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D_2-Receptor Upregulation is Dependent upon Temporal Course of D_2-Occupancy: A Longitudinal [^{11}C]-Raclopride PET Study in Cats

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Long-term occupancy of dopamine D_2-receptors, as achieved by chronic treatment with antipsychotics, leads to D_2-receptor upregulation, and this upregulation is thought to be responsible for loss of efficacy and development of tardive dyskinesia. However, little is known about the parameters of D_2-receptor blockade (duration and percentage of blockade) that lead to upregulation. In this study, we investigated the effects of different degrees (60 *vs* >80%) and durations (a transient peak *vs* 24 h/day) of D_2-receptor blockade on inducing this upregulation. These different patterns of D_2-receptor occupancy kinetics were produced in cats using bolus *vs* constant infusion of haloperidol for 4 weeks. D_2-receptors were measured using positron emission tomography and Scatchard analyses of [^{11}C]raclopride binding, before and after withdrawal of treatment. Continuously high (80% for 24 h/day) D_2-receptor blockade led to a robust upregulation of striatal D_2-receptors that was maximal at 1-week withdrawal (35 ± 5%) and still detectable at 2-week withdrawal (20 ± 3%). This pattern of D_2-receptor blockade also induced behavioral tolerance to the effect of haloperidol on spontaneous locomotor activity. Continuously moderate (60% for 24 h/day) or transiently high (80% for a few hours/day) D_2-receptor blockade did not produce any of these effects. The long-term effect of haloperidol on D_2-receptor density and behavioral tolerance thus appears to be dependent not only on a critical threshold of D_2-receptor blockade but also on the daily duration of D_2-receptors blockade. This suggests that as far as antipsychotics are concerned, not only dose but disbursment throughout the day have an impact on eventual pharmacodynamic and behavioral outcomes.

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INTRODUCTION

The therapeutic effects of antipsychotic drugs are thought to be mediated through the central blockade of dopamine (DA) D_2-receptors (Seeman *et al*, 1976). One of the most striking and consistent consequences of chronic antipsychotic exposure is an increase in striatal D_2-receptor density, a finding that was first described in animals (Burt *et al*, 1977) and subsequently confirmed in the brains of patients with schizophrenia (Lee *et al*, 1978; Mackay *et al*, 1982). This D_2-receptor upregulation is thought to be responsible for loss of efficacy of antipsychotics and the development of tardive dyskinesia (TD) in humans and may represent a central mechanism in mediating the long-term, and potentially irreversible, consequences of antipsychotic treatment (Klawans and Rubovits, 1972; Tarsy and Baldessarini, 1977; Chouinard and Jones, 1980; Creese and Snyder, 1980). More recently, it has been shown in rodents that this upregulation can lead to breakthrough DA supersensitivity, which can weaken treatment efficacy despite the presence of an adequate level of D_2-occupancy by the antipsychotic (Samaha *et al*, 2007). Such a decrease in drug effect is concomitant with the development of a behavioral tolerance to the acute cataleptic effect of the drug seen during chronic exposure to haloperidol (Asper *et al*, 1973; Ezrin-Waters and Seeman, 1977; Rastogi *et al*, 1982). This behavioral tolerance is suspected to arise directly from striatal D_2-receptor upregulation and is considered to correspond, at least partially, to the emergence of TD observed in clinical patients on long-term antipsychotic medication (Sayers *et al*, 1975; Burt *et al*, 1977; Tarsy and Baldessarini, 1977; Clow *et al*, 1979; Rastogi *et al*, 1982).

There is evidence that the upregulation of D_2-receptors is dose dependent—it has been suggested that the temporal pattern of antipsychotic administration is important for the development of tolerance (Post, 1980). For instance, several studies have shown that although tolerance develops when

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antipsychotics are administered continuously, reverse tolerance (sensitization) is observed when antipsychotics are administered intermittently so that the drug is allowed to wear off between repeated administrations (Ezrin-Waters and Seeman, 1977; Carey and DeVeaugh-Geiss, 1984; Barnes *et al*, 1990; Csernansky *et al*, 1990; See and Ellison, 1990; Ahlenius *et al*, 1991). The few studies that have aimed to assess the importance of antipsychotic treatment schedule on alteration of D_2-receptor density gave conflicting results. Two studies in rats have shown that haloperidol given by continuous infusion or by depot leads to a receptor upregulation, whereas the same dose given over the same overall period but administered by daily bolus injections does not (Kashihara *et al*, 1986; Akiyama *et al*, 1987). In contrast, two other studies (Bannet *et al*, 1980; Koller, 1984) suggested that both sustained and intermittent haloperidol administration caused similar effects on D_2-receptor upregulation. However, a problem with the aforementioned studies is that they used high doses of haloperidol (0.5–5 mg/kg per day) and there was no measure of drug plasma levels or drug-induced receptor D_2-occupancy. When such saturation doses are used they may lead to cumulative drug dosage so that both intermittent and sustained administration methods can give rise to continuously high D_2-receptor blockade (Kapur *et al*, 2000a). Furthermore, none of these studies tried to correlate the changes in D_2-receptor density with a behavioral alteration or with evidence of behavioral tolerance.

The aim of this study was to compare, *in vivo* using PET, the effects of continuous *vs* intermittent treatment with haloperidol and to better understand the parameters (duration, magnitude) of receptor blockade controlling D_2-receptor upregulation. We sought to determine whether high ($\sim$80%) and transient (few hours a day) D_2-receptor blockade produce similar D_2-receptor alterations to continuously high ($\sim$80%, 24 h a day) and continuously moderate ($\sim$60%, 24 h a day) D_2-receptor blockades. To enhance the clinical relevance of these findings, we used doses of haloperidol which produce levels of D_2-receptor occupancy similar to those measured in patients (Farde *et al*, 1992; Nyberg *et al*, 1995; Kapur *et al*, 2000b) and hypothesized that it is the pattern of D_2-receptor occupancy kinetics that determine D_2-upregulation which, in turn, leads to behavioral tolerance. For each treatment schedule, dose selection was based on dosing regimens established for animal studies by Kapur *et al* (2003) and aiming at producing D_2-occupancies comparable to the clinical setting. To our knowledge, this study constitutes the first systematic examination of the parameters of D_2-receptor occupancy leading to upregulation and relating it to behavior using clinically relevant levels of occupancy values.

MATERIALS AND METHODS

Animals and Haloperidol Treatment Schedules

Animal studies and experimental procedures conformed to the guidelines established by the Canadian Council on Animal Care, and were approved by the local Animal Care Committees.

In an effort to limit the number of animals to the minimum necessary to produce reliable scientific data, five cats (Liberty Research Inc., Waverly, USA) were used in this study. Cats were assigned to three experimental groups with different haloperidol treatment schedules. In a first group (noted TRANShigh), cats ($n=3$) were given daily bolus injections of fixed doses of haloperidol (0.05 mg/kg per day; s.c.). Bolus injections were given every morning (0900 hours) for 4 weeks. This intermittent treatment schedule aimed at producing a peak striatal D_2-receptor occupancy of about 80% only transiently during the day (a few hours). In a second group (noted CONThigh), cats ($n=3$) were implanted s.c. with four osmotic minipumps (model 2ML4; Alzet). The minipumps continuously infused a total dose of 0.25 mg/kg per day at a rate of 2.5 μl/h each for 4 weeks. This treatment schedule aimed at producing a striatal D_2-receptor occupancy of about 80% continuously throughout the day. In a third group (noted CONTmod), cats ($n=2$) were implanted s.c. with two osmotic minipumps (model 2ML4; Alzet), which continuously infused a total haloperidol dose of 0.05 mg/kg per day for 4 weeks. This treatment schedule was aimed at producing a striatal D_2-receptor occupancy of about 50–60% continuously throughout the day.

Three of the five cats used were each alternatively examined in two of the three treatment schedule groups. Cat A was first examined in the TRANShigh group and then in the CONThigh group. Cat B was first examined in the CONThigh group and then in the TRANShigh group. Cat C was first examined in the TRANShigh group and then in the CONTmod group. For each cat, the period between the two treatments was at least 3 months. Cats D and E were examined only in the CONThigh and in the CONTmod groups, respectively.

Spontaneous Locomotor Activity Measurements

Cats' spontaneous locomotor activity was monitored for 4 weeks at baseline conditions, during the 4 weeks of chronic haloperidol treatment, and for 4 weeks following withdrawal from the drug using an Actiwatch activity monitor (model-AW16; Mini Mitter Company Inc., USA). The Actiwatch device is a long-term activity monitor containing an accelerometer capable of sensing motion in any direction. By integrating the speed and degree of motion, it produces a voltage that correlates positively with changes in speed and degree of motion. This voltage is stored as activity counts in the form of discrete integers. The Actiwatch was mounted, via an animal harness, on the back of the cats' torso. The device was programmed to store activity counts in 30-s intervals over 24 h.

As D_2-receptor occupancy was expected to be high for only a few hours a day in the TRANShigh group, spontaneous locomotor activity in this group was evaluated during two different time periods of the day: a peak occupancy period and a trough occupancy period. Locomotor activity in the peak occupancy period was calculated using activity counts stored from 1300 hours to 1700 hours (ie from 4 to 8 h following the 0900 hours daily dose of haloperidol), a time interval including the peak of drug action in brain. Locomotor activity in the trough occupancy period was evaluated using activity counts stored from 1900 hours to 0700 hours (ie from 10 to 22 h following the 0900

hours daily dose of haloperidol), a time interval when D_2-receptor occupancy declined to trough levels. For cats in the CONThigh and CONTmod groups, in which D_2-receptor blockade was expected to remain stable throughout the day, daily locomotor activity was calculated using activity counts stored from 0700 hours to 1900 hours. Activity data stored on days when cats had to be examined using PET were excluded.

The total activity count data from cats in each treatment condition (TRANShigh, CONThigh, and CONTmod) were combined for analysis. For each cat, the mean daily activity at baseline was calculated using data collected over the 4 weeks of measurement and was set at 100% of activity. Daily activity data obtained during treatment and at withdrawal were then expressed as a percentage of this mean daily activity at baseline. Daily activities were averaged over 1-week intervals for each period (baseline, treatment, and withdrawal) and each treatment condition.

PET STUDIES

PET Scanning Procedures

Studies were performed on a head-dedicated PET camera (Scanditronix GEMS 2048-15B). Anesthesia was induced with isoflurane (4%). As soon as deep anesthesia was obtained, an endotracheal intubation was performed and anesthesia was maintained by insufflation of 2.5% isoflurane in oxygen. End-tidal carbon dioxide pressure ($ETCO_2$), heart rhythm, and body temperature were continuously monitored during the PET experiments. Ventilation was assisted using an ADS 1000 microprocessor-controlled ventilator (Engler Engineering, Hialeah, FL, USA). Standard ventilation parameters (flow rate: 10–12 l/min; 8–10 breaths per minute; peak inspiratory pressure: 14–16 cm/H_2O) were used. During the experiment, ventilation was modified within the above standard values to target $ETCO_2$ values of 28–31 mmHg. A head fixation system was used to secure a fixed and reproducible position of the cat's head during the PET measurements, as previously described. This system consists of a stereotaxic frame similar to frames used for standard cat surgical stereotactic procedures, except that it is constructed with Plexiglas to reduce the attenuation and scatter of photons during PET scans. For positioning of the cat into the frame, ear bars were inserted into the external auditory meatus of the animal to orient its interaural line. A tooth bar and orbital bars were used to place the animal head in the orientation defined in the cat Atlas of Jasper and Ajmone-Marsan (1954). The frame is attached directly to the computer-controlled bed of the PET scanner so that the animal can be precisely placed in the same axial position of the scanner field of view. [^{11}C]raclopride was synthesized as previously described by methylation of the desmethyl precursor using [^{11}C]methyl iodide (Wilson *et al*, 2000). [^{11}C]raclopride (2–2.2 mCi) was injected i.v. as a bolus. The total time for measurement of radioactivity in the brain was 60 min.

Test–Retest Analysis of D_2-Receptor Binding Measurements at Baseline Conditions

The within-animal reliability of radioligand D_2-receptor density (B_{max}) and apparent affinity (K'_D) values obtained from *in vivo* Scatchard analyses of [^{11}C]raclopride binding was tested using a test–retest paradigm. Cats were scanned on two PET sessions with an interval of 1–6 weeks between the first (Test 1) and the second session (Test 2). Three of the five cats (cat A, cat B, and cat C) were scanned on a third PET session with an interval of 4–8 months between the first (Test 1) and the third session (Test 3). On each scan session, B_{max} and K'_D were determined *in vivo* from the Scatchard analysis of two consecutive [^{11}C]raclopride experiments performed at high (>1000 Ci/mmol) and low specific radioactivity (~ 50 Ci/mmol). In addition, data obtained from the high specific radioactivity experiments were used to calculate [^{11}C]raclopride binding potentials.

D_2-Receptor Occupancy Measurements During Chronic Treatment with Haloperidol

At 1 week following the start of haloperidol treatment, PET experiments were performed in each animal to determine the effective striatal D_2-receptor occupancy achieved by the treatment. Cats in the TRANShigh group were examined at 4 h (peak) and 24 h (trough) after the last morning dose of haloperidol. In two of the three TRANShigh cats, additional PET experiments were performed at 3 weeks of chronic treatment to determine striatal D_2-receptor occupancy at peak and trough. Cats in the CONThigh and CONTmod groups were examined at 1 and 3 weeks of constant infusion of the drug.

On the day preceding PET measurements, a 2 ml blood sample was withdrawn from nonanaesthetized cats for determination of haloperidol levels in plasma (ng/ml). For cats in the TRANShigh group, blood samples were withdrawn at 4 h (peak) and 24 h (trough) after the last morning dose of haloperidol. Haloperidol levels were measured by using a liquid/liquid extraction followed by a liquid chromatography-mass spectrometry analysis (St Joseph's Health Centre, London, Canada).

Determination of [^{11}C]Raclopride Binding Parameters During Withdrawal

Each cat was examined using PET at 1, 2, and 3 weeks following cessation of the daily injections or removal of the minipumps. On each occasion, D_2-receptor density and apparent affinity were determined from the Scatchard analysis of two consecutive PET experiments performed 1.5 h apart using [^{11}C]raclopride at high (>1000 Ci/mmol) and low specific radioactivity (~ 50 Ci/mmol).

Data Analysis

Regions of interest analysis. The stereotaxic frame used in this study enables a reproducible positioning of the cat's head within the scanner field of view and allows for the same regions of interest (ROIs) to be used across serial studies of a same animal. ROIs for the right and left striatum and the cerebellum were drawn on summation PET images obtained with [^{11}C]raclopride at baseline conditions. The summation images used represented radioactivity accumulation from 10 to 60 min post-radioligand injection. ROIs for the striatum included the caudate and the putamen and were defined on the two central PET slices where the

caudate-putamen had its maximal extension. For each cat, the same set of ROIs was used across studies. When analyzing serial scans of a same animal, inspection of the ROIs positioning was systematically done and found close agreement across studies. ROIs were then transferred onto the dynamic PET images and regional radioactivity concentration (nCi/ml) was determined for each frame, corrected for decay, and plotted *vs* time.

Binding potential quantification and scatchard analyses. In the Scatchard approach, quantification of [^{11}C]raclopride binding potentials (BP) was performed using the transient equilibrium method and the cerebellum as the reference region (Farde *et al*, 1989). The time curve for radioactivity in cerebellum, a region with low density of D_2-receptors, was used as an estimate of free plus nonspecifically bound radioligand concentration in brain ($F+\mathrm{NS}(t)$). The time curve for specific radioligand binding ($B(t)$) was defined as the radioactivity in the striatum minus that in the cerebellum. A set of three exponential functions was fitted to the time curves for $B(t)$ and $F+\mathrm{NS}(t)$. Time for transient equilibrium was defined as the moment when $B(t)$ peaked, ie $dB/dt=0$. The $B/F+\mathrm{NS}$ ratio was calculated using the values obtained at transient equilibrium. The value of B obtained at transient equilibrium was divided by the SR of injected [^{11}C]raclopride to obtain the specifically bound radioligand concentration (B; pmol/ml). The $B/F+\mathrm{NS}$ ratio obtained for each experiment was plotted *vs* B in a Scatchard graph and a straight line was drawn through the plotted points. The B_{max} was defined at the intercept with the x axis and the K'_D by the inverse of the slope.

Quantification of [^{11}C]raclopride BP was also performed using the simplified reference tissue model (Lammertsma and Hume, 1996). BP values obtained with this method were referred as BP_{ND} (Innis *et al*, 2007) and were calculated from each high SR experiment of the Scatchard analyses. BP_{ND} were then compared with the B_{max} values obtained from the saturation approach.

Haloperidol-induced occupancies. For each treatment schedule, haloperidol-induced occupancy of striatal D_2-receptors was defined as the percentage reduction of the BP_{ND} obtained after drug treatment as compared to the BP_{ND} obtained at baseline (Farde *et al*, 1992).

RESULTS

Test–Retest Analysis of BP_{ND}, B_{max}, and K'_D at Baseline Conditions

All test–retest measurements performed with a 1- to 6-week interval showed a high reliability of BP_{ND}, B_{max}, and K'_D values (Table 1). The absolute variabilities between Test 1 and Test 2 were equal to 5.5% (range: −7 to +8%) for BP_{ND}, 3.6% (range: −5 to +3%) for B_{max}, and 5.3% (range: −11 to +5%) for K'_D.

After 4–8 months, the absolute test–retest variability increased by 2–4% for all measures (Table 2). However, the same variability pattern persisted when compared with that obtained with a 1- to 6-week interval. The difference between measures obtained in Test 1 and Test 2 and between measures obtained in Test 1 and Test 3 did not reach statistical significance using the paired t-test.

Table 1 Test–Retest on D_2-Receptor Binding Measurements with a 1- to 6-Week Interval ($n=5$)

	BP_{ND}	B_{max}	K'_D
Test 1			
Mean ± SD	1.86 ± 0.12	24.9 ± 1.0	12.7 ± 1.3
COV (%)	6.6	3.9	9.9
Test 2			
Mean ± SD	1.89 ± 0.22	24.5 ± 0.9	12.3 ± 1.2
COV (%)	11.5	3.8	10.1
Absolute variability (%)[a]			
Mean ± SD	5.5 ± 2.2	3.6 ± 1.9	5.3 ± 4.0
Range of % difference[b]	−7 to +8	−5 to +3	−11 to +5

Abbreviations: B_{max}, D_2-receptor density; K'_D, D_2-receptor apparent affinity; BP_{ND}, binding potential as calculated with the simplified reference tissue model; COV, coefficient of variation (SD/mean × 100).
[a]Test 1−Test 2/(Test 1+Test 2)/2 × 100% (disregarding the direction of change).
[b]Test 1−Test 2/Test 1 × 100%.

Table 2 Test–Retest on D_2-Receptor Binding Measurements with a 4- to 8-Month Interval ($n=3$)

	BP_{ND}	B_{max}	K'_D
Test 1			
Mean ± SD	1.87 ± 0.09	24.8 ± 0.5	12.7 ± 1.1
COV (%)	4.7	1.9	8.7
Test 2			
Mean ± SD	1.79 ± 0.27	23.3 ± 1.2	12.4 ± 1.6
COV (%)	15.1	5.1	13.3
Absolute variability (%)[a]			
Mean ± SD	9.3 ± 3.1	6.2 ± 4.9	6.8 ± 2.0
Range of % difference[b]	−12 to +6	−10 to −1	−9 to +6

Abbreviations: B_{max}, D_2-receptor density; K'_D, D_2-receptor apparent affinity; BP_{ND}, binding potential as calculated with the simplified reference tissue model; COV, coefficient of variation (SD/mean × 100).
[a]Test 1−Test 2/(Test 1+Test 2)/2 × 100% (disregarding the direction of change).
[b]Test 1−Test 2/Test 1 × 100%.

Measures of D_2-Receptor Occupancy During Haloperidol Treatment

Measures of D_2-receptor occupancy in the TRANShigh group showed that daily bolus injections of haloperidol at 0.05 mg/kg per day gave rise to daily transiently high D_2-receptor blockade during the whole treatment (Figure 1a). At 1 week of treatment, D_2-receptor occupancies were 83 ± 4% and 40 ± 8% at 4 h (peak) and at 24 h (trough) after

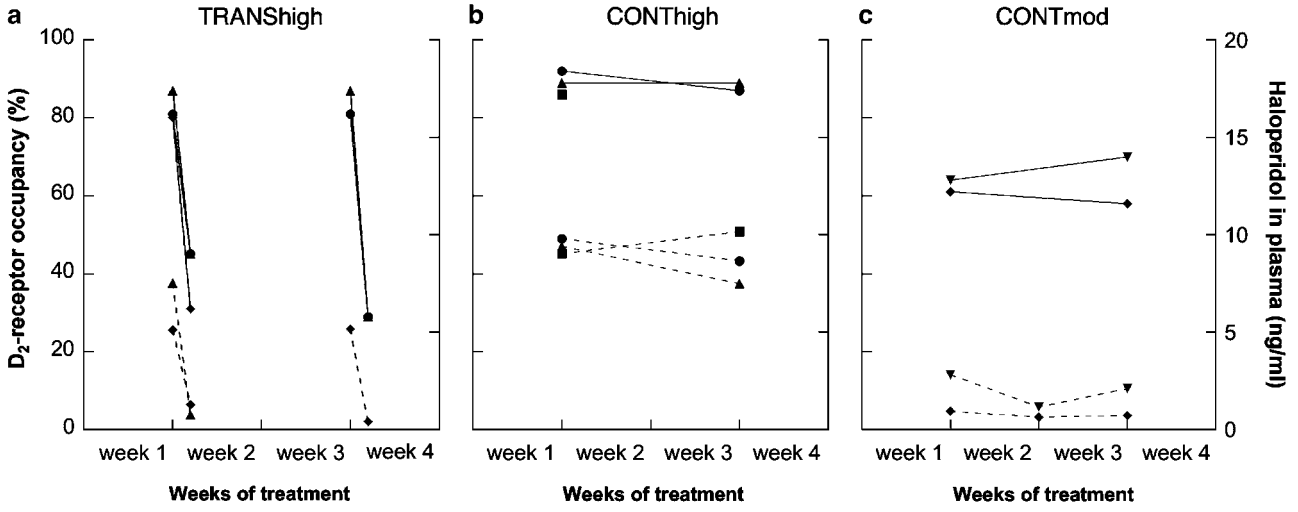

Figure 1 D_2-receptor occupancy measures (solid lines) obtained during a 4-week treatment with haloperidol administered either (a) as daily bolus injections at 0.05 mg/kg per day (TRANShigh group), (b) as a continuous infusion at 0.25 mg/kg per day (CONThigh group), or (c) as a continuous infusion at 0.05 mg/kg per day (CONTmod group). In each cat, measures were performed after 1 and 3 weeks of treatment. Cats in the TRANShigh group were examined at 4 h (peak) and 24 h (trough) after the last morning dose of haloperidol. On the day preceding the PET occupancy measurement, the level of haloperidol in plasma (ng/ml) was measured (dashed lines). The occupancy and haloperidol level values measured in a given cat are represented as a given symbol: cat A, ●; cat B, ▲; cat C, ◆; cat D, ■; and cat E, ▼.

the last dose, respectively. At three weeks of treatment, D_2-receptor occupancies were $84 \pm 4\%$ and $29 \pm 1\%$ at 4 and 24 h after the last dose, respectively. These transiently high levels of D_2-receptor blockade in brain were associated with transiently high levels of haloperidol in plasma, with haloperidol plasma levels ranging from 5.2 to 7.5 ng/ml at peak and from 0.4 to 1.2 ng/ml at trough (Figure 1a).

In the CONThigh group, continuous infusion of haloperidol at 0.25 mg/kg per day led to steady and high levels of D_2-receptor blockade during the whole treatment (Figure 1b). D_2-receptor occupancies were $89 \pm 3\%$ and $88 \pm 1\%$ at 1 and 3 weeks of treatment, respectively (Figure 1b). Haloperidol levels in plasma were high and stable during the 4 weeks of treatment and ranged from 9.0–9.8 ng/ml at 1 week to 7.5–10.1 ng/ml at 3 weeks of treatment (Figure 1b).

In the CONTmod group, continuous infusion of haloperidol at 0.05 mg/kg per day led to steady and moderate levels D_2-receptor blockade during the whole treatment (Figure 1c). D_2-receptor occupancies were $63 \pm 2\%$ and $64 \pm 8\%$ at 1 week and at 3 weeks of treatment, respectively (Figure 1c). Haloperidol levels in plasma remained stable and ranged from 0.8 to 2.8 ng/ml throughout the treatment (Figure 1c).

These data thus showed that it is possible to produce either transient or steady levels of D_2-receptor blockade using the same drug but different administration schedules.

Determination of D_2-Receptor Density, Apparent Affinity, and BP_{ND} During Withdrawal

After the 4 weeks of chronic treatment with haloperidol, cats were withdrawn from the drug and D_2-receptor density and apparent affinity was determined in each animal at 1 , 2, and 3 weeks following withdrawal. Scatchard analyses of [^{11}C]raclopride binding revealed that D_2-receptor blockade exceeding 80% for only a few hours a day (TRANShigh group) did not lead to any significant changes in either B_{max} (Figure 2a) or K'_D (Figure 3a). In contrast, D_2-receptor blockade exceeding 80% continuously throughout the day (CONThigh group) led to a significant increase in D_2-receptor density that was maximal at 1-week withdrawal ($35 \pm 5\%$; $p < 0.01$; $n = 3$; Student's t-test; Figure 2b) and still detectable at 2-week withdrawal ($20 \pm 3\%$; $P < 0.05$; $n = 2$; Student's t-test; Figure 2b). D_2-receptor densities were within the ranges of baseline values at 3-week withdrawal (Figure 2b). No significant changes in the affinity of the receptors for [^{11}C]raclopride was detected at any time following the withdrawal (Figure 3b). Moderate ($\sim 60\%$) and continuous D_2-receptor blockade (CONTmod group) did not lead to any significant changes in either B_{max} or K'_D (Figures 2c and 3c).

The levels of haloperidol in plasma were below the limit of detection (0.4 ng/ml) at 1, 2, and 3-week withdrawal in the three treatment conditions.

As K'_D remained relatively stable in each treatment condition, BP_{ND} were calculated from each high SR experiment of the Scatchard analyses and compared with the B_{max} values obtained from saturation. In the TRANShigh (Figure 2d) and CONTmod (Figure 2f) groups, no significant change in BP_{ND} was detected throughout the treatment. In the CONThigh group, a significant increase in BP_{ND} was detected at 1-week withdrawal ($38 \pm 10\%$; $p = 0.01$; $n = 3$; Student's t-test; Figure 2e). At 2-week withdrawal, BP_{ND} were still increased but the difference did not reach statistical significance ($18 \pm 6\%$; $p > 0.05$; $n = 2$; Student's t-test; Figure 2e).

Effects of Chronic Treatment with Haloperidol on Spontaneous Locomotor Activity

The changes in spontaneous locomotor activity induced by each haloperidol treatment schedule are shown in Figure 4.

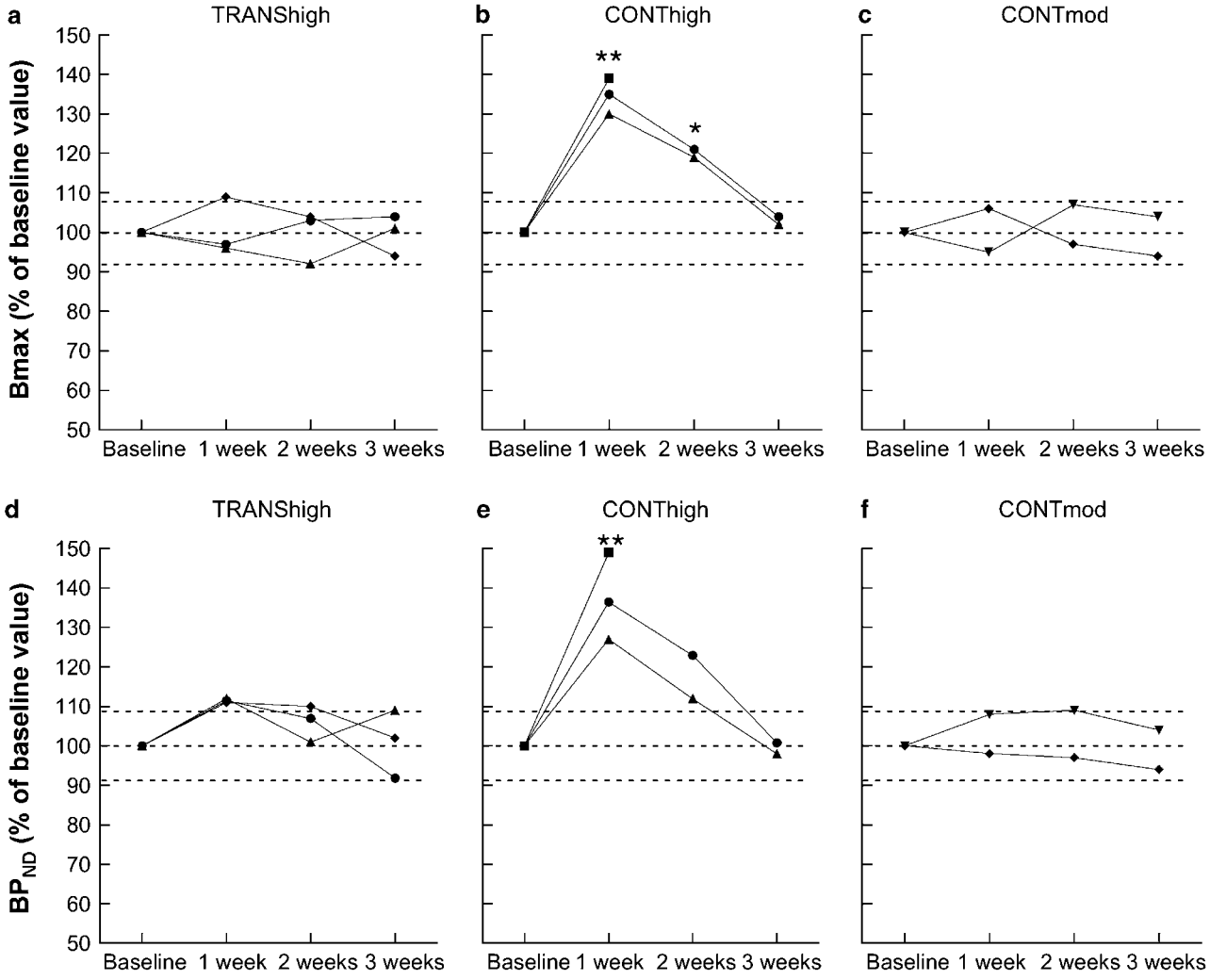

Figure 2 Changes in D_2-receptor density (B_{max}) and BP_{ND} during withdrawal from chronic treatment with haloperidol in the TRANShigh (a and d; $n=3$), CONThigh (b and e; $n=3$), and CONTmod (c and f; $n=2$) groups. Panels (a), (b), and (c) show the longitudinal changes in B_{max} whereas panels (d), (e) and (f) show the corresponding changes in BP_{ND}. In each cat, B_{max} and BP_{ND} were measured at baseline conditions and at 1, 2, and 3-week withdrawal from haloperidol. For each cat, the B_{max} or BP_{ND} value obtained at baseline was set at 100%, and the values obtained during withdrawal were expressed as a percentage of this baseline value. Dashed lines represent the 95% confidence limits of the mean B_{max} and BP_{ND} values measured in the test–retest study. The B_{max} values obtained in a given cat are represented as a given symbol: cat A, ●; cat B, ▲; cat C, ◆; cat D, ■; and cat E, ▼. *$p<0.05$ and **$p<0.01$.

In the TRANShigh group, locomotor activity levels measured during the light phase (ie from 4 to 8 h post injection of haloperidol) were decreased by 62, 58, 60, and 60% at 1, 2, 3, and 4 weeks of treatment, respectively, when compared with the mean baseline measurement (Figure 4, top). Cats had virtually the same low levels of spontaneous activity throughout the 4 weeks of treatment with no significant change in motor activity observed throughout the course of the treatment (Figure 4, top). Locomotor activity returned to baseline levels as soon as daily injections of haloperidol were stopped (Figure 4, top). During the dark phase (ie from 10 to 22 h post injection of haloperidol), activity levels in the TRANShigh group were decreased by only 14, 11, 15, and 12% at 1, 2, 3, and 4 weeks of treatment, respectively, when compared with the mean baseline measurement (data not shown). The mean activity level measured during the light phase was significantly ($p<0.001$; $n=3$; Student's t-test) lower than that measured during the dark phase, suggesting a maximal reduction of spontaneous activity during the few hours following drug administration and a progressive recovery thereafter during the dark phase.

The CONThigh group also showed a dramatic decrease (reaching 70%) in spontaneous locomotor activity during the first week of treatment (Figure 4, middle). In contrast to what was observed in the TRANShigh group, this effect on locomotor activity was significantly attenuated after 3, and 4 weeks of treatment ($p<0.001$; $n=3$; Student's t-test) when compared with the first week of treatment. This result suggests that behavioral tolerance to the effect of the drug on spontaneous locomotor activity developed when haloperidol was administered continuously at high dose. Locomotor activity normalized to baseline values after withdrawal and was not significantly different than that measured at baseline.

Only moderate decreases in locomotor activity were observed in the CONTmod group during the treatment (Figure 4, bottom). Activity levels were decreased by 36, 37,

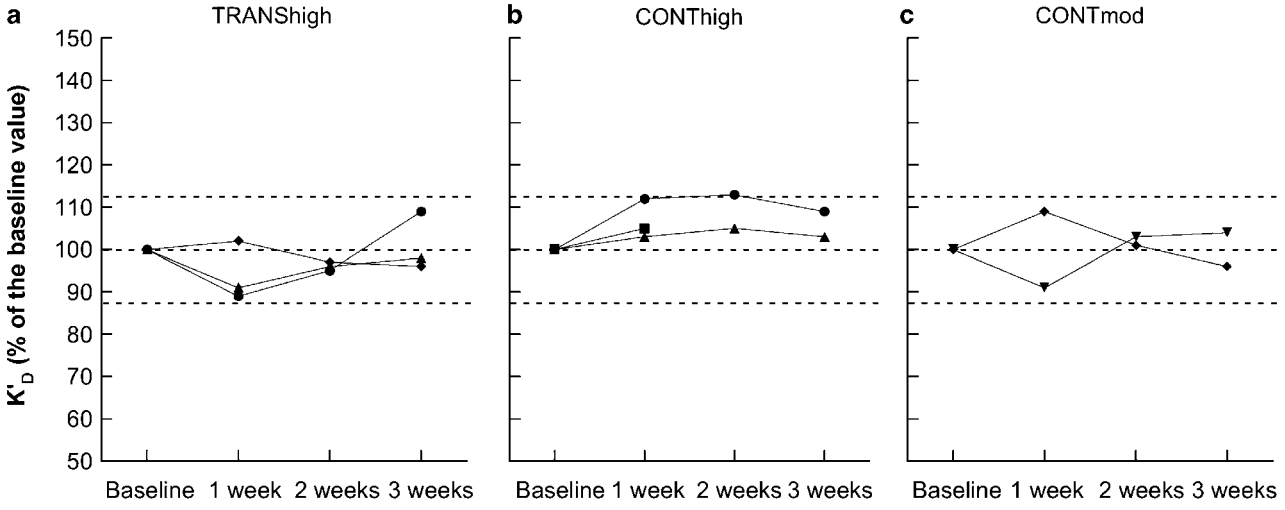

Figure 3 Changes in D_2-receptor apparent affinity (K'_D) during withdrawal from chronic treatment with haloperidol in the TRANShigh (a; $n=3$), CONThigh (b; $n=3$), and CONTmod (c; $n=2$) groups. In each cat, K'_D was measured at baseline conditions and at 1-, 2-, and 3-week withdrawal from haloperidol. For each cat, the K'_D value obtained at baseline was set at 100%, and the values obtained during withdrawal were expressed as a percentage of this baseline value. Dashed lines represent the 95% confidence limits of the mean K'_D values measured in the test–retest study. The K'_D values obtained in a given cat are represented as a given symbol: cat A, ●; cat B, ▲; cat C, ◆; cat D, ■; and cat E, ▼.

31, and 38% at 1, 2, 3, and 4 weeks of treatment, respectively, when compared with baseline measurements. No significant change in motor activity was observed throughout the course of the treatment (Figure 4, bottom). After removal of the osmotic minipumps, the mean locomotor activity did not fully normalize to the mean baseline value and was still 12% significantly lower ($p<0.01$; $n=2$; Student's *t*-test) than that measured at baseline.

DISCUSSION

This study shows that the temporal pattern of D_2-receptor blockade induced by a specific antipsychotic drug, haloperidol, is an important determinant for the induction of D_2-receptor upregulation and for the development of behavioral tolerance.

Although several studies have examined D_2-receptor upregulation and/or behavioral tolerance in response to an antipsychotic, none has systematically controlled for the effects of dose, duration, and *in-vivo* occupancy—leading to inconsistent results (Bannet *et al*, 1980; Carey and DeVeaugh-Geiss, 1984; Koller, 1984; Kashihara *et al*, 1986; Akiyama *et al*, 1987; Csernansky *et al*, 1990). Inconsistencies are possibly explained by differences in dosing, interval between last dose and D_2-receptor measurement, and type of radioligand employed for D_2 measurement. For example, all aforementioned studies have used haloperidol doses ranging from 0.5 to 5 mg/kg per day and no reason was given for using such high doses. As the dose of haloperidol giving rise to a 50% D_2-receptor occupancy in the rat brain is about 0.01 mg/kg (Kapur *et al*, 2000a), such highly saturating doses, even when given as daily injections, likely induced prolonged D_2-receptor blockade in brain.

In this study, we first aimed at using a haloperidol treatment regimen in cats as representative as possible of the human treatment condition. Pharmacokinetic equivalency in cats was established by selecting a dose of haloperidol that produced peak levels of D_2-receptor occupancy similar to those measured in humans and by selecting a dosing schedule that produced a similar pattern of occupancy kinetics within a 24 h dosing interval. Successful treatment of schizophrenic patients with haloperidol is achieved with levels of D_2-receptor blockade exceeding 70% with extrapyramidal symptoms arising when D_2-receptor blockade exceeds 78% (Farde *et al*, 1992; Nyberg *et al*, 1995; Kapur *et al*, 2000b). Such high levels of receptor blockade are maintained continuously during treatment as demonstrated by PET studies, showing that a single clinical dose of haloperidol is sufficient to occupy more than 75% of the receptors for at least 27 h (Nordstrom *et al*, 1992). Based on these considerations, continuously high (>80% throughout the day) levels of D_2-receptor blockade were produced in cats by constant infusion of haloperidol at 0.25 mg/kg per day through osmotic minipumps. Such a treatment regimen in cats produced stable levels of haloperidol in plasma and high and stable (exceeding 78% throughout the day) levels of D_2-receptor occupancy in brain. This reproduces the pattern of D_2-receptor blockade achieved in clinical treatment. This pattern of D_2-receptor blockade produced a robust upregulation of striatal D_2-receptors and induced the development of behavioral tolerance to the effect of haloperidol on spontaneous locomotor activity. These consequences are similar to those reported in patients on haloperidol medication, in whom stable haloperidol plasma concentrations and high levels of D_2-receptor occupancies are maintained, and who show D_2-upregulation in the striatum (Lee *et al*, 1978; Mita *et al*, 1986; Silvestri *et al*, 2000). Although this continuous pattern of high D_2-receptor blockade produced the undesirable effects of the drug, continuous but only moderate (~60% throughout the day) levels of D_2-receptor blockade did not produce any of these effects. This suggests that there is a threshold of D_2-receptor blockade at which upregulation and tolerance occur.

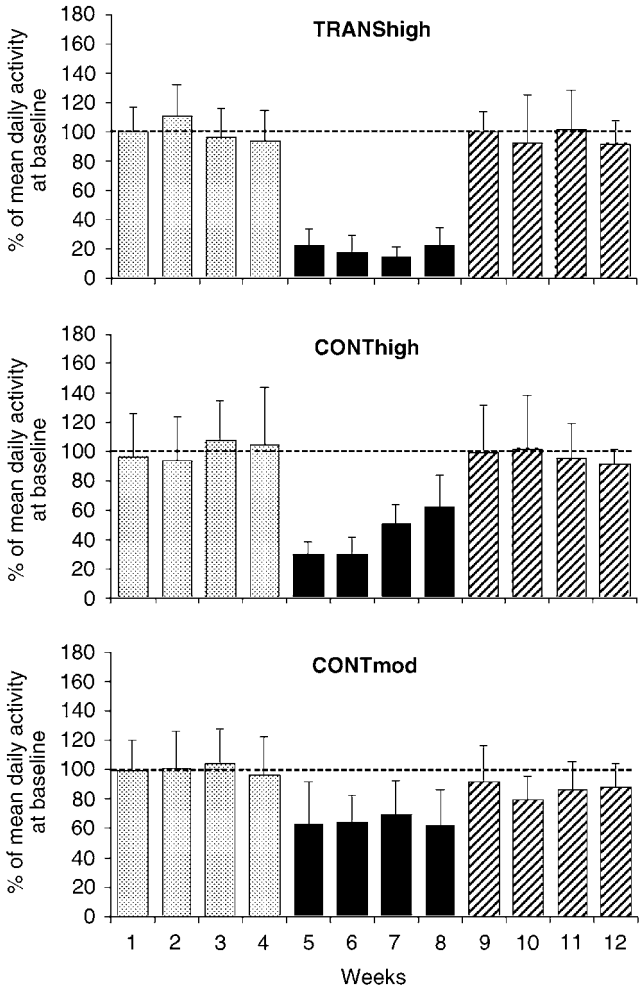

Figure 4 Spontaneous locomotor activity in the TRANShigh (top panel; $n=3$), CONThigh (middle panel; $n=3$), and CONTmod (bottom panel; $n=2$) groups. In each group of cats, spontaneous locomotor activity was monitored for 4 weeks at baseline conditions (weeks 1–4), during the 4-week treatment with haloperidol (weeks 5–8), and for 4 weeks following withdrawal from the drug (weeks 9–12). In the TRANShigh group, daily locomotor activity was evaluated using data collected from 1300 hours to 1700 hours (ie from 4 to 8 h following the 0900 hours daily dose of haloperidol), a time interval including the peak of drug action in brain. In the CONThigh and CONTmod groups, in which D_2-receptor blockade remained stable throughout the day, daily locomotor activity was evaluated using data collected from 0700 hours to 1900 hours. For each cat, the mean daily activity at baseline was calculated using data collected over the 4 week of measurement and was set at 100% of activity. Daily activity data obtained during treatment and after withdrawal were expressed as a percentage of the mean daily activity at baseline, and averaged over 1-week intervals.

Importantly, our study also showed that high but only transient levels of D_2-receptor blockade (exceeding 80% but for only a few hours a day) did not result in significant alterations of D_2-receptor density. This in turn suggests that reaching a certain threshold of D_2-receptor blockade is necessary but not sufficient to promote upregulation and tolerance. The long-term effect of haloperidol treatment on D_2-receptor density and behavioral tolerance thus appears to be dependent not only on a critical threshold of D_2-receptor blockade but also on the daily duration D_2-receptor blockade. Either too short or too low levels of daily D_2-receptor blockade failed to induce receptor upregulation and tolerance. Our results are also consistent with the idea of a close causal relationship between D_2-receptor upregulation and locomotor activity, and support the view that increases in the number of D_2 receptors are associated with increases in D_2-receptor-mediated behaviors.

In the CONThigh group, haloperidol increased the number of D_2-receptors without changes in their affinity for the antagonist (increase in [^{11}C]raclopride B_{max} without changes in K'_D). However, this lack of change in [^{11}C]raclopride affinity does not preclude that chronic haloperidol altered the affinities or magnitudes of the high- and low-affinity components associated with agonist binding to the D_2 receptor. Furthermore, although [^{11}C]raclopride binds with similar affinities to both the D_2 and D_3 receptors (Malmberg *et al*, 1993), it is unlikely that the increased [^{11}C]raclopride binding seen after haloperidol reflects increased D_3-receptor densities. Indeed, previous studies have shown that whereas chronic haloperidol increases D_2-receptor density, it does not affect D_3-receptor density in the rat striatum (Levesque *et al*, 1995; Florijn *et al*, 1997; Joyce, 2001).

Our results coincide nicely with a recent report by Turrone *et al* (2003) who showed that continuously high (*vs* transiently high) levels of D_2-receptor blockade lead to an increased risk for the development of vacuous chewing movements in rodents, a behavior that is routinely regarded as an animal model of TD. In their continuously high group they used doses that led to $>70\%$ occupancy throughout the day, and in their transiently high group they used doses that led to $>80\%$ occupancy at peak and $<17\%$ occupancy at trough. Thus, converging evidence from both rat and cat studies suggest that the pharmacokinetics of D_2-receptor blockade during chronic treatment with haloperidol is important when evaluating the development of D_2-receptor upregulation, related tolerance, and motor side effects.

The reason why continuously high, but not transiently high, D_2-receptor blockade produced D_2-upregulation is not clear. One possibility could be that these postsynaptic adaptive changes are mediated via effects at the signal transduction level. Blocking DA D_2-receptors with haloperidol or other typical antipsychotics has been shown to increase intracellular cAMP levels *in vivo* (Berndt and Schwabe, 1973; Palmer *et al*, 1978; Dias *et al*, 1979; Kaneko *et al*, 1992). On the other hand, long-lasting maintenance of high cAMP levels in Ltk cells transfected with the human D_2-receptor has been shown to induce upregulation of these receptors (Johansson and Westlind-Danielsson, 1994; Wanderoy and Westlind-Danielsson, 1997; Wanderoy *et al*, 1997). One possibility could be that it is the persistently elevated cAMP levels induced by the continuously high treatment schedule that causes D_2-upregulation. Blocking the receptors only transiently during the day would preserve some access of endogenous DA to the receptors. This pulsatory agonist stimulation could be sufficient to lower the total cell exposure to high cAMP levels and prevent a compensatory postsynaptic upregulation. A differential effect of different D_2-occupancy kinetics on cAMP signaling could ultimately result in different changes in synaptic plasticity.

The results from this study might be of clinical relevance. A recent study performed in rodents indicates that the development of D_2-receptor upregulation and supersensitivity

(induced by continuously high levels of D_2-receptor blockade) is directly related to the loss of efficacy of antipsychotic treatment (Samaha *et al*, 2007). As our study showed that transiently high D_2-receptor blockade did not induce receptor upregulation and behavioral tolerance, such a pattern of D_2-receptor occupancy kinetics might have a lower incidence of therapeutic tolerance and supersensitivity psychosis seen in patients placed on a long-term medication regimen. D_2-occupancy transience during clinical treatment might be achieved either by reducing the dose of haloperidol used or by using antipsychotics with fast kinetics. Reducing the dose of haloperidol is however not clinically feasible or advisable because of the drug narrow therapeutic index and marked interindividual variabilities in plasma level and D_2-occupancy attained at a given dose (Kapur *et al*, 1996, 1997; Kapur and Seeman, 2001). Although it is too early to conclude precisely which temporal pattern of D_2-occupancy is desirable and what the clinical trade offs might be, the preclinical data presented here however provide a very strong rationale for examination of these axes (daily transient *vs* daily continuous) as a mechanism of clinical relevance.

In conclusion, this study constitutes the first systematic examination of the parameters of D_2-occupancy that lead to upregulation in clinically relevant doses and occupancies. It suggests that transiently high peaks of D_2-occupancies are less likely to cause D_2-receptor upregulation than continuous high levels of occupancies. In addition, our data also clearly indicate a direct relationship between development of tolerance and D_2-receptor upregulation. This highlights the importance of studying the within-day profile of occupancy (in addition to its peak levels) when evaluating the effects of D_2-based antipsychotics.

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DISCLOSURE/CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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