between striatal *Nur77* expression and TD-like movements previously documented in rodents, supporting the validity of these observations to the human condition. We have previously shown that haloperidol-induced expression of neuromodulatory neuropeptides enkephalin and neurotensin is blunted in *Nur77* knockout mice (Ethier *et al.*, 2004a), suggesting that this transcription factor might contribute to the modulation of gene expression involved in the control of dopamine neurotransmission homeostasis. By promoting adaptive gene expression patterns, activation of transcriptional factors of the nuclear receptor family may provide an interesting novel approach for drug intervention in the prevention and palliation of TD.

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Abbreviations

3'-UTR	3'-untranslated regulatory region
Acc	nucleus accumbens
AIMS	abnormal involuntary movement scale
CL	caudate lateral
CM	caudate medial
cRNA	complementary RNA
GPe	external globus pallidus
GPI	internal globus pallidus
NGFI-B	Nerve-Growth Factor Inducible gene B
PDL	putamen dorsolateral
PDM	putamen dorsomedial
PVL	putamen ventrolateral
PVM	putamen ventromedial
SNP	single nucleotide polymorphism

TD	tardive dyskinesia
VCM	vacuous chewing movement

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Figure 1. Representative photographs of tardive dyskinesia manifestations in two female capuchins displaying forehead muscle retraction, neck twisting and lip retraction (top panel), and tongue protrusion (lower panel). The figure was adapted with permission from (Blanchet *et al.*, 2012).

Figure 2.

Representative autoradiograms showing the *in situ* hybridization signals obtained with the monkey radiolabeled *Nur77* mRNA probe. Representative examples of monkey brain coronal sections taken at anterior **A**) and posterior **B**) levels of the caudate-putamen in control (CTL), haloperidol (HAL)- and clozapine (CLZ)-treated groups. The left panels illustrate schematic representations of caudate nucleus and putamen subdivision areas used for quantification. Abbreviations are: Acc, nucleus accumbens, lateral (CL) and medial (CM) portions of the caudate nucleus, dorsolateral (PDL), dorsomedial (PDM), ventrolateral (PVL) and ventromedial (PVM) portions of the putamen, GPe, external globus pallidus and GPi, internal globus pallidus.

Figure 3.

Modulation of *Nur77* mRNA levels in antipsychotic drug-treated monkeys in the anterior caudate-putamen. *Nur77* mRNA levels were measured in control (CTL), haloperidol (HAL) and clozapine (CLZ)-treated monkeys in the anterior caudate nucleus and putamen. *Nur77* mRNA levels were evaluated in lateral (CL) and medial (CM) portions of the caudate nucleus as well as in dorsolateral (PDL), dorsomedial (PDM), ventrolateral (PVL) and ventromedial (PVM) portions of the putamen. Values are expressed in percent (%) of control values. Absolute values in nCi/g tissue of controls are presented in Table 1. Histogram bars represent means $\pm$ SEM (N=5–11 per group) (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs

CTL group). Inset graphs represent *Nur77* mRNA levels in haloperidol-treated animals that have been subdivided into dyskinetic (Dysk) and non-dyskinetic (No-dysk) groups.

Figure 4.

Modulation of *Nur77* mRNA levels in antipsychotic drug-treated monkeys in the posterior caudate-putamen. *Nur77* mRNA levels were measured in control (CTL), haloperidol (HAL) and clozapine (CLZ)-treated monkeys in the posterior caudate nucleus and putamen. *Nur77* mRNA levels were evaluated in lateral (CL) and medial (CM) portions of the caudate nucleus as well as in dorsolateral (PDL), dorsomedial (PDM), ventrolateral (PVL) and ventromedial (PVM) portions of the putamen. Values are expressed in percent (%) of control values. Absolute values in nCi/g tissue of controls are presented in Table 1. Histogram bars represent means $\pm$ SEM (N=5–11 per group) (* $p < 0.05$ and ** $p < 0.01$ vs CTL group).

Inset graphs represent *Nur77* mRNA levels in haloperidol-treated animals that have been subdivided into dyskinetic (Dysk) and non-dyskinetic (No-dysk) groups (## $p < 0.01$ vs Dysk group).

Table 1Basal *Nur77* mRNA levels in monkey caudate nucleus and putamen in control animals

	<u><i>Nur77</i> mRNA levels (nCi/g tissue)</u>	
	Anterior level	Posterior level
Caudate nucleus		
CM	366 ± 38	149 ± 10
CL	325 ± 27	197 ± 10
Putamen		
PDM	317 ± 47	174 ± 4
PDL	344 ± 37	149 ± 11
PVM	204 ± 21	111 ± 8
PVL	256 ± 13	110 ± 15

Data represent means ± SEM. Abbreviations: lateral (CL) and medial (CM) portions of the caudate nucleus as well as in dorsolateral (PDL), dorsomedial (PDM), ventrolateral (PVL) and ventromedial (PVM) portions of the putamen. These values were used to establish 100 % control values of *Nur77* mRNA levels presented in Figures 3 and 4.