

# Occupancy of Striatal and Extrastriatal Dopamine D<sub>2</sub> Receptors by Clozapine and Quetiapine

Robert M Kessler<sup>\*1</sup>, M Sib Ansari<sup>1</sup>, Patrizia Riccardi<sup>1</sup>, Rui Li<sup>2</sup>, Karuna Jayatilake<sup>3</sup>, Benoit Dawant<sup>2</sup> and Herbert Y Meltzer<sup>3</sup>

<sup>1</sup>Department of Radiology, Vanderbilt University School of Medicine, Nashville, TN, USA; <sup>2</sup>Department of Electrical Engineering and Computer Science, Vanderbilt University School of Medicine, Nashville, TN, USA; <sup>3</sup>Department of Psychiatry, Vanderbilt University School of Medicine, Nashville, TN, USA

Clozapine and quetiapine have a low incidence of extrapyramidal side effects at clinically effective doses, which appears to be related to their significantly lower occupancy of striatal dopamine D<sub>2</sub> receptors (DA D<sub>2</sub>r) compared to typical antipsychotic drugs (APDs). Animal studies have indicated that clozapine and quetiapine produce selective effects on cortical and limbic regions of the brain and in particular on dopaminergic neurotransmission in these regions. Previous PET and SPECT studies have reported conflicting results regarding whether clozapine produces preferential occupancy of cortical DA D<sub>2</sub>r. To examine whether clozapine and/or quetiapine produce preferential occupancy of DA D<sub>2</sub>r in cortex and limbic regions, we studied the occupancy of putamenal, ventral striatal, thalamic, amygdala, substantia nigra, and temporal cortical DA D<sub>2</sub>r using PET with [<sup>18</sup>F]fallypride in six schizophrenic subjects receiving clozapine monotherapy and in seven schizophrenic subjects receiving quetiapine monotherapy. Doses were chosen clinically to minimize psychopathology at tolerable levels of side effects such as drowsiness. All had minimal positive symptoms at the time of the study. Regional receptor occupancies were estimated using mean regional DA D<sub>2</sub>r levels calculated for 10 off-medication schizophrenic subjects. Both clozapine and quetiapine produced lower levels of putamenal DA D<sub>2</sub>r occupancy than those reported for typical APDs, 47.8 and 33.5%, respectively. Clozapine produced preferential occupancy of temporal cortical vs putamenal DA D<sub>2</sub>r, 59.8% ( $p = 0.05$ , corrected for multiple comparisons), and significantly lower levels of occupancy in the substantia nigra, 18.4% ( $p = 0.0015$ , corrected for multiple comparisons). Quetiapine also produced preferential occupancy of temporal cortical DA D<sub>2</sub>r, 46.9% ( $p = 0.03$ , corrected for multiple comparisons), but did not spare occupancy of substantia nigra DA D<sub>2</sub>r. The therapeutic effects of clozapine and quetiapine appear to be achieved at less than the 65% threshold for occupancy seen with typical APDs, consistent with the involvement of non-DA D<sub>2</sub>r mechanisms in at least partially mediating the therapeutic effects of these drugs. Preferential occupancy of cortical DA D<sub>2</sub>r, sparing occupancy of substantia nigra receptors, and non-DA D<sub>2</sub>r-mediated actions may contribute to the antipsychotic actions of these and other atypical APDs.

Neuropsychopharmacology (2006) 31, 1991–2001. doi:10.1038/sj.npp.1301108; published online 31 May 2006

**Keywords:** PET; clozapine; quetiapine; antipsychotic drugs; dopamine D<sub>2</sub> receptor occupancy

## INTRODUCTION

Clozapine and quetiapine are atypical antipsychotic drugs (APDs) (Meltzer, 1992; Arvanitis and Miller, 1997; Kane *et al*, 2001; Davis *et al*, 2003), which have equivalent and sometimes superior antipsychotic effects to those seen with typical APDs. They produce significantly lower levels of striatal dopamine D<sub>2</sub> receptor (DA D<sub>2</sub>r) occupancy

compared to typical APDs at comparable clinical doses (Nordstrom *et al*, 1993; Kapur *et al*, 1996; Farde *et al*, 1994; Gefvert *et al*, 1998). The lesser occupancy of striatal DA D<sub>2</sub>r is believed to be responsible at least in part for the low incidence of extrapyramidal side effects (EPS) seen with these drugs. Studies of cfos (Robertson *et al*, 1994; Robertson and Fibiger, 1992), chronic deltafosB induction (Vahid-Ansari *et al*, 1996), cortical DA D<sub>2</sub>r (Janowsky *et al*, 1992; Florijn *et al*, 1997), cortical DA D<sub>2</sub>r mRNA (Damask *et al*, 1996; Lidow and Goldman-Rakic, 1997), and regional DA release (Yamamoto and Cooperman, 1994; Youngren *et al*, 1999; Kuroki *et al*, 1999; Ichikawa *et al*, 2002) suggest that the atypical profile of clozapine and quetiapine may be mediated by preferential effects on mesocortical and mesolimbic vs nigrostriatal dopaminergic projections. Chronic treatment with clozapine and quetiapine produces

\*Correspondence: Dr RM Kessler, Department of Radiology, Vanderbilt University School of Medicine, 21st and Garland Avenue, Nashville, TN 37232, USA, Tel: +1 615 343 3938, Fax: +1 615 343 6531, E-mail: Robert.kessler@vanderbilt.edu

Received 4 November 2005; revised 23 March 2006; accepted 25 April 2006

Online publication: 26 April 2006 at <http://www.acnp.org/citations/Npp042606050666/default.pdf>

depolarization inactivation of ventral tegmental dopaminergic neurons, sparing those in the substantia nigra, whereas haloperidol inactivates both (Chiodo and Bunney, 1983; Goldstein *et al*, 1993). Whereas clozapine and quetiapine bind to multiple cerebral neurotransmitter receptors (Schotte *et al*, 1996), the above studies suggest that the atypical profile of these drugs may be mediated, at least in part, by preferential effects on DA D<sub>2</sub>r-mediated neurotransmission in cortex and limbic regions, compared to the dorsal striatum.

Some but not all imaging studies of occupancy of extrastriatal DA D<sub>2</sub>r by clozapine and quetiapine report preferential occupancy of cortical DA D<sub>2</sub>r compared to striatal DA D<sub>2</sub>r, consistent with the hypothesis that the therapeutic effects of these drugs are mediated by cortical and/or limbic DA D<sub>2</sub>r. There have been four studies comparing the occupancy of extrastriatal DA D<sub>2</sub>r by clozapine to typical APDs (Pilowsky *et al*, 1997; Talvik *et al*, 2001; Xiberas *et al*, 2000; Grunder *et al*, 2006) that have produced conflicting results regarding whether clozapine produces preferential occupancy of cortical DA D<sub>2</sub>r and a single study of the occupancy of extrastriatal DA D<sub>2</sub>r by quetiapine that reported preferential occupancy of cortical DA D<sub>2</sub>r (Stephenson *et al*, 2000). The studies of clozapine's occupancy of extrastriatal DA D<sub>2</sub>r from Pilowsky *et al*, Talvik *et al*, and Xiberas *et al* have been criticized on methodological grounds (Kessler and Meltzer, 2002; Olsson and Farde, 2001; Erlandsson *et al*, 2003). Grunder's recent study utilized normal control subjects to compute regional occupancies in schizophrenic subjects; this may bias the results, as a number of studies have reported decreased DA D<sub>2</sub>r levels in the thalamus and temporal cortex in schizophrenics (Talvik *et al*, 2003; Yasuno *et al*, 2004; Tuppurainen *et al*, 2003; Buchsbaum *et al*, 2004). The quetiapine study (Stephenson *et al*, 2000) used the same methodology criticized by Olsson (Olsson and Farde, 2001). In a study of olanzapine-treated schizophrenic patients, we have reported no preferential occupancy of cortical DA D<sub>2</sub>r but sparing of nigral DA D<sub>2</sub>r occupancy using the same methods utilized here (Kessler *et al*, 2005).

To evaluate whether clozapine and/or quetiapine produce preferential or nonuniform occupancy of DA D<sub>2</sub>r in extrastriatal regions, we used PET with [<sup>18</sup>F]fallypride (Kessler *et al*, 2000; Mukherjee *et al*, 2002) to measure the levels of DA D<sub>2</sub>r occupancy in putamen, ventral striatum, thalamus, amygdala, temporal cortex, and substantia nigra in schizophrenic subjects who were treated with either clozapine or quetiapine monotherapy. [<sup>18</sup>F]Fallypride is a high-affinity radioligand for DA D<sub>2</sub> and D<sub>3</sub> receptors that can be used to quantitate levels of DA D<sub>2</sub>/3r in man in both striatal and extrastriatal regions with a single tracer injection. The results of this study have been previously communicated in an abstract (Kessler *et al*, 2002).

## METHODS

### Subjects

This study was conducted under protocols approved by the Vanderbilt University and Centerstone Mental Health Center Institutional Review Boards. All subjects provided

informed consent for this study. Subjects meeting the Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition (DSM IV) (American Psychiatric Association, 1994) criteria, and Research Diagnostic Criteria (Andreasen *et al*, 1977) for the diagnosis of schizophrenia without a history of significant medical illness or trauma between the ages of 18 and 50 were recruited. Subjects were evaluated using the Brief Psychiatric Rating Scale (BPRS). All subjects were judged capable of giving informed consent by a senior research psychiatrist and provided informed consent for this study. The diagnosis of schizophrenia was established by the Structured Clinical Interview for DSM IV Axis I disorders (SCID-I) (First *et al*, 1996) and checklist; significant medical conditions and substance abuse other than nicotine use were criteria for exclusion. All subjects had a medical history and physical examination, complete blood count with differential, plasma electrolytes, glucose, blood urea nitrogen, creatinine, calcium, total protein, albumin, bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, cholesterol, triglyceride, and uric acid determinations, a urine analysis, and urine drug screen. Subjects participating in this study were either never treated or were off medication for at least 3 weeks (*N* = 10, five male, five female; mean age of 31.8 ± 8.5 (SD) years and age range of 20–45 years), or chronically treated with clozapine monotherapy (*N* = 6, four male, two female; mean age of 31.8 ± 10.2 (SD) years and age range of 18–46 years) or quetiapine monotherapy (*N* = 7, four male, three female; mean age of 37.9 ± 7.5 (SD) years and age range of 22–45 years). Four of the off-medication subjects were never treated with any APD. Treated subjects had their doses determined using open dosing to achieve optimal therapeutic effects and had been treated for a minimum of 6 months at the time of the PET study. EPS were assessed using the AIMS (Fann *et al*, 1977) and were minimal in both clozapine- and quetiapine-treated subjects (identical mean ratings of 0.40 ± 0.89, range 0–2.0 for each group). Clozapine-treated subjects were studied approximately 14–15 h after their last daily dose, that is, 250 mg (*N* = 1), 450 mg (*N* = 2), 500 mg (*N* = 1), 700 mg (*N* = 1), or 900 mg (*N* = 1). Quetiapine-treated subjects were studied 2 h following the last oral dose, that is, 200 mg (*N* = 3), 350 mg (*N* = 1), 400 mg (*N* = 2), or 700 mg (*N* = 1), owing to transient occupancy of cerebral DA D<sub>2</sub>r by quetiapine (Kapur *et al*, 2000).

### Data Acquisition and Analysis

An MRI of the brain was performed using a GE 1.5 T Signa LXi echo-speed MRI scanner. Acquisitions included thin section high-resolution T1-weighted gradient echo acquisitions (IR prepared FSPGR, 512 × 224 matrix, TE = 1.8, TR = 10–13, IR = 400) in the sagittal plane (1.3 mm thick slices) and coronal planes (1.4–1.5 mm thick slices); axial spin density weighted (fast spin echo, TE = 19, TR = 5,000, 3 mm thick slices) and T2-weighted (fast spin echo, TE = 102, TR = 5,000, 3 mm thick slices) acquisitions were obtained as well. PET scans were performed using a GE Advance PET scanner in the 3-D acquisition mode. [<sup>18</sup>F]Fallypride (4–5 mCi, specific activity > 2000 Ci/mmol) was injected intravenously over a 20 s period and serial

scans of increasing duration were obtained from 180 to 240 min, depending on the medication status of the subject; unmedicated subjects were scanned for 240 min, whereas medicated subjects were scanned for 180–240 min depending on the residual counts. A measured attenuation correction was utilized for all scans. All female subjects had a plasma beta HCG determination performed within the 24 h preceding the PET study.

Serial PET scans were coregistered to each other using a rigid-body mutual information algorithm (Maes *et al*, 1997; Wells *et al*, 1996). Both PET and MRI scans were interpolated to a 256 × 256 × 256 matrix, coregistered using the same rigid-body mutual information algorithm, and reoriented to the ACPC line. Regions of interest were identified on the IR prepared SPGR thin section T1-weighted MRI images, and automatically transferred to the coregistered PET studies. The putamen and thalamus were manually drawn by a neuroradiologist with considerable PET experience (RMK) on multiple axial slices from 2 to 12 mm above the ACPC line. The ventral striatum was defined using the criteria of Mawlawi *et al* (2001). The substantia nigra can be stereotactically localized in the ventral midbrain 9–14 mm below the ACPC line (Schaltenbrand and Wahren, 1977) and can be easily visualized in the midbrain on PET [<sup>18</sup>F]fallypride scans (see Figure 2). The amygdala can be easily visualized in the anteromedial temporal lobe on MRI scans just anterior to the tip of the temporal horn of the lateral ventricle and deep to the uncus (Kessler *et al*, 2005); stereotactically, the amygdala is localized approximately 6–20 mm below the ACPC line, from 12 to 28 mm lateral to the midline, and from 2 to 12 mm behind the plane of the anterior commissure (Schaltenbrand and Wahren, 1977). To decrease partial voluming from the striatum, regions of interest for the amygdala were drawn on the MRI images from 10 to 16 mm below the plane of the ACPC. Regions of interest for the temporal cortex were manually drawn on axial MRI images from 35 to 25 mm below the ACPC. Our previous studies have shown excellent inter-subject reliability for these regions of interest, that is, inter-subject coefficients of variation of 6.8–15.9% (Riccardi *et al*, 2005). Regional levels of DA D<sub>2</sub>r were estimated using the reference region method with the cerebellum used as the reference region (Lammertsma *et al*, 1996). We and others have shown that the cerebellum is an appropriate reference region (Kessler *et al*, 2000, 2005; Grunder *et al*, 2006; Siessmeier *et al*, 2005) and that reference region method estimates of binding potentials are highly correlated with ( $r > 0.99$ ) and not statistically different from those obtained using modeled estimates with a metabolite corrected plasma input function (Kessler *et al*, 2000, 2005; Siessmeier *et al*, 2005).

### Statistical Analysis

The regional occupancies of DA D<sub>2</sub>r were calculated using the appropriate regional mean value for off-medication schizophrenic subjects as follows:

$$\text{percent occupancy in region}_i = \left[ 1 - \frac{\text{DA D}_{2r_i}(\text{medicated})}{\text{DA D}_{2r_i}(\text{unmedicated})} \right] \times 100$$

where DA D<sub>2r<sub>i</sub></sub> represents the available level of DA D<sub>2</sub>r in region 'i' in the indicated state. To evaluate whether clozapine and/or quetiapine produced selective and/or nonuniform occupancy of DA D<sub>2</sub>r in extrastriatal brain regions compared to that in the putamen, an analysis of variance for regional DA D<sub>2</sub>r occupancy with region as a factor and covaried for putamenal occupancy was performed. Bonferroni corrections were used to adjust significance levels for multiple comparisons. Correlations of DA D<sub>2</sub>r occupancy in the substantia nigra with other brain regions were performed using Pearson product moment correlations.

### RESULTS

Individual and mean regional levels of DA D<sub>2</sub>r occupancy for clozapine and quetiapine are shown in Table 1 and Figure 1. An analysis of variance for DA D<sub>2</sub>r occupancy with region as a factor and covaried for putamenal occupancy was performed for clozapine-treated subjects to determine whether there was preferential or nonuniform occupancy in extrastriatal regions in comparison to the putamen. DA D<sub>2</sub>r occupancy for the temporal cortex, 59.8%, was significantly higher than that seen in the putamen, 47.8% ( $p = 0.0033$  uncorrected for multiple comparisons,  $p = 0.05$  corrected for multiple comparisons). DA D<sub>2</sub>r occupancy in the substantia nigra was significantly lower than in all other regions sampled ( $p = 0.0001$  uncorrected for multiple comparisons,  $p = 0.0015$  corrected for all multiple comparisons). Mean DA D<sub>2</sub>r occupancy was 18.4% in the substantia nigra vs 47.8% in the putamen for clozapine-treated subjects. No other region demonstrated a significantly lower occupancy compared to the putamen. These results are illustrated in Figure 2 as well as in the time activity curves shown in Figure 3, and suggest that at clinically therapeutic doses, clozapine produces preferential occupancy of temporal cortical DA D<sub>2</sub>r and spares occupancy of substantia nigra receptors in comparison to that seen in the putamen.

An analysis of variance for DA D<sub>2</sub>r occupancy in quetiapine-treated subjects was performed using region as a factor and covaried for putamenal occupancy. The results demonstrated significantly higher DA D<sub>2</sub>r occupancy in the temporal cortex, 46.9%, than in the putamen, 33.5% ( $p = 0.002$ , uncorrected for multiple comparisons,  $p = 0.03$  corrected for multiple comparisons). Unlike clozapine, no significant difference in occupancy was found between the substantia nigra and the putamen. Mean DA D<sub>2</sub>r occupancy in the substantia nigra was 34.3%, whereas that in the putamen was 33.5%. No other regions showed a significant difference with the putamen after correction for multiple comparisons. Quetiapine appeared to produce preferential occupancy of temporal cortical DA D<sub>2</sub>r in comparison to that in the putamen, as demonstrated in Table 1 and Figure 1.

Given the difference between clozapine and quetiapine in sparing DA D<sub>2</sub>r occupancy in the substantia nigra, the relationships of DA D<sub>2</sub>r occupancies in the substantia nigra to other brain regions were examined for each drug using inter-regional correlations (Table 2). Occupancies in the substantia nigra were not significantly correlated with

**Table 1** Regional Occupancies of Striatal and Extrastriatal DA D<sub>2</sub>r by Clozapine and Quetiapine

Dose (mg)	Percent regional occupancy					
	Putamen	Ventral striatum	Thalamus	Amygdala	Temporal cortex	Substantia nigra
<i>Clozapine</i>						
250	28.5	24.5	35.1	40.1	41.0	18.1
450	67.5	66	73.8	64.9	73.4	34.6
450	32.7	27.2	42.4	36.9	53.3	18.2
500	56.6	51.9	63.8	56.9	69.9	11.9
700	48.2	52.9	58.4	53.7	62.7	2.8
900	53.0	53.7	54.8	60.7	58.4	24.9
Mean ± SD	47.8 ± 14.8	46.0 ± 16.5	54.7 ± 14.1	52.2 ± 11.3	59.8 ± 11.8*	18.4 ± 10.8 <sup>†</sup>
<i>Quetiapine</i>						
200	17.1	15.8	17.2	42.2	37.8	16.7
200	22.6	23.6	26.7	31.5	22.2	29.1
200	30.0	33.7	46.7	34.3	60.3	30.5
350	19.9	15.8	38.4	37.9	40.5	15.8
400	50.4	63.5	56.7	64.3	54.4	57.2
400	44.1	39.1	46.2	41.1	53.0	34.5
700	49.7	49.4	48	51.4	60.0	49.4
Mean ± SD	33.5 ± 14.5	34.4 ± 17.8	40.0 ± 13.7	43.2 ± 16.3	46.9 ± 14.0 <sup>‡</sup>	34.3 ± 12.9

\*Significantly different from putamenal occupancy for clozapine ( $p \geq 0.05$ , corrected for multiple comparisons).

<sup>†</sup>Significantly different from putamenal occupancy for clozapine ( $p = 0.0015$ , corrected for multiple comparisons).

<sup>‡</sup>Significantly different from putamenal occupancy for quetiapine ( $p = 0.03$ , corrected for multiple comparisons).

occupancies in other brain regions for clozapine-treated subjects; correlation coefficients ranged from 0.17 to 0.37. For quetiapine-treated subjects, occupancy in the substantia nigra was significantly correlated with occupancy in all other regions except for the temporal cortex; correlation coefficients ranged from 0.81 to 0.95 for the putamen, ventral striatum, thalamus, and amygdala, but fell to 0.52 for the temporal cortex (see Table 2). These findings suggest that clozapine produces occupancy of nigral DA D<sub>2</sub>r in a manner different from that in other brain regions. In quetiapine-treated subjects, nigral occupancy appears to be regulated similarly to that in most other brain regions.

Comparing mean regional occupancies for quetiapine-treated subjects to those seen in clozapine-treated subjects reveals that, except for the substantia nigra, occupancies were lower in quetiapine-treated subjects. For subjects receiving 200–350 mg single doses of quetiapine, the mean DA D<sub>2</sub>r occupancies were 22.4 and 40.2% in the putamen and temporal cortex respectively, whereas at doses of 400–700 mg mean occupancies were 48.2 and 55.8%, respectively—similar to the occupancies seen in clozapine-treated subjects, that is, 47.8 and 59.8%, respectively. These data suggest that for quetiapine to achieve occupancies in striatum and cortex comparable to those seen with clozapine, single doses of 400 mg or greater may be required. The total BPRS scores were  $11.8 \pm 10.9$  in clozapine-treated subjects and  $16.6 \pm 7.9$  in quetiapine-treated subjects; this difference was not significant for the groups studied. Subjects treated with higher doses of quetiapine, 400–700 mg, had lower total BPRS scores than

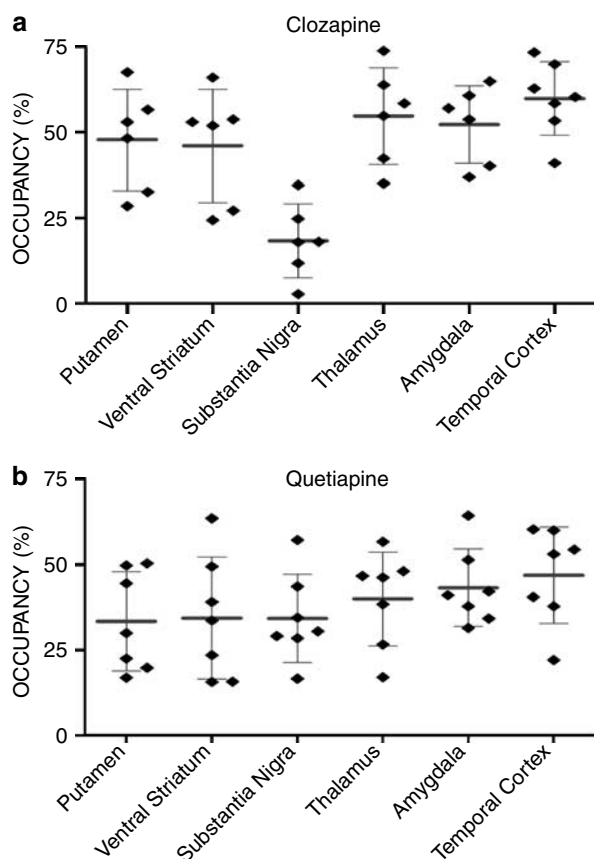
subjects treated with lower doses, 200–350 mg, being 13.7 vs 18.8; this difference was not significant in this small sample.

## DISCUSSION

The major findings of this study are (1) that both clozapine and quetiapine produce higher, that is, preferential, occupancy of temporal cortical DA D<sub>2</sub>r in comparison to putamenal DA D<sub>2</sub>r occupancy; (2) that clozapine but not quetiapine produces significantly lower DA D<sub>2</sub>r occupancy in the substantia nigra compared to all other regions examined, that is, sparing of nigral DA D<sub>2</sub>r occupancy; and (3) that both clozapine and quetiapine produce significant therapeutic effects at DA D<sub>2</sub>r occupancies in all regions examined less than the 65–70% threshold seen with typical APDs (Nordstrom *et al*, 1993; Kapur *et al*, 1996). The putamenal occupancies reported in this study for clozapine and quetiapine are similar to those reported for [<sup>11</sup>C]raclopride PET studies of DA D<sub>2</sub>r occupancy (Farde *et al*, 1994; Gefvert *et al*, 1998; Tauscher-Wisniewski *et al*, 2002).

Preferential occupancy of temporal cortical vs striatal DA D<sub>2</sub>r has been suggested as a mechanism by which clozapine achieves an atypical profile of APD effects (Pilowsky *et al*, 1997; Grunder *et al*, 2006).

As discussed above, the issue of whether clozapine and/or quetiapine produces preferential occupancy of cortical DA D<sub>2</sub>r has been an area of disagreement in the literature (Pilowsky *et al*, 1997; Xiberas *et al*, 2000; Grunder *et al*, 2006; Stephenson *et al*, 2000; Talvik *et al*, 2001); previous

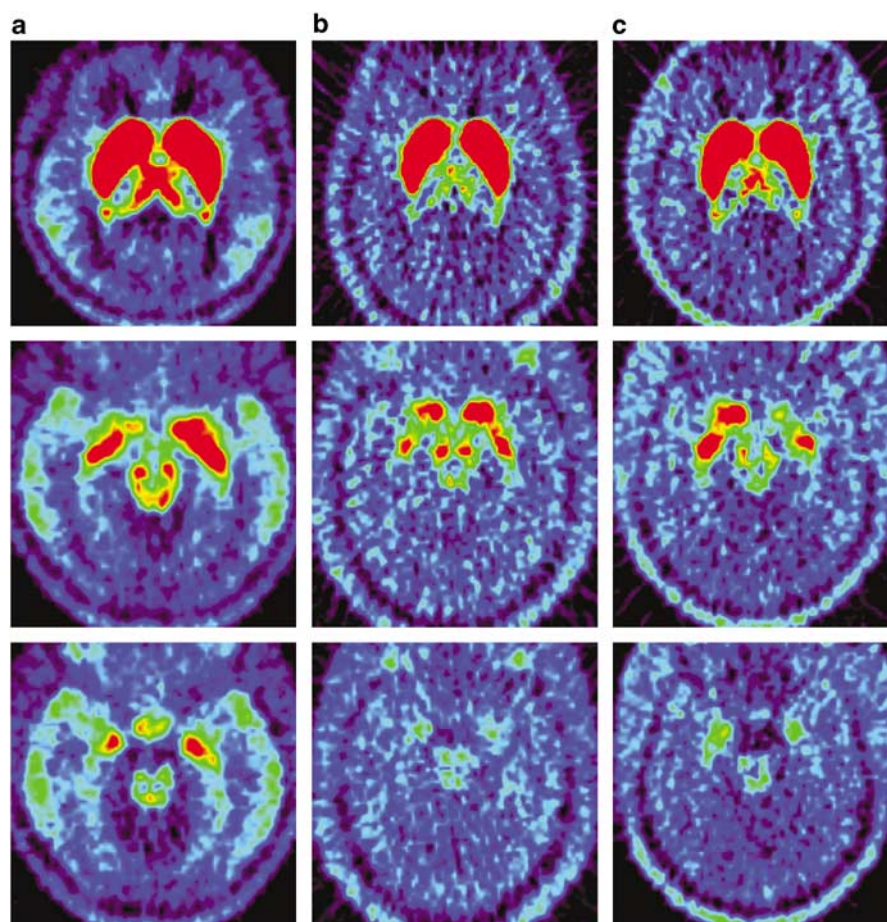


**Figure 1** Scattergrams of individual regional DA D<sub>2</sub>r occupancies are shown for clozapine-treated (a) and quetiapine-treated (b) subjects. Clozapine-treated subjects demonstrate preferential occupancy of temporal cortical DA D<sub>2</sub>r in comparison to putamenal DA D<sub>2</sub>r occupancy and sparing of substantia nigra DA D<sub>2</sub>r occupancy. Quetiapine demonstrated preferential occupancy of temporal cortical DA D<sub>2</sub>r, but did not demonstrate sparing of substantia nigra DA D<sub>2</sub>r.

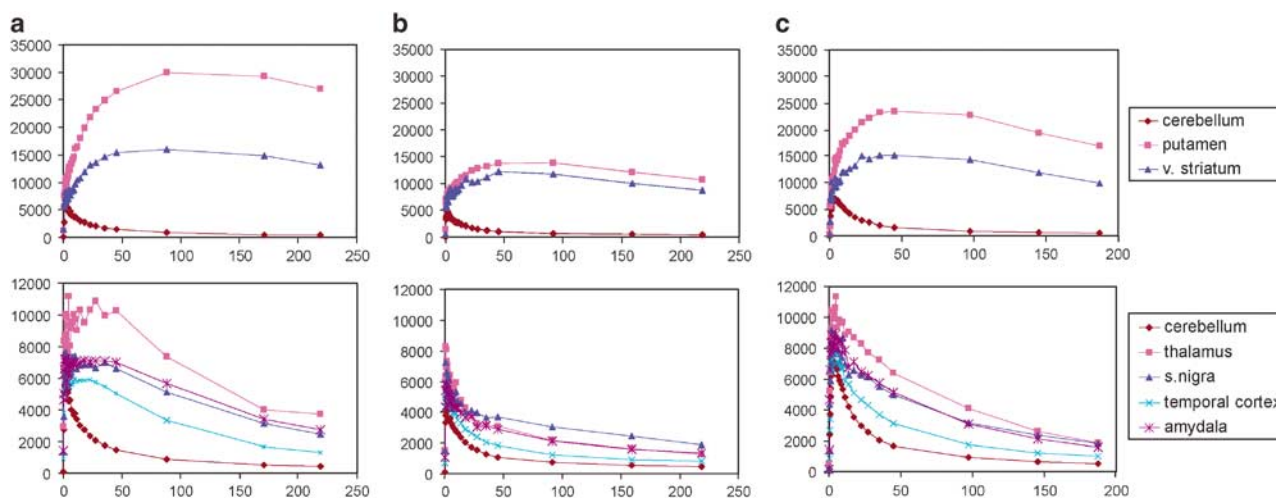
studies may be criticized on methodological grounds (Kessler and Meltzer, 2002; Olsson and Farde, 2001; Erlandsson *et al*, 2003; Yasuno *et al*, 2004; Tuppurainen *et al*, 2003; Buchsbaum *et al*, 2004). The results of the current study indicate that both clozapine and quetiapine produce preferential occupancy of temporal cortical DA D<sub>2</sub>r. The one study that did not report preferential occupancy of temporal cortical receptors by clozapine (Talvik *et al*, 2001) has been criticized because of the use of two different radioligands, that is, [<sup>11</sup>C]raclopride to estimate striatal and [<sup>11</sup>C]FLB457 to estimate cortical DA D<sub>2</sub>r occupancies, and possible effects of low levels of cerebellar DA D<sub>2</sub>r on estimates of cortical occupancy (Kessler and Meltzer, 2002). Occupancies were calculated using the simplified reference region method (Gunn *et al*, 1997). The results showed that clozapine produced similar DA D<sub>2</sub>r occupancies in cortex and striatum, that is, 42.5% in cortex and 43.3% in striatum. Haloperidol produced a mean occupancy of 80.7% in striatum and 61.7% in cortical regions. Although the authors did not report this difference in striatal vs cortical occupancy to be significant for haloperidol, reanalysis of this data shows a significant difference; haloperidol appears to preferentially occupy

striatal DA D<sub>2</sub>r (Kessler and Meltzer, 2002). This is contrary to our previous results using a single radioligand, [<sup>18</sup>F]fallypride, which reported similar DA D<sub>2</sub>r occupancies in the putamen and temporal cortex for haloperidol (Kessler *et al*, 2005). Although both [<sup>11</sup>C]FLB457 and [<sup>11</sup>C]raclopride are benzamides, differences in radioligands may result in different apparent levels of receptor occupancy. Estimates of striatal receptor DA D<sub>2</sub>r occupancy obtained using [<sup>11</sup>C]raclopride with the simplified reference region method are well validated (Lammertsma *et al*, 1996; Lammertsma and Hume, 1996). The presence of low levels of DA D<sub>2</sub>r in the cerebellum (Delforge *et al*, 2001; Olsson *et al*, 1999) causes an underestimation of the level of occupancy, particularly in cortical regions, when using [<sup>11</sup>C]FLB457 with the simplified reference region method (Christian *et al*, 2004; Olsson *et al*, 2004); this underestimation is greater at lower occupancies such as are seen with clozapine and quetiapine. An underestimation of cortical DA D<sub>2</sub>r occupancies could account for both the failure to observe preferential occupancy of cortical DA D<sub>2</sub>r by clozapine and the unexpected lower occupancy of cortical DA D<sub>2</sub>r seen with haloperidol. The difference between studies using [<sup>18</sup>F]fallypride and Talvik *et al*'s study may be related to the use of two different radioligands to estimate striatal and cortical DA D<sub>2</sub>r occupancies. In the present study, we utilized [<sup>18</sup>F]fallypride and employed the reference region method (Lammertsma *et al*, 1996) similar to the study of Talvik *et al* (2001). The use of a single tracer capable of quantitating receptor occupancies in striatum and extrastriatal regions circumvents the potential confounds associated with the use of two radioligands. Although the use of the reference region method with [<sup>18</sup>F]fallypride may also be criticized because of the presence of low levels of cerebellar DA D<sub>2</sub>r (Gunn *et al*, 1997), the level of specific [<sup>18</sup>F]fallypride binding in the cerebellar reference region is low enough, about 3%, not to significantly bias the estimates of regional DA D<sub>2</sub>r occupancy (Kessler *et al*, 2000, 2005; Siessmeier *et al*, 2005).

The [<sup>123</sup>I]epidepride SPECT studies of clozapine and quetiapine (Pilowsky *et al*, 1997; Stephenson *et al*, 2000) have been criticized because of the use of a ratio method before the attainment of a transient equilibrium in striatum, which could lead to underestimation of striatal DA D<sub>2</sub>r receptor occupancy (Olsson and Farde, 2001). This underestimation is greater for regions with higher receptor levels and could spuriously produce an apparent pattern of preferential occupancy of cortical DA D<sub>2</sub>r owing to underestimation of striatal DA D<sub>2</sub>r occupancy. Modeling studies of [<sup>123</sup>I]epidepride have produced conflicting results regarding whether the 3–4 h period used for measurement of temporal cortical–cerebellar ratios by Pilowsky *et al* (1997) and Stephenson *et al* (2000) permits accurate estimation of receptor levels in this region (Fujita *et al*, 1999; Erlandsson *et al*, 2003). Xiberas *et al* (2000), using PET with [<sup>76</sup>Br]FLB457, have reported greater occupancy of temporal cortical than striatal DA D<sub>2</sub>r by clozapine and olanzapine using a ratio measure. Xiberas *et al* reported that 10–20 mg doses of olanzapine produced an apparent striatal DA D<sub>2</sub>r occupancy of 44%; other studies report a 70–80% striatal occupancy and no preferential occupancy of temporal cortical DA D<sub>2</sub>r with these doses of olanzapine (Kessler *et al*, 2005; Nordstrom *et al*, 1998; Kapur *et al*,



**Figure 2** Images of  $[^{18}\text{F}]$ fallypride uptake in (a) an off-medication schizophrenic subject, (b) a clozapine-treated subject, and (c) a quetiapine-treated subject at the level of the thalamus (top row), substantia nigra (middle row), and amygdala (bottom row) at 90 min following  $[^{18}\text{F}]$ fallypride administration. Images for each subject are identically windowed to show the pattern of uptake in extrastriatal regions. The clozapine-treated subject (daily dose of 500 mg) demonstrated 57% DA D<sub>2</sub>r occupancy in the putamen, 64% in the thalamus, 57% in the amygdala, 12% in the substantia nigra, and 70% in the temporal cortex; comparable occupancies for the quetiapine-treated subject (700 mg dose 2 h before scanning) were 50, 48, 51, 49, and 60%, respectively. The pattern of uptake demonstrates sparing of nigral DA D<sub>2</sub>r occupancy for the clozapine- but not the quetiapine-treated subject. There is little diminution of uptake in the substantia nigra for the clozapine-treated subject in comparison to the thalamus, amygdala, and other extrastriatal brain regions.



**Figure 3** Time activity curves of regional brain  $[^{18}\text{F}]$ fallypride uptake are shown for the off-medication schizophrenic subject (a), clozapine-treated subject (b), and quetiapine-treated subject (c) shown in Figure 2. In the normal and quetiapine-treated subjects, the order of uptake is thalamus > substantia nigra = amygdala, whereas in the clozapine-treated subject, the order of uptake is substantia nigra > thalamus = amygdala consistent with the sparing of nigral occupancy seen in clozapine-treated subjects.



**Table 2** Pearson Product Moment Correlations of DA D<sub>2</sub>r Occupancy in Substantia Nigra with Occupancies in Other Brain Regions

Region	Clozapine (p-value)	Quetiapine (p-value)
Putamen	0.37 (0.61)	0.87 (0.01)
Ventral striatum	0.26 (0.61)	0.95 (0.001)
Thalamus	0.26 (0.61)	0.86 (0.01)
Amygdala	0.35 (0.50)	0.81 (0.03)
Temporal cortex	0.17 (0.75)	0.52 (0.23)

The associated *p*-values are shown in parentheses.

1998). This underestimation of striatal DA D<sub>2</sub>r occupancy is consistent with the use of a ratio measure before attainment of a transient equilibrium. In the current study, subjects were scanned for at least 3 h, which is sufficient to produce stable estimates of DA D<sub>2</sub>r binding potentials in all brain regions with [<sup>18</sup>F]fallypride (Kessler *et al*, 2000, 2005; Siessmeier *et al*, 2005).

As noted above, Grunder *et al* (2006) used normal subjects to calculate temporal cortical DA D<sub>2</sub>r occupancies in clozapine-treated schizophrenic subjects. Three (Yasuno *et al*, 2004; Tuppurainen *et al*, 2003; Buchsbaum *et al*, 2004) of the four (Talvik *et al*, 2003; Yasuno *et al*, 2004; Tuppurainen *et al*, 2003; Buchsbaum *et al*, 2004) previously reported studies of extrastriatal DA D<sub>2</sub>r levels in unmedicated schizophrenic subjects have reported lower levels of DA D<sub>2</sub>r in the temporal cortex compared to normal subjects and this difference achieved significance in two of these studies (Tuppurainen *et al*, 2003; Buchsbaum *et al*, 2004). Lower levels of temporal cortical DA D<sub>2</sub>r in schizophrenic subjects could artifactually produce the appearance of preferential occupancy of temporal cortical DA D<sub>2</sub>r by clozapine. To avoid this potential confound, receptor occupancies in this study were calculated using regional levels of DA D<sub>2</sub>r in off-medication schizophrenic subjects. Using off-medication schizophrenic subjects as controls does raise the issue of treatment effects. Although a comparison of the off-medication schizophrenic subjects used in this study to age-matched normal controls is the subject of a separate manuscript in preparation, the mean putamenal binding potentials were nearly identical for the 10 off-medication schizophrenic and 10 age-matched normal control subjects, that is,  $36.08 \pm 4.53$  vs  $37.34 \pm 2.53$ , consistent with previous studies (Farde *et al*, 1990; Hietala *et al*, 1994), and showing no medication effects.

There are a number of possible mechanisms by which clozapine and quetiapine may produce preferential occupancy of cortical DA D<sub>2</sub>r. These include the higher fraction of DA D<sub>2s</sub>r in cortex than striatum (Khan *et al*, 1998), the greater release of DA by clozapine in cortex than striatum (Yamamoto and Cooperman, 1994; Youngren *et al*, 1999; Kuroki *et al*, 1999), differences in modes and level of cortical vs striatal dopaminergic neurotransmission (Garris and Wightman, 1994), and differential upregulation of cortical vs striatal DA D<sub>2</sub>r by clozapine (Damask *et al*, 1996; Lidow and Goldman-Rakic, 1997). Although there is a

higher fraction of DA D<sub>2s</sub>r in cortex than striatum (Khan *et al*, 1998), the relative affinities of clozapine, olanzapine, and haloperidol for the DA D<sub>2s</sub> vs D<sub>2L</sub> receptors are similar (Schotte *et al*, 1996); as olanzapine and haloperidol do not produce preferential cortical occupancy, it is unlikely that this could explain clozapine's preferential cortical DA D<sub>2</sub>r occupancy (Kessler *et al*, 2005). Similarly, it has been shown that [<sup>18</sup>F]fallypride has similar *in vivo* affinity for the DA D<sub>2</sub>r in striatum and extrastriatal regions (Slifstein *et al*, 2004). In primate studies, acute clozapine administration produces a 225% increase in cortical DA release and a 170% increase in striatal DA release (Youngren *et al*, 1999; Kuroki *et al*, 1999). Chronic clozapine administration elevates cortical extracellular DA levels by 74%, but produces no significant change in striatum (Yamamoto and Cooperman, 1994). Given the large increase in striatal DA release, 44%, needed to produce a 1% decrease in striatal [<sup>11</sup>C]raclopride binding potential (Breier *et al*, 1997), it is unlikely that clozapine-induced DA release could lead to the preferential occupancy seen with clozapine or quetiapine. As noted above, chronic clozapine administration upregulates DA D<sub>2</sub>r binding in cortex but not in striatum (Janowsky *et al*, 1992; Florijn *et al*, 1997). Higher levels of cortical DA D<sub>2</sub>r with chronic clozapine therapy would produce spuriously low levels of cortical DA D<sub>2</sub>r occupancy compared to striatum. Differential upregulation of cerebral DA D<sub>2</sub>r does not explain the preferential occupancy of cortical DA D<sub>2</sub>r seen with clozapine.

The difference in modes and levels of dopaminergic neurotransmission in striatum vs cortex is another potential explanation (Garris and Wightman, 1994) for the preferential cortical DA D<sub>2</sub>r occupancy observed with clozapine and quetiapine. Both of these atypical APDs have a low affinity for the DA D<sub>2</sub>r (Schotte *et al*, 1996). Studies of striatal DA D<sub>2</sub>r indicate that, although a significant fraction are extrasynaptic, the majority of striatal DA D<sub>2</sub>r are located at synapses (Levey *et al*, 1993; Descarries *et al*, 1996). In the cortex, dopaminergic neurotransmission appears to be largely extrasynaptic or a volume mode (Garris and Wightman, 1994; Smiley *et al*, 1994; Sesack *et al*, 1998). In striatum, synaptic DA D<sub>2</sub>r will be exposed to transiently high levels of DA during phasic firing; this has been measured as high as 250 nM in the vicinity of the synapse and estimated to be 1.6 μM within the synapse (Kawagoe *et al*, 1992; Venton *et al*, 2003; Garris *et al*, 1994). Between phasic firing of DA neurons, the level of tonic extracellular DA is 10-fold higher in striatum than cortex (Yamamoto and Cooperman, 1994; Youngren *et al*, 1999). APDs with high affinity for the DA D<sub>2</sub>r such as haloperidol will compete more effectively with the high levels of extracellular striatal DA than low-affinity APDs such as clozapine and quetiapine (Schotte *et al*, 1996). In the cortex where DA D<sub>2</sub>r are exposed to much lower levels of extracellular DA owing to both the lower extracellular levels of DA and the volume mode of neurotransmission, the affinity of the APD has less effect on occupancy of the DA D<sub>2</sub>r. This may be one mechanism by which an atypical profile is achieved and is consistent with previous studies relating an atypical profile to low affinity for the DA D<sub>2</sub>r (Meltzer *et al*, 1989; Roth *et al*, 1995; Seeman and Tallerico, 1998).

In regard to the finding of sparing of nigral DA D<sub>2</sub>r occupancy by clozapine, the results of the current study

differ from those reported by Grunder *et al* (2006). This difference is likely owing to differences in resolution and partial voluming in these two studies. The substantia nigra is a small structure, which is sensitive to partial volume effects (Kessler *et al*, 1984). Grunder's study utilized stereotactic normalizations of parametric DA D<sub>2</sub>r images that were smoothed to a resolution of 12 mm. Regions of interest were then located using a template. Images with 12 mm resolution do not allow adequate quantitation of the substantia nigra, as ideally a resolution of 4 mm or higher is needed to completely quantify the substantia nigra (Kessler *et al*, 1984). In the current study, the resolution at the center of the field of view where the substantia nigra is located was about 5 mm and regions of interest were delineated on individual coregistered high-resolution MRI studies and transferred to coregistered PET studies. As can be seen in Figure 2, there is good visualization of the substantia nigra in the current study. Although some loss of quantitation likely occurs in the current study, a partial volume correction was not used, as the exact borders of the substantia nigra are not well defined on high-resolution T1-weighted MRI scans. The identifiability of DA D<sub>2</sub>r levels in this structure when using a PET scanner with high resolution is supported by a number of observations, that is, low mean test-retest error of DA D<sub>2</sub>r levels for this structure—5.2% (Mukherjee *et al*, 2002) the high inter-subject reliability for the substantia nigra we have reported in normal subjects—an 8% coefficient of variation across subjects (Riccardi *et al*, 2006), and the high correlation between PET [<sup>18</sup>F]fallypride binding potentials in extrastriatal regions of human brain, including the substantia nigra, with quantitative autoradiographic measurements of DA D<sub>2</sub>r levels in post-mortem human brain (Rieck *et al*, 2004). The sensitivity of DA D<sub>2</sub>r occupancy measurements for the substantia nigra is demonstrated by the differences in occupancies seen for clozapine and quetiapine in the current study as well as the significant differences in nigral occupancy, which we have previously reported for haloperidol and olanzapine (Kessler *et al*, 2005).

Clozapine, like olanzapine but unlike quetiapine or haloperidol (Kessler *et al*, 2005), produces significantly less occupancy of nigral than putamenal DA D<sub>2</sub>r. The relative sparing of DA D<sub>2</sub>r occupancy seen in the substantia nigra may be another mechanism by which a low incidence of extrapyramidal motor side effects may be achieved. There are a number of observations indicating that nigral dopaminergic neurotransmission plays an important role in the regulation of motor tone and function. In rats, selective blockade of nigral DA D<sub>2</sub>r has been shown to produce increased muscle tone that was not reversed by intravenous apomorphine, despite the presence of intact striatal DA D<sub>1</sub> and D<sub>2</sub> receptors (Double and Crocker, 1995). Age-related motor deficits in rats have been correlated with nigral but not striatal extracellular DA and DA metabolite levels (Gerhardt *et al*, 2002). Recovery of motor function after 6-hydroxydopamine treatment followed by GDNF administration correlated with increased levels of DA release in the substantia nigra even in the absence of change in striatal DA release (Gerhardt *et al*, 1999). Antipsychotic-induced EPS may require a critical level of DA D<sub>2</sub>r blockade in both the striatum and substantia nigra (Crocker and Hemsley, 2001). The lesser blockade of nigral

region DA D<sub>2</sub>r seen with clozapine may be protective against EPS.

The factors mediating the sparing of DA D<sub>2</sub>r occupancy in the substantia nigra, which is seen with clozapine and olanzapine (Kessler *et al*, 2005) but not quetiapine, are unclear. Given the known modulation of substantia nigra dopaminergic neuronal function by 5-HT<sub>2A</sub>-mediated neurotransmission (Sorenson *et al*, 1993; Cobb and Abercrombie, 2003; Bruggeman *et al*, 2000), the correlation of a high 5-HT<sub>2A</sub>:DA D<sub>2</sub> affinity ratio with an atypical profile (Meltzer *et al*, 1989; Roth *et al*, 1995), and the three- to seven-fold higher 5-HT<sub>2A</sub>:DA D<sub>2</sub>r affinity ratio seen with olanzapine and clozapine compared to quetiapine (Schotte *et al*, 1996), the role of 5-HT<sub>2A</sub>-mediated serotonergic neurotransmission in sparing of nigral DA D<sub>2</sub>r occupancy requires further investigation.

In addition to preferential occupancy of cortical and sparing of nigral DA D<sub>2</sub>r occupancy, non-DA D<sub>2</sub>r-mediated mechanisms may be involved in the production of an atypical antipsychotic profile. Previous studies have suggested that a threshold of DA D<sub>2</sub>r occupancy of 65–70% is required for APDs to produce therapeutic effects (Kapur *et al*, 1996; Nordstrom *et al*, 1993). Although clozapine and quetiapine have been shown to have lower occupancy in the striatum at therapeutic doses than seen with typical APDs (Farde *et al*, 1994; Pilowsky *et al*, 1997; Gefvert *et al*, 1998), it has been suggested that clozapine and quetiapine produce preferential occupancy of cortical DA D<sub>2</sub>r, resulting in levels of cortical occupancy similar to those seen with typical APDs (Pilowsky *et al*, 1997; Stephenson *et al*, 2000). While quetiapine produces preferential occupancy of temporal cortical DA D<sub>2</sub>r, all regional DA D<sub>2</sub>r occupancies seen in quetiapine-treated subjects were significantly lower than those previously reported in haloperidol-treated subjects ( $p = 0.02$ – $0.006$ , ANOVA with drug and region as factors, corrected for multiple comparisons) (Kessler *et al*, 2005); there was no significant difference in BPRS total scores for quetiapine- vs haloperidol-treated subjects, that is, scores of 16.6 vs 15.0 (RM Kessler, unpublished data), consistent with previous studies showing similar therapeutic effects for quetiapine and haloperidol (Davis *et al*, 2003; Arvanitis and Miller, 1997). Clozapine has been shown to be superior to typical APDs in neuroleptic-resistant patients (Kane *et al*, 2001; Davis *et al*, 2003). These superior therapeutic effects occur at DA D<sub>2</sub>r occupancies that are significantly lower than those seen with haloperidol-treated subjects in all regions except the temporal cortex ( $p = 0.02$ – $0.0006$ , ANOVA with drug and region as factors, corrected for multiple comparisons), which has nonsignificantly lower occupancy compared to haloperidol, that is, 59.8 vs 70.9% (Kessler *et al*, 2005). These findings suggest that receptor interactions beyond DA D<sub>2</sub>r blockade are involved, at least in part, in the therapeutic effects of these drugs. Non-DA D<sub>2</sub>r-mediated mechanisms may include interactions at 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, NK<sub>3</sub>,  $\alpha_1$  and/or  $\alpha_2$  receptors (Meltzer, 1999; Meltzer *et al*, 2004; Svensson, 2003).

In comparing the levels of DA D<sub>2</sub>r occupancy seen in quetiapine- and clozapine-treated subjects, the results, although preliminary, suggest that single doses of 400 mg or greater may be needed to produce levels of putamenal and temporal cortical DA D<sub>2</sub>r occupancy similar to those seen with clozapine. BPRS scores were lower with single



doses of quetiapine greater than 400 mg. A recent study of quetiapine, 400 mg/day, in treatment-resistant patients with schizophrenia found it to be no more effective than typical APDs (Conley *et al*, 2005) although the current results suggest that the dose utilized may not have been high enough.

In conclusion, preferential occupancy of cortical DA D<sub>2</sub>r, sparing of DA D<sub>2</sub>r occupancy in the substantia nigra, as well as interactions at sites beyond the DA D<sub>2</sub>r may all be mechanisms by which clozapine and quetiapine achieve an atypical antipsychotic profile. As previously suggested (Roth *et al*, 2003; Meltzer *et al*, 2004), an atypical profile of APD action may be achieved by multiple mechanisms.

## ACKNOWLEDGEMENTS

This work was supported by NIMH Grant number 1R01MH60898, by funding from Astra Zeneca, from the William K Warren Medical Research Foundation, the Ritter Foundation, and Centerstone Mental Health Center.

## REFERENCES

- Andreasen NC, Endicott J, Spitzer RL, Winokur G (1977). The family history method using diagnostic criteria. Reliability and validity. *Arch Gen Psychiat* 34: 1229–1235.
- Arvanitis LA, Miller BG (1997). Multiple fixed doses of 'seroquel' (quetiapine) in patients with acute exacerbation of schizophrenia: a comparison with haloperidol and placebo. The seroquel trial 13 study group. *Biol Psychiat* 42: 233–246.
- Breier A, Su T-P, Saunders R, Carson RE, Kolachana BS, De Bartolomeis A *et al* (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc Natl Acad Sci USA* 94: 2569–2574.
- Bruggeman RM, Heeringa M, Westerink BHC, Timmerman W (2000). Combined 5-HT<sub>2</sub>/D<sub>2</sub> receptor blockade inhibits the firing rate of SNR neurons in the rat brain. *Prog Neuro-Psychopharm Biol Psychiat* 24: 579–593.
- Buchsbaum MS, Christian BT, Lehrer DS, Mukherjee J, Siu B, Mantil J (2004). D<sub>2</sub>/D<sub>3</sub> dopamine receptor binding in the thalamus of medication-naïve schizophrenics. *Int J Neuro-psychopharmacol* 7: S430.
- Chiodo LA, Bunney BS (1983). Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *J Neurosci* 3: 1607–1619.
- Christian BT, Narayanan T, Shi B, Morris ED, Mantil J, Mukherjee J (2004). Measuring the *in vivo* binding parameters of [<sup>18</sup>F]-fallypride in monkeys using a PET multiple-injection protocol. *J Cereb Blood Flow Metab* 24: 309–322.
- Cobb WS, Abercrombie ED (2003). Differential regulation of somatodendritic and nerve terminal dopamine release by serotonergic innervation of substantia nigra. *J Neurochem* 84: 576–584.
- Conley RR, Kelly DL, Nelson MW, Richardson CM, Feldman S, Benham R *et al* (2005). Risperidone, quetiapine and fluphenazine in the treatment of patients with therapy-refractory schizophrenia. *Clin Neuropharmacol* 28: 163–168.
- Crocker AD, Hemsley KM (2001). An animal model of extrapyramidal side effects induced by antipsychotic drugs: relationship with a D<sub>2</sub> dopamine receptor occupancy. *Prog Neuro-Psychopharm Biol Psychiat* 25: 573–590.
- Damask SP, Bovenkerk KA, De la Pena G, Hoversten KM, Peters DB, Valentine AM *et al* (1996). Differential effects of clozapine and haloperidol dopamine receptor mRNA expression in rat striatum and cortex. *Brain Res Mol Brain Res* 41: 241–249.
- Davis JM, Chen N, Glick ID (2003). A meta-analysis of the efficacy of second-generation antipsychotics. *Arch Gen Psychiat* 60: 553–564.
- Delforge J, Bottlaender M, Loc'h C, Dolle F, Syrota A (2001). Parametric images of the extrastriatal d<sub>2</sub> receptor density obtained using a high-affinity ligand (FLB 457) and a double-saturation method. *J Cereb Blood Flow Metab* 21: 1493–1503.
- Descarries L, Watkins KC, Garcia S, Bosler O, Doucet G (1996). Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. *J Comp Neurol* 375: 167–186.
- American Psychiatric Association 1994. *Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition (DSM-IV)*. American Psychiatric Association: Washington, DC.
- Double KL, Crocker AS (1995). Dopamine receptors in the substantia nigra are involved in the regulation of muscle tone. *Proc Natl Acad Sci USA* 92: 1669–1673.
- Erlandsson K, Bressan RA, Mulligan RS, Ell PJ, Cunningham VJ, Pilowsky LS (2003). Analysis of D<sub>2</sub> dopamine receptor occupancy with quantitative SPET using the high-affinity ligand [<sup>123</sup>I]epidepride: resolving conflicting findings. *NeuroImage* 19: 1205–1214.
- Fann WE, Stafford JR, Malone RL, Frost Jr JD, Richman BW (1977). Clinical research techniques in tardive dyskinesia. *Am J Psychiat* 134: 759–762.
- Farde L, Nordstrom NS, Halldin C, Sedvall G (1994). D<sub>1</sub>-, D<sub>2</sub>- and 5-HT<sub>2</sub>-receptor occupancy in clozapine-treated patients. *J Clin Psychiat* 9(Suppl BJ): 67–69.
- Farde L, Wiesel FA, Stone-Elander S, Halldin C, Nordstrom AL, Hall H *et al* (1990). D<sub>2</sub> dopamine receptors in neuroleptic-naïve schizophrenic patients: a positron emission tomography study with [<sup>11</sup>C] raclopride. *Arch Gen Psychiat* 47: 213–219.
- First MB, Spitzer RL, Gibbon M, Williams JBW 1996. *Structured Clinical Interview for Axis I DSM-IV Disorders—Patient Edition (with Psychotic Screen) (SCID-I/P) (Version 2.0)*. Biometrics Research Department, New York State Psychiatric Institute: New York.
- Florijn WJ, Tarazi FI, Creese I (1997). Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. *J Pharm Exp Ther* 280: 561–569.
- Fujita M, Selbyl JP, Verhoeff PILG, Ichise M, Baldwin RM, Zoghbi SS *et al* (1999). Kinetic and equilibrium analyses of [<sup>123</sup>I]epidepride binding to striatal and extrastriatal dopamine D<sub>2</sub> receptors. *Synapse* 34: 290–304.
- Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994). Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *J Neurosci* 14: 6084–6093.
- Garris PA, Wightman RM (1994). Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an *in vivo* voltammetric study. *J Neurosci* 14: 442–450.
- Gefvert O, Bergström M, Långström, Lundberg T, Lindström L, Yates R (1998). Time course of central nervous dopamine-D<sub>2</sub> and 5-HT<sub>2</sub> receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel®) in patients with schizophrenia. *Psychopharmacology* 135: 119–126.
- Gerhardt GA, Cass WA, Huettl P, Brock S, Zhang ZM, Gash DM (1999). GDNF improves dopamine function in the substantia nigra but not the putamen of unilateral MPTP-lesioned rhesus monkeys. *Brain Res* 817: 163–171.
- Gerhardt GA, Cass WA, Yi A, Zhang Z, Gash DM (2002). Changes in somatodendritic but not terminal dopamine regulation in aged rhesus monkeys. *J Neurochem* 80: 168–177.

- Goldstein JM, Litwin LC, Sutton EB, Malick JB (1993). Seroquel: electrophysiological profile of a potential atypical antipsychotic. *Psychopharmacology (Berl)* 112: 293–298.
- Grunder G, Landvogt C, Vernalenken, Buchholz H-G, Ondracek J, Siessmeier (2006). The striatal and extrastriatal D2/3 receptor-binding profile of clozapine in patients with schizophrenia. *Neuropsychopharmacology* 31: 1027–1035.
- Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997). Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *NeuroImage* 6: 279–287.
- Hietala J, Syvalahti E, Vuorio K, Nagren K, Lehtikoinen P, Ruotsalainen U et al (1994). Striatal D2 dopamine receptor characteristics in neuroleptics-naïve schizophrenic patients studied with positron emission tomography. *Arch Gen Psychiat* 51: 116–123.
- Ichikawa J, Dai J, Meltzer HY (2002). Atypical antipsychotic drugs, quetiapine, iloperidone, and melperone, preferentially increase dopamine and acetylcholine release in rat medial prefrontal cortex: role of 5HT<sub>1A</sub> receptor agonism. *Brain Res* 956: 349–357.
- Janowsky A, Neve KA, Kinzie JM, Taylor B, dePaulis T, Belknap JK (1992). Extrastriatal dopamine D2 receptors: distribution pharmacological characterization and region-specific regulation by clozapine. *J Pharm Exp Ther* 261: 1282–1290.
- Kane JM, Marder SR, Schooler NR, Wirshing WC, Umbricht D, Baker RW (2001). Clozapine and haloperidol in moderately refractory schizophrenia. *Arch Gen Psychiat* 58: 965–972.
- Kapur S, Remington G, Jones C, Wilson A, DaSilva J, Houle S et al (1996). High levels of dopamine D<sub>2</sub> receptor occupancy with low-dose haloperidol treatment: a PET study. *Am J Psychiat* 153: 948–950.
- Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P (2000). A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D<sub>2</sub> receptor occupancy. *Arch Gen Psychiat* 57: 553–559.
- Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J, Wilson AA et al (1998). 5-HT<sub>2</sub> and D<sub>2</sub> receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Am J Psychiat* 155: 921–928.
- Kawagoe KT, Garris PA, Wiedmann DJ, Wightman RM (1992). Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. *Neuroscience* 51: 55–64.
- Kessler RM, Ansari MS, Li R, Lee M, Schmidt D, Dawant B et al (2002). Occupancy of cortical and substantia nigra DA D2 receptors by typical and atypical antipsychotic drugs. *NeuroImage* 16: S9.
- Kessler RM, Ansari MS, Riccardi P, Li R, Jayatilake K, Dawant B et al (2005). Occupancy of striatal and extrastriatal DA D2/3 receptors by olanzapine and haloperidol. *Neuropsychopharmacology* 30: 2283–2289.
- Kessler RM, Ellis Jr JR, Eden M (1984). Analysis of emission tomographic scan data: limitations imposed by resolution and background. *J Comput Assist Tomogr* 8: 514–522.
- Kessler RM, Mason NS, Jones C, Ansari MS, Manning RF, Price RR (2000). [<sup>18</sup>F]N-allyl-5-fluoropropylpeptide (fallypride): radiation dosimetry, quantification of striatal and extrastriatal dopamine receptors in man. *NeuroImage* 11: S32.
- Kessler RM, Meltzer HY (2002). Regional selectivity in clozapine treatment. *Am J Psy* 159: 1064–1065.
- Khan ZU, Mrzljak L, Gutierrez A, DeLa Calle A, Goldman-Rakic PS (1998). Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proc Natl Acad Sci Neurobiol* 95: 7731–7736.
- Kuroki T, Meltzer HY, Ichikawa J (1999). Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J Pharm Exp Ther* 288: 774–781.
- Lammertsma AA, Bench CJ, Hume SP, Osman S, Gunn K, Brooks DJ et al (1996). Comparison of methods of analysis of clinical [<sup>11</sup>C]raclopride studies. *J Cereb Blood Flow Metab* 16: 42–52.
- Lammertsma AA, Hume SP (1996). Simplified reference tissue model for PET receptor studies. *NeuroImage* 4(3 Part 1): 153–158.
- Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA et al (1993). Localization of D<sub>1</sub> and D<sub>2</sub> dopamine receptors in brain with subtype-specific antibodies. *Proc Natl Acad Sci USA* 90: 8861–8865.
- Lidow MS, Goldman-Rakic PS (1997). Differential regulation of D<sub>2</sub> and D<sub>4</sub> dopamine receptor mRNAs in the primate cerebral cortex vs neostriatum: effects of chronic treatment with typical and atypical antipsychotic drugs. *J Pharmacol Exp Therapeut* 283: 939–946.
- Maes F, Collignon A, Vandermuele D, Marchal G, Suetens P (1997). Multimodality image registration by maximization of mutual information. *IEEE Trans Med Imag* 16: 187–198.
- Mawlawi O, Martinez D, Slifstein M, Broft A, Chatterjee R, Hwang DR et al (2001). Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D (2) receptor parameter measurements in ventral striatum. *J Cereb Blood Flow Metab* 21: 1034–1057.
- Meltzer HY, Matsubara S, Lee J-C (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin<sub>2</sub> pK<sub>i</sub> values. *J Pharmacol Exp Therapeut* 251: 238–246.
- Meltzer HY (1992). The mechanism of action of clozapine in relation to its clinical advantage. In: Meltzer HY (ed). *Novel Antipsychotic Drugs*. Raven Press: New York. pp 1–13.
- Meltzer HY. (1999). The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* 21: 106S–115S.
- Meltzer HY, Arvanitis L, Bauer D, Rein W (2004). Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *Am J Psychiat* 161: 975–984.
- Mukherjee J, Christian BT, Dunigan KA, Shi B, Narayanan TK, Satter M et al (2002). Brain images of <sup>18</sup>F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse* 46: 170–188.
- Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C et al (1993). Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects—a double-blind PET study of schizophrenic patients. *Biol Psychiat* 33: 227–235.
- Nordstrom AL, Nyberg S, Olsson H, Farde L (1998). Positron emission tomography finding of a high striatal D<sub>2</sub> receptor occupancy in olanzapine-treated patients. *Arch Gen Psychiat* 55: 283–284.
- Olsson H, Farde L (2001). Potentials and pitfalls using high affinity radioligands in PET and SPET determinations on regional drug induced D2 receptor occupancy—a simulation study based on experimental data. *NeuroImage* 14: 945–946.
- Olsson H, Halldin C, Farde L (2004). Differentiation of extrastriatal dopamine D2 receptor density and affinity in the human brain using PET. *NeuroImage* 22: 794–803.
- Olsson H, Halldin C, Swahn CG, Farde L (1999). Quantification of [<sup>11</sup>C] FLB 457 binding to extrastriatal dopamine receptors in the human brain. *J Cereb Blood Flow Metab* 10: 1164–1173.
- Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa D, Kerwin RW (1997). Limbic selectivity of clozapine. *Lancet* 350: 490–491.
- Riccardi P, Li R, Ansari MS, Zald D, Park S, Dawant B et al (2006). Amphetamine induced displacement of [<sup>18</sup>F] fallypride in striatum and extrastriatal regions in humans. *Neuropsychopharmacology* 31: 1016–1026.
- Rieck RW, Ansari MS, Whetsell Jr WO, Deutch AY, Kessler RM (2004). Distribution of dopamine D2-like receptors in the human

- thalamus: autoradiographic and PET studies. *Neuropsychopharmacology* 29: 362–372.
- Robertson GS, Fibiger HC (1992). Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. *Neuroscience* 46: 315–328.
- Robertson GS, Matsumura H, Fibiger HC (1994). Induction patterns of fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. *J Pharmacol Exp Ther* 271: 1058–1066.
- Roth BL, Sheffler D, Potkin SG (2003). Atypical antipsychotic drug actions: unitary or multiple mechanisms for 'atypicality'? *Clin Neurosci Res* 3: 108–117.
- Roth BL, Tandra S, Burgess LH, Sibley DR, Meltzer HY (1995). D<sub>4</sub> dopamine receptor binding affinity does not distinguish between typical and atypical antipsychotic drugs. *Psychopharmacology* 120: 365–368.
- Schaltenbrand G, Wahren W (1977). *Atlas for Stereotaxy of the Human Brain*. Yearbook Medical Publisher Inc.: Chicago.
- Schotte A, Janssen PFM, Gommeren WM, Lyuten WHML, Van Gompel P, Lesage AS et al (1996). Risperidone compared with new and reference antipsychotic drugs: *in vivo* and *in vivo* receptor binding. *Psychopharmacology* 124: 57–73.
- Seeman P, Tallerico T (1998). Antipsychotic drugs which elicit little or no Parkinsonism bind more loosely than dopamine to brain D<sub>2</sub> receptors, yet occupy high levels of these receptors. *Mol Psychiat* 3: 123–134.
- Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI (1998). Dopamine axon varicosities in the prefrontal division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci* 18: 2697–2708.
- Siessmeier T, Zhou Y, Buchholz HG, Landvogt C, Vernaleken I, Piel M et al (2005). Parametric mapping of binding in human brain of D<sub>2</sub> receptor ligands of different affinities. *J Nucl Med* 46: 964–972.
- Slifstein M, Hwang D-R, Huang Y, Guo NN, Sudo Y, Narendran R et al (2004). *In vivo* affinity of [<sup>18</sup>F]fallypride for striatal and extrastriatal dopamine D<sub>2</sub> receptors in nonhuman primates. *Psychopharmacology* 175: 274–286.
- Smiley JF, Levey AI, Ciliax BJ, Goldman-Rakic PS (1994). D<sub>1</sub> dopamine receptor immunoreactivity in human and monkey cerebral cortex: predominant and extrasynaptic localization in dendritic spines. *Proc Natl Acad Sci USA Neurobiol* 91: 5720–5724.
- Sorenson SM, Kehne JH, Fadayel GM, Humphreys TM, Ketteler HJ, Sullivan CK et al (1993). Characterization of the 5-HT<sub>2</sub> receptor antagonist MLD 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. *J Pharm Exp Ther* 266: 684–691.
- Stephenson CME, Bigliani V, Jones HM, Mulligan RS, Acton PD, Visvikis D et al (2000). Striatal and extra-striatal D<sub>2</sub>/D<sub>3</sub> dopamine receptor occupancy by quetiapine *in vivo*. *Br J Psychiat* 177: 408–415.
- Svensson TH (2003). Adrenoceptor modulation hypothesis of antipsychotic atypicality. *Prog Neuro-Psychopharmacol Biol Psychiat* 27: 1145–1158.
- Talvik M, Nordstrom AL, Olsson H, Halldin C, Farde L (2003). Decreased thalamic D<sub>2</sub>/D<sub>3</sub> receptor binding in drug-naïve patients with schizophrenia: a PET study with [<sup>11</sup>C]FLB 457. *Int J Neuropsychopharmacol* 6: 361–370.
- Talvik M, Nordström AL, Nyberg S, Olsson H, Halldin C, Lars F (2001). No support for regional selectivity in clozapine-treated patients: a PET study with [<sup>11</sup>C]raclopride and [<sup>11</sup>C] FLB 457. *Am J Psychiat* 158: 926–930.
- Tauscher-Wisniewski S, Kapur S, Tauscher J, Jones C, Daskalakis ZJ, Papatheodorou G et al (2002). Quetiapine: an effective antipsychotic in first-episode schizophrenia despite only transiently high dopamine-2 receptor blockade. *J Clin Psychiat* 63: 992–997.
- Tuppurainen H, Kuikka J, Viinamäki H, Husso-Saastamoinen M, Bergström K, Tiihonen J (2003). Extrastriatal dopamine D<sub>2</sub>/D<sub>3</sub> receptor density and distribution in drug-naïve schizophrenic patients. *Mol Psychiat* 8: 453–455.
- Vahid-Ansari F, Nakabeppu Y, Robertson GS (1996). Contrasting effects of chronic clozapine, Seroquel (TM) (ICI 204,636) and haloperidol administration of delta FosB-like immunoreactivity in the rodent forebrain. *Eur J Neurosci* 8: 927–936.
- Venton BJ, Zhang H, Garris PA, Phillips PEM, Sulzer D, Wightman RM (2003). Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. *J Neurochem* 87: 1284–1295.
- Wells III WM, Viola P, Atsumi H, Nakajima S, Kikinis R (1996). Multimodal volume registration by maximization of mutual information. *Med Imag Anal* 1: 35–51.
- Xiberas X, Marinot JL, Mallet L, Artiges E, Loc'h C, Mazière B et al (2000). Extrastriatal and striatal D<sub>2</sub> dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. *Br J Psychiat* 179: 503–508.
- Yamamoto BK, Cooperman MA (1994). Differential effects of chronic antipsychotic drug treatment on extracellular glutamate and dopamine concentrations. *J Neurosci* 14: 4159–4166.
- Yasuno F, Suhara T, Okubo Y, Sudo Y, Inoue M, Ichimiya T et al (2004). Low dopamine d(2) receptor binding in subregions of the thalamus in schizophrenia. *Am J Psychiat* 161: 1016–1022.
- Youngren KD, Inglis FM, Pivrotto PH, Jedema HP, Bradberry CW, Goldman-Rakic PS et al (1999). Clozapine preferentially increases dopamine release in the rhesus monkey prefrontal cortex compared with the caudate nucleus. *Neuropsychopharmacology* 20: 403–412.