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Occupancy of Striatal and Extrastriatal Dopamine D_2 Receptors by Clozapine and Quetiapine

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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_2 receptors (DA D_2r) 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_2r. To examine whether clozapine and/or quetiapine produce preferential occupancy of DA D_2r in cortex and limbic regions, we studied the occupancy of putamenal, ventral striatal, thalamic, amygdala, substantia nigra, and temporal cortical DA D_2r using PET with [^{18}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_2r levels calculated for 10 off-medication schizophrenic subjects. Both clozapine and quetiapine produced lower levels of putamenal DA D_2r occupancy than those reported for typical APDs, 47.8 and 33.5%, respectively. Clozapine produced preferential occupancy of temporal cortical *vs* putamenal DA D_2r, 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_2r, 46.9% ($p = 0.03$, corrected for multiple comparisons), but did not spare occupancy of substantia nigra DA D_2r. 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_2r mechanisms in at least partially mediating the therapeutic effects of these drugs. Preferential occupancy of cortical DA D_2r, sparing occupancy of substantia nigra receptors, and non-DA D_2r-mediated actions may contribute to the antipsychotic actions of these and other atypical APDs.

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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_2 receptor (DA D2r) 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_2r 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_2r (Janowsky *et al*, 1992; Florijn *et al*, 1997), cortical DA D_2r 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

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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_2r-mediated neurotransmission in cortex and limbic regions, compared to the dorsal striatum.

Some but not all imaging studies of occupancy of extrastriatal DA D_2r by clozapine and quetiapine report preferential occupancy of cortical DA D_2r compared to striatal DA D_2r, consistent with the hypothesis that the therapeutic effects of these drugs are mediated by cortical and/or limbic DA D_2r. There have been four studies comparing the occupancy of extrastriatal DA D_2r 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_2r and a single study of the occupancy of extrastriatal DA D_2r by quetiapine that reported preferential occupancy of cortical DA D_2r (Stephenson *et al*, 2000). The studies of clozapine's occupancy of extrastriatal DA D_2r 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_2r 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_2r but sparing of nigral DA D_2r 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_2r in extrastriatal regions, we used PET with [^{18}F]fallypride (Kessler *et al*, 2000; Mukherjee *et al*, 2002) to measure the levels of DA D_2r occupancy in putamen, ventral striatum, thalamus, amygdala, temporal cortex, and substantia nigra in schizophrenic subjects who were treated with either clozapine or quetiapine monotherapy. [^{18}F]Fallypride is a high-affinity radioligand for DA D_2 and D_3 receptors that can be used to quantitate levels of DA D2/3r 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_2r 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 = 5000, 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. [^{18}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 $256 \times 256 \times 256$ 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 [^{18}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_2r 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_2r were calculated using the appropriate regional mean value for off-medication schizophrenic subjects as follows:

$$\text{percent occupancy in region}_i = \left[1 - \frac{\text{DA D2r}_i\,(\text{medicated})}{\text{DA D2r}_i\,(\text{unmedicated})}\right] \times 100$$

where DA D_2r_i represents the available level of DA D_2r in region '*i*' in the indicated state. To evaluate whether clozapine and/or quetiapine produced selective and/or nonuniform occupancy of DA D_2r in extrastriatal brain regions compared to that in the putamen, an analysis of variance for regional DA D_2r 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_2r 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_2r occupancy for clozapine and quetiapine are shown in Table 1 and Figure 1. An analysis of variance for DA D_2r 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_2r 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_2r 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_2r 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_2r and spares occupancy of substantia nigra receptors in comparison to that seen in the putamen.

An analysis of variance for DA D_2r occupancy in quetiapine-treated subjects was performed using region as a factor and covaried for putamenal occupancy. The results demonstrated significantly higher DA D_2r 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_2r 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_2r 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_2r occupancy in the substantia nigra, the relationships of DA D_2r 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_2r by Clozapine and Quetiapine

	Percent regional occupancy					
Dose (mg)	**Putamen**	**Ventral striatum**	**Thalamus**	**Amygdala**	**Temporal cortex**	**Substantia nigra**
Clozapine						
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†
Quetiapine						
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‡	34.3 ± 12.9

*Significantly different from putamenal occupancy for clozapine ($p \geqslant 0.05$, corrected for multiple comparisons).
†Significantly different from putamenal occupancy for clozapine ($p = 0.0015$, corrected for multiple comparisons).
‡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_2r 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_2r 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 ± 10.9 in clozapine-treated subjects and 16.6 ± 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_2r in comparison to putamenal DA D_2r occupancy; (2) that clozapine but not quetiapine produces significantly lower DA D_2r occupancy in the substantia nigra compared to all other regions examined, that is, sparing of nigral DA D_2r occupancy; and (3) that both clozapine and quetiapine produce significant therapeutic effects at DA D_2r 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 [^{11}C]raclopride PET studies of DA D_2r occupancy (Farde *et al*, 1994; Gefvert *et al*, 1998; Tauscher-Wisniewski *et al*, 2002).

Preferential occupancy of temporal cortical *vs* striatal DA D_2r 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_2r 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

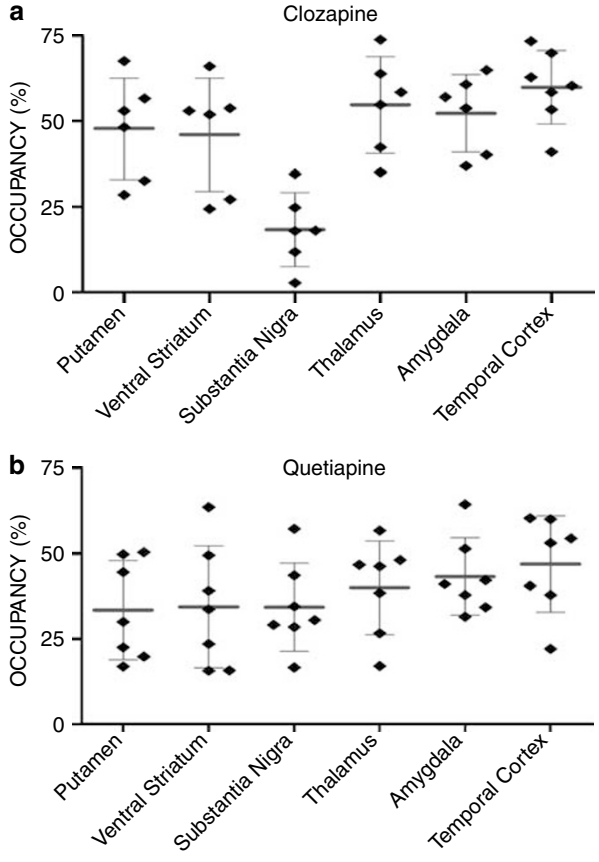

Figure 1 Scattergrams of individual regional DA D_2r occupancies are shown for clozapine-treated (a) and quetiapine-treated (b) subjects. Clozapine-treated subjects demonstrate preferential occupancy of temporal cortical DA D_2r in comparison to putamenal DA D_2r occupancy and sparing of substantia nigra DA D_2r occupancy. Quetiapine demonstrated preferential occupancy of temporal cortical DA D_2r, but did not demonstrate sparing of substantia nigra DA D_2r.

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_2r. 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, [^{11}C]raclopride to estimate striatal and [^{11}C]FLB457 to estimate cortical DA D_2r occupancies, and possible effects of low levels of cerebellar DA D_2r 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_2r 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_2r (Kessler and Meltzer, 2002). This is contrary to our previous results using a single radioligand, [^{18}F]fallypride, which reported similar DA D_2r occupancies in the putamen and temporal cortex for haloperidol (Kessler *et al*, 2005). Although both [^{11}C]FLB457 and [^{11}C]raclopride are benzamides, differences in radioligands may result in different apparent levels of receptor occupancy. Estimates of striatal receptor DA D_2r occupancy obtained using [^{11}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_2r 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 [^{11}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_2r occupancies could account for both the failure to observe preferential occupancy of cortical DA D_2r by clozapine and the unexpected lower occupancy of cortical DA D_2r seen with haloperidol. The difference between studies using [^{18}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_2r occupancies. In the present study, we utilized [^{18}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 [^{18}F]fallypride may also be criticized because of the presence of low levels of cerebellar DA D_2r (Gunn *et al*, 1997), the level of specific [^{18}F]fallypride binding in the cerebellar reference region is low enough, about 3%, not to significantly bias the estimates of regional DA D_2r occupancy (Kessler *et al*, 2000, 2005; Siessmeier *et al*, 2005).

The [^{123}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_2r 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_2r owing to underestimation of striatal DA D_2r occupancy. Modeling studies of [^{123}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 [^{76}Br]FLB457, have reported greater occupancy of temporal cortical than striatal DA D_2r 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_2r occupancy of 44%; other studies report a 70–80% striatal occupancy and no preferential occupancy of temporal cortical DA D_2r with these doses of olanzapine (Kessler *et al*, 2005; Nordstrom *et al*, 1998; Kapur *et al*,

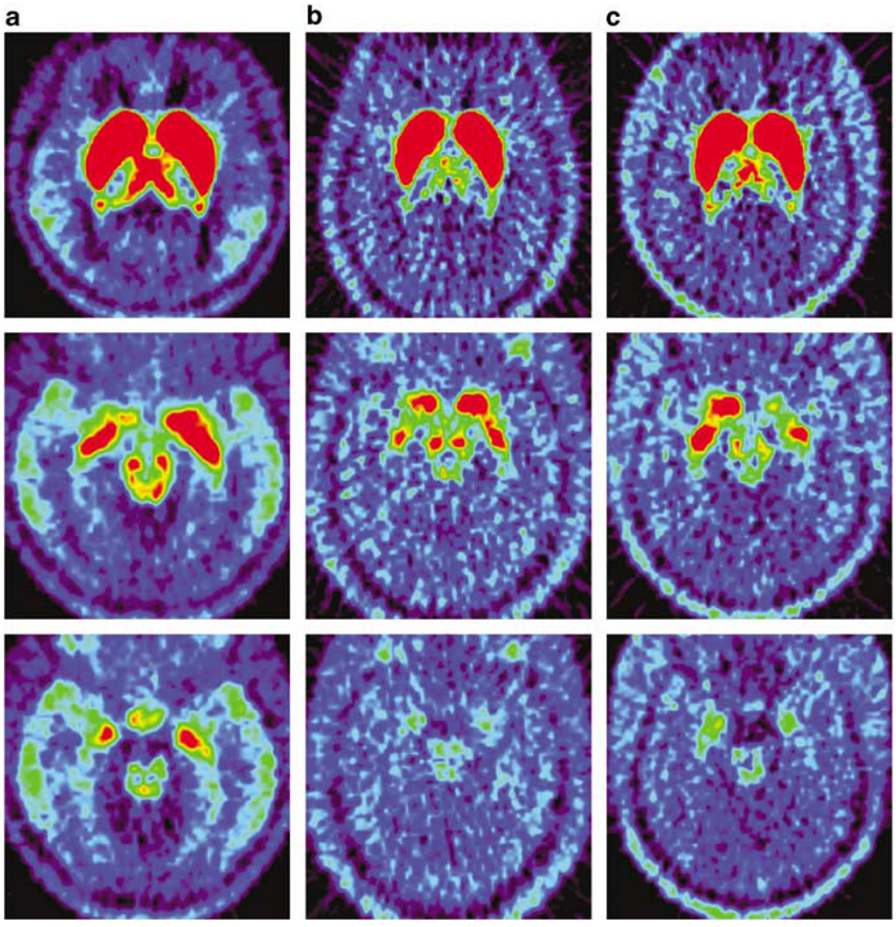

Figure 2 Images of [^{18}F]fallypride uptake in (a) an off-medication schizophrenic subject, (b) a clozapine-treated subject, and (c) a quetiapine-treated subject at the level of the thalamus (top row), substantia nigra (middle row), and amygdala (bottom row) at 90 min following [^{18}F]fallypride administration. 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_2r 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_2r 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.

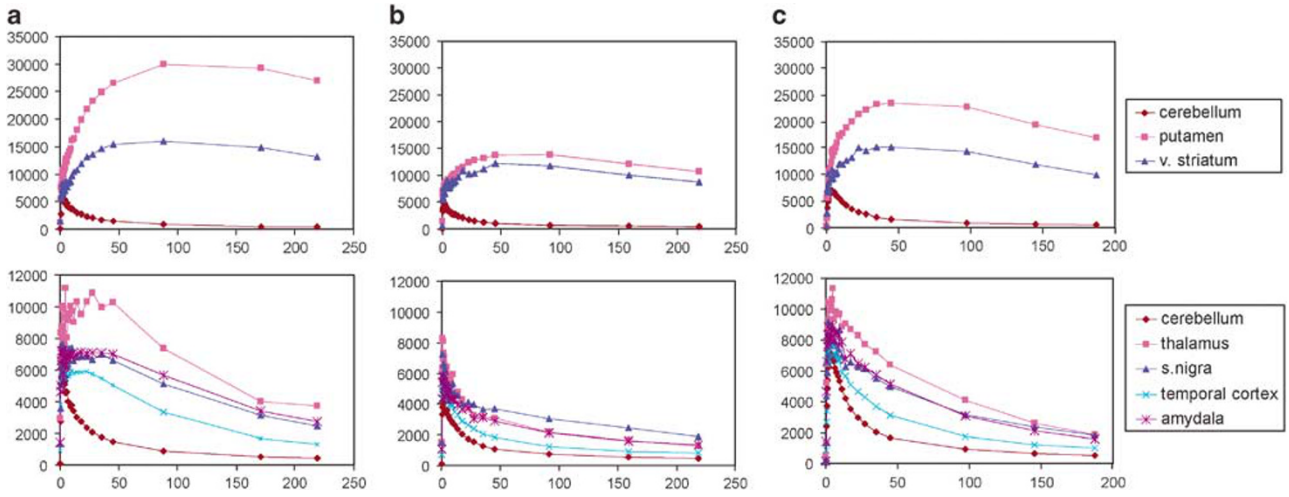

Figure 3 Time activity curves of regional brain [^{18}F]fallypride uptake are shown for the off-medication schizophrenic subject (a), clozapine-treated subject (b), and quetiapine-treated subject (c) shown in Figure 2. In the normal and quetiapine-treated subjects, the order of uptake is thalamus > substantia nigra = amygdale, 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_2r 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_2r 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_2r binding potentials in all brain regions with [^{18}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_2r 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_2r levels in unmedicated schizophrenic subjects have reported lower levels of DA D_2r 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_2r in schizophrenic subjects could artifactually produce the appearance of preferential occupancy of temporal cortical DA D_2r by clozapine. To avoid this potential confound, receptor occupancies in this study were calculated using regional levels of DA D_2r 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 ± 4.53 *vs* 37.34 ± 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_2r. These include the higher fraction of DA D_{2s}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_2r by clozapine (Damask *et al*, 1996; Lidow and Goldman-Rakic, 1997). Although there is a higher fraction of DA D_{2s}r in cortex than striatum (Khan *et al*, 1998), the relative affinities of clozapine, olanzapine, and haloperidol for the DA D2s *vs* D_{2L} 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_2r occupancy (Kessler *et al*, 2005). Similarly, it has been shown that [^{18}F]fallypride has similar *in vivo* affinity for the DA D_2r 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 [^{11}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_2r binding in cortex but not in striatum (Janowsky *et al*, 1992; Florijn *et al*, 1997). Higher levels of cortical DA D_2r with chronic clozapine therapy would produce spuriously low levels of cortical DA D_2r occupancy compared to striatum. Differential upregulation of cerebral DA D_2r does not explain the preferential occupancy of cortical DA D_2r 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_2r occupancy observed with clozapine and quetiapine. Both of these atypical APDs have a low affinity for the DA D_2r (Schotte *et al*, 1996). Studies of striatal DA D_2r indicate that, although a significant fraction are extrasynaptic, the majority of striatal DA D_2r 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_2r 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_2r 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_2r 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_2r. 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_2r (Meltzer *et al*, 1989; Roth *et al*, 1995; Seeman and Tallerico, 1998).

In regard to the finding of sparing of nigral DA D_2r 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_2r 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_2r 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_2r 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 [^{18}F]fallypride binding potentials in extra-striatal regions of human brain, including the substantia nigra, with quantitative autoradiographic measurements of DA D_2r levels in post-mortem human brain (Rieck *et al*, 2004). The sensitivity of DA D_2r 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_2r. The relative sparing of DA D_2r 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_2r has been shown to produce increased muscle tone that was not reversed by intravenous apomorphine, despite the presence of intact striatal DA D_1 and D_2 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_2r blockade in both the striatum and substantia nigra (Crocker and Hemsley, 2001). The lesser blockade of nigral region DA D_2r seen with clozapine may be protective against EPS.

The factors mediating the sparing of DA D_2r 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_{2A}-mediated neurotransmission (Sorenson *et al*, 1993; Cobb and Abercrombie, 2003; Bruggeman *et al*, 2000), the correlation of a high 5-HT_{2A} : DA D_2 affinity ratio with an atypical profile (Meltzer *et al*, 1989; Roth *et al*, 1995), and the three- to seven-fold higher 5-HT_{2A} : DA D_2r affinity ratio seen with olanzapine and clozapine compared to quetiapine (Schotte *et al*, 1996), the role of 5-HT_{2A}-mediated serotonergic neurotransmission in sparing of nigral DA D_2r occupancy requires further investigation.

In addition to preferential occupancy of cortical and sparing of nigral DA D_2r occupancy, non-DA D_2r-mediated mechanisms may be involved in the production of an atypical antipsychotic profile. Previous studies have suggested that a threshold of DA D_2r 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_2r, 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_2r, all regional DA D_2r 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_2r 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_2r blockade are involved, at least in part, in the therapeutic effects of these drugs. Non-DA D_2r-mediated mechanisms may include interactions at 5-HT_{2A}, 5-HT_{2C}, NK_3, α_1 and/or α_2 receptors (Meltzer, 1999; Meltzer *et al*, 2004; Svensson, 2003).

In comparing the levels of DA D_2r 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_2r 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_2r, sparing of DA D_2r occupancy in the substantia nigra, as well as interactions at sites beyond the DA D_2r 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.

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