Receptor Occupancy and Brain Free Fraction

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Abbreviations: BBB, blood-brain barrier; CSF, cerebrospinal fluid; C_{u,brain}, brain unbound concentration; C_{CSF}, cerebrospinal fluid concentration; C_{u,blood}, blood unbound concentration
Abstract

This study was designed to investigate whether brain unbound concentration (C_{u,brain}) provides a better prediction of dopamine D_2 receptor occupancy as compared to using total brain concentration, cerebrospinal fluid concentration (C_{CSF}) or blood unbound concentration (C_{u,blood}). The \textit{ex vivo} D_2 receptor occupancy and concentration-time profiles in CSF, blood and brain of 6 marketed antipsychotics were determined following oral administration in rats at a range of dose levels. The C_{u,brain} was estimated from the product of total brain concentration and unbound fraction, which was determined using a brain homogenate method. In conclusion, the C_{u,brain} of selected antipsychotics is a good predictor of D_2 receptor occupancy in rats. Furthermore, C_{u,brain} appears to provide a better prediction of D_2 receptor occupancy than C_{CSF} or C_{u,blood} for those compounds whose mechanism of entry into brain tissue is influenced by factors other than simple passive diffusion.
Introduction

In CNS drug discovery, the extent of brain penetration of new compounds has historically been determined in small rodents, measuring the total brain concentration and comparing this with the total blood concentration, usually once a pseudo-steady-state is achieved, to give a brain to blood ratio. Moreover the trend in lead optimisation has focused on selecting compounds with high in vitro potency and high total brain to blood ratio. Whilst the measure of total brain concentration is relatively simple to obtain, it is often difficult to correlate with receptor occupancy / pharmacodynamic (PD) readout.

It is thought that only unbound drug in the interstitial spaces in the brain (C_{u,brain}) is available to interact with the majority of CNS receptors (de Lange and Danhof, 2002). Therefore, in an attempt to obtain better correlation with receptor occupancy / PD readouts, it may be essential to determine C_{u,brain} for brain-targeted compounds. However, this has posed a significant challenge as current methodologies to directly estimate C_{u,brain} are not amenable to the demands of an early drug discovery setting.

Microdialysis has been used to measure C_{u,brain} but this technique is resource-demanding and is not broadly applicable across discovery programmes for CNS targets due to probe recovery problems for very lipophilic compounds. Cerebrospinal fluid concentration (C_{CSF}) has also often been measured as it may (with various caveats) provide a surrogate measure for C_{u,brain}. Even if better correlations can be obtained with receptor occupancy / PD readouts, reliable CSF measurements are, however, very difficult to obtain routinely. This is mainly due to technical problems such as blood contamination, poor recovery, and compound adsorption to the collection apparatus providing very low levels of compound for analysis. Additionally, the value of measuring C_{CSF} has been challenged, particularly as the expression of many transporters at the blood cerebrospinal fluid
barrier (BCSFB) are now known to be different from those at the blood-brain barrier (BBB),
supporting the opinion that $C_{\text{CSF}}$ can significantly deviate from $C_{u,\text{brain}}$ (Soontornmalai et al. 2006; Liu et al. 2006). This opinion is further supported by the work of Shen et al. (2004) where they demonstrated marked differences between interstitial fluid concentration (as measured using microdialysis) and CSF concentration for a set of 22 compounds.

Considering the free drug hypothesis (Tillement et al. 1988) it may not be unreasonable to consider the unbound drug concentration in blood ($C_{u,\text{blood}}$) as a surrogate for $C_{u,\text{brain}}$ for compounds that passively permeate across the BBB. However, considering the high number of compounds within CNS drug discovery programmes that are substrate for an efflux transporter at the BBB, $C_{u,\text{blood}}$ will not be equal to $C_{u,\text{brain}}$ in many cases. There is therefore a need for a more direct measure of $C_{u,\text{brain}}$ and this could be achieved using equilibrium dialysis to determine the free fraction of a compound in brain homogenate (Kalvass and Maurer, 2002) and then using this free fraction to correct total brain concentration for $C_{u,\text{brain}}$.

The objective of this work was to examine whether $C_{u,\text{brain}}$ estimated from the product of total brain concentration and unbound fraction offers improved prediction of receptor occupancy over a simple measure of total brain concentration, $C_{\text{CSF}}$ or $C_{u,\text{blood}}$. The molecular target chosen was the dopamine D$_2$ receptor given that the occupancy of test ligands, at these receptors, can be readily estimated using ex vivo autoradiography (Kapur et al., 2002).

Materials and Methods
Ziprasidone and quetiapine were synthesized by GlaxoSmithKline, Harlow (U.K.). All other drugs and reagents were purchased from Sigma-Aldrich (Poole, U.K.), Calbiochem (Nottingham, U.K.), Bio-Rad (Hemel Hempstead, U.K.), Fisons Scientific Equipment (Loughborough, U.K.), Research Biochemicals International (Poole, U.K.), Tocris Cookson Ltd. (Bristol, U.K.) Gibco (Paisley,
U.K.), Biomol International, and MP Biomedicals. [3H]Raclopride and [3H]FLB-457 were supplied by Amersham Pharmacia Biotech UK Ltd. (Little Chalfont, U.K.).

**Equilibrium Dialysis measurements.** The methodology employed in this study was a modification of that reported by Summerfield et al. (2006). In brief, a 96 well equilibrium dialysis apparatus was used to determine the free fraction in the blood and brain for each drug (HT Dialysis LLC, Gales Ferry, CT). Membranes (3-kDA cut-off) were conditioned in deionised water for 60 minutes, followed by conditioning in 80:20 deionised water:ethanol for 20 minutes, and then rinsed in deionised water before use. Rat blood was obtained fresh on the day of experiment and diluted 1:1 with phosphate buffer pH7.4. Rat brain was removed from the freezer and allowed to thaw on the day of experiment. Thawed brain tissue was homogenised with phosphate buffer pH7.4 to a final composition of 1:2 brain:phosphate buffer using a Tomtec autogiser (Receptor Technologies, Adderbury, Oxon, UK). Diluted blood and brain homogenate was spiked with the test compound (1μg/mL), and 150μL aliquots (n=6 replicate determinations) were loaded into the 96-well equilibrium dialysis plate. Dialysis vs phosphate buffer, pH7.4 (150μL) was carried out for 6 hours in a temperature controlled incubator at ca. 37°C (Stuart Scientific, Watford) using an orbital microplate shaker at 125 revolutions/minute (Stuart Scientific). At the end of the incubation period, aliquots of blood or brain homogenate or phosphate buffer were transferred to micronic tubes (Micronic B.V., the Netherlands) and the composition in each tube was balanced with control fluid, such that the volume of phosphate buffer to blood or brain was the same. Sample extraction was performed by the addition of 300μL of 95:5 acetonitrile:ethanol containing 0.1% formic acid and an internal standard. Samples were allowed to mix for 5 minutes and then centrifuged at 3000rpm in 96-well blocks for 15 minutes (Heraeus Multifuge 3 L-R, Kendro Laboratory Products, Germany). The unbound fraction was determined as the ratio of the peak area in buffer to that in blood or brain, with correction for dilution factor according to eq.1 (Kalvass and Maurer, 2002),
Undiluted $f_u = \frac{1}{D} \left( \frac{1}{f_{u,\text{apparent}}} - 1 \right) + \frac{1}{D} \quad (1)$

where $D = \text{dilution factor in blood or brain homogenate}$ and $f_{u,\text{apparent}}$ is the measured free fraction of diluted blood or brain homogenate.

**Analysis of Test Compounds in Equilibrium Dialysis.** All samples were analysed by means of HPLC/MS/MS on a Micromass Quattro Premier Mass Spectrometer (Water’s Ltd., UK). Samples (20μL) were injected using a CTC analytics HTS Pal autosampler (Presearch, Hitchin, UK) onto a Phenomenex Luna C18 50 x 2.1 mm, 5-μm column (Phenomenex, UK) operated at 40ºC and at an eluant flow rate of 0.8mL/min. Analytes were eluted using a high pressure linear gradient program by means of an HP1100 binary HPLC system (Agilent Technologies, Stockport, Cheshire, UK). Relative peak areas between the phosphate buffer and tissue half-wells were used to determine the respective free fractions.

**[^H]FLB-457 binding in rat striatal membranes.** Striatum was dissected from male Sprague-Dawley rats (Charles River, UK) and placed into 10 volumes ice-cold TRIS buffer (50 mM TRIS pre-set, pH 7.4). Striatal samples were then homogenised using a polytron homogeniser and centrifuged for 15 minutes, 20,000 RPM at 5ºC. The supernatant was discarded and the pellet re-suspended in approximately 10 mls TRIS buffer and incubated for 15-20 minutes at 37ºC to ensure endogenous dopamine degradation. Subsequently, the membrane suspension was homogenised and centrifuged twice, as described above. The final pellet was re-suspended in 5-10 mls TRIS buffer, homogenised and a protein assay carried out using the Bradford method. Samples (1mL) were stored at approximately -80ºC until used. In radioligand binding studies, striatal membranes (50 μg protein / well) were incubated in assay buffer (50 mM TRIS pre-set, 120 mM NaCl, 5 mM...
KCl, 1 mM MgCl₂, 2 mM CaCl₂, pH 7.4) in the presence of 0.2 nM [³H]-FLB-457 with or without increasing concentrations of test compounds, at 37°C for 45 minutes. Non-specific binding was determined using 10 μM Haloperidol. The reaction was terminated by rapid filtration through GF/B filter mats, pre-soaked in 0.3 % PEI, followed by three 1 ml washes with ice cold TRIS buffer. Radioactivity bound was determined by scintillation spectrometry using a Packard beta scintillation counter.

**Determination of CNS Penetration in the Rat after Acute Oral Dosing.** Male Sprague-Dawley rats (250-300 g, Charles River) were randomly assigned to each of five treatment groups (5-7 animals per group) for each compound. The five treatment groups for each compound consisted of vehicle and four dose levels. Ziprasidone (1, 3, 30, 100 mg/kg); quetiapine (3, 10, 30, 100 mg/kg); risperidone (0.1, 0.3, 3, 10 mg/kg); haloperidol (0.1, 0.3, 1, 10 mg/kg); clozapine (10, 30, 50, 100 mg/kg) and chlorpromazine (1, 3, 10, 30 mg/kg) were administered orally using 1% methyl cellulose made up in deionised water as vehicle. The dose solution for each test compound was prepared on the day of dosing. At 1 hour following oral administration, at pseudo-steady-state, a cisternal magna cerebrospinal fluid (CSF) sample was taken from each animal, the rats exsanguinated and decapitated and the brains were removed. CSF (ca. 60μL) and blood samples (100μL) were collected into micronic tubes and stored at approximately -80°C to await analysis. Brains were removed immediately from the skull, placed into a brain mould and divided into two halves in the coronal plain. The forebrain was then divided along the mid-line and one half, to be used for [³H] Raclopride Autoradiographic binding in rat striatum, was placed on a circular piece of cork with the region of interest uppermost, covered thoroughly with Tissue-Tek, rapidly frozen in isopentane (cooled to between -20°C to -30°C with dry ice) and then stored at approximately -20°C prior to sectioning. The second half was placed in a tube and stored frozen to await HPLC/MS/MS analysis.
[\^3H]Raclopride Autoradiographic binding in rat striatum. Coronal sections (20 µm) containing striata, (1–1.7 mm from bregma, Paxinos and Watson, 2nd Ed., 1986) were cut and thaw mounted onto silane coated microscope slides. Three adjacent brain slices from the same animal were collected per microscope slide. Two brain slices were used to measure total binding and the third one to evaluate non-specific binding. Sections were stored at -20°C until the day of assay.

Slide mounted sections were allowed to equilibrate to room temperature for approx 1 hour and encircled using a Pap Pen. Sections were incubated at room temperature for 10 minutes in Tris HCl assay buffer (trizma pre-set crystals, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, pH 7.4) containing either 1 nM [\^3H]raclopride (total binding) or 1 nM [\^3H]raclopride and 1 µM (-)sulpiride (non-specific binding). Assay solutions were removed by aspiration. All sections were then washed for 2 x 2 minutes with 200 µl ice-cold assay buffer. Slides were then dipped briefly in ice-cold distilled water to remove buffer salts and allowed to dry at room temperature. The slides were then placed in the chamber of the \(\beta\)-imager and data collected from the brain sections for 12 h. The levels of bound radioactivity in striatum (cpm/mm²) was directly determined by counting the number of \(\beta\)-particles emerging from the delineated area using the \(\beta\)-vision program (BioSpace).

Bioanalysis of CSF, Blood and Brain Homogenate Extracts. Each brain sample was homogenised with 50:50 (v/v) methanol:water (2.15mL/g brain), using a Tomtec autogiser (Receptor Technologies, Adderbury, Oxon, UK) In all cases, the sample extraction of blood and brain homogenate was performed by a method based on protein precipitation, using an aliquot of 95:5 (v/v) acetonitrile:ethanol containing 0.1% formic acid and a structural analogue of the analyte of interest as internal standard. Blood and brain extracts were vortex mixed for 5 minutes followed by centrifugation at 3000rpm for 15 minutes (Heraeus Multifuge 3 L-R, Kendro Laboratory Products, Germany). CSF sample analysis was performed by dilution (1:1) with 95:5 (v/v) acetonitrile:ethanol containing 0.1% formic acid and internal standard. Blood, CSF and brain
extracts were analysed by HPLC/MS/MS using a Micromass Quattro Premier mass spectrometer. Samples (20μL) were injected using a CTC Analytics Pal autosampler and chromatographed by means of an HP1100 binary HPLC system. Specific chromatographic conditions were developed for each analyte to complement their physicochemical properties. The flowing conditions were common to all assays: column temperature of 40ºC, eluant flow rate of 0.8mL/min and assay run times of 2.3 minutes/sample. Calibration curves were constructed in blood, artificial CSF and brain homogenate to cover at least 3 orders of magnitude (ie 0.1-100 ng/mL).

**Data Analysis**

Data from membrane radioligand binding assays were analysed using a 4 parameter iterative curve fit package (VICE) to yield pIC$_{50}$ values. These were then used to determine pKi values using the equation described by Cheng and Prusoff (1973). In autoradiographic binding assays, non-specific binding was subtracted from total binding to obtain specific binding values and subsequently expressed as percentage of vehicle specific binding.

**Results**

The antipsychotic compounds selected in this study are marketed D$_2$ receptor antagonists. These compounds displaced [$^3$H]FLB-457 from rat striatal membranes, in a monophasic manner to yield pKi values ranging from 6.7 to 8.6 (Table 1). Unbound fraction in brain tissue (Table 2), determined by equilibrium dialysis of spiked brain homogenate, varied over 50 fold from chlorpromazine ($f_u$ (brain) = 0.002) to risperidone ($f_u$ (brain) = 0.099). The mean brain, blood and CSF concentrations following acute oral dosing at a range of dose levels are listed in Table 3. Inhibition of [$^3$H]Raclopride specific binding in rat striatum at each dose level for haloperidol, risperidone, quetiapine, ziprasidone, chlorpromazine and clozapine are shown in Figures 1 to 6 respectively. Each compound significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D$_2$ receptor occupancy.
The influence of the unbound fraction in brain tissue on prediction of D₂ receptor occupancy

Figure 7 shows the increase in D₂ receptor occupancy with increasing total brain concentration for each compound. The total brain concentration has been normalised for the in vitro affinity of each compound for rat striatal D₂ receptors. Consequently, if there is a good relationship between the total brain concentration and the D₂ receptor occupancy each dose response curve should overlap each other. However, it is clearly seen from Figure 7 that this is not the case as there remains approximately 100 fold difference in total brain concentration required to achieve approximately 50% D₂ receptor occupancy even though each compound has been normalised for in vitro affinity for rat striatal D₂ receptors. Clearly, total brain concentration does not predict the receptor occupancy of these D₂ antagonists.

Figure 8 shows the increase in D₂ receptor occupancy with increasing unbound brain concentration for each compound. The unbound brain concentration has been determined by multiplying the total brain concentration by the Fu in brain and has been normalised for the in vitro affinity of each compound for rat striatal D₂ receptors. In contrast to Figure 7, Figure 8 clearly demonstrates that there is a good relationship between receptor occupancy and unbound brain concentration for these compounds. Each dose response curve is closer to overlap and at ~50% receptor occupancy there is only approximately a 1-2 fold difference in the unbound brain concentration required to achieve this level of occupancy when normalised for the rat striatal D₂ affinity.

Figure 9 shows a plot of the unbound brain concentration versus the unbound blood concentration. With the exception of Risperidone and Ziprasidone there appears to be a good relationship between C_u,blood and C_u,brain. Considering the free drug hypothesis this finding should not be too surprising considering that clozapine, chlorpromazine, haloperidol and quetiapine are not a substrate for Pgp whereas risperidone is a Pgp substrate (Doran et al., 2005; Summerfield et al., 2006).
observation that ziprasidone demonstrates higher unbound blood concentrations compared to unbound brain concentrations is somewhat surprising as ziprasidone appears not to be a substrate of Pgp (Summerfield et al., 2006). Clearly, interaction of ziprasidone with efflux transporters at the BBB warrants further investigation and the possibility that efflux transporters in addition to, or other than, Pgp might be involved in the transport of this compound. A further somewhat surprising observation is the plot of unbound blood concentration versus measured CSF concentration (Figure 10). It would be expected that risperidone and ziprasidone would demonstrate higher $C_{u,blood}$ than $C_{CSF}$ but this does not appear to be the case suggesting that there are routes into the CSF compartment that by-pass drug efflux mechanisms.

Figure 11 shows the increase in $D_2$ receptor occupancy with increasing unbound blood concentration for each compound. The unbound blood concentration has been determined by multiplying the total blood concentration by the $F_u$ in blood and has been normalised for the *in vitro* affinity of each compound for rat striatal $D_2$ receptors. Considering the findings in Figure 9 a good relationship should be observed between $D_2$ receptor occupancy and normalised unbound blood concentration for clozapine, chlorpromazine, haloperidol and quetiapine. This was indeed found to be the case but the relationship is improved further when unbound brain concentration is considered (Figure 8), highlighting the importance of assessing unbound concentration close to the target receptors. Furthermore prediction of $D_2$ receptor occupancy from $C_{u,blood}$ is likely to be inaccurate for all compounds entering brain tissue by mechanisms other than simple passive diffusion.

Figure 12 shows the good relationship observed between the measured CSF concentration and the unbound brain concentration. Considering clozapine, chlorpromazine, haloperidol and quetiapine, $C_{CSF}$ or $C_{u,blood}$ (Figure 9) appear to represent $C_{u,brain}$ equally well. For risperidone and ziprasidone, $C_{CSF}$ appears to be equivalent to or more accurate than $C_{u,blood}$ to represent $C_{u,brain}$. This finding is in
good agreement with the work of Lui et al. (2006). Lui and his co-workers concluded, following assessment of 7 model compounds, that for quick brain penetration with a simple diffusion mechanism compounds, $C_{CSF}$ or $C_{u,plasma}$ represent $C_{u,brain}$ equally well whereas for efflux substrates or slow brain penetration compounds, $C_{CSF}$ appears to be equivalent to or more accurate than $C_{u,plasma}$ to represent $C_{u,brain}$. From the knowledge we have on the mechanism of brain penetration for our test set, this conclusion is supported.

**Discussion**

The ability to determine the occupancy of molecular targets by neuropsychiatric drugs is an important aspect in confirming CNS penetration and subsequently understanding their pharmacodynamic / efficacious action. For example, clinical PET studies have suggested that slight differences in striatal dopamine D$_2$ receptor occupancy (from 75% to 85%), by antipsychotic drugs, is associated with an increase extrapyramidal side-effects (Kapur et al., 2000). These data highlight the importance of understanding the relationship between antipsychotic dose and D$_2$ receptor occupancy. In the drug discovery process, ideally, one would use the same imaging technique, preclinically, as is used in humans (i.e. PET) to link drug exposure to target occupancy but this is not a viable drug screening option predominantly due to expense and limited availability of experimental slots. To this end, other animal models which have been used to measure receptor occupancy include either *in vivo* or *ex vivo* radioligand binding. However, the former method is not without it’s limitations in that suitable radioligands for such a technique (i.e. with adequate specific binding) are not always available for every drug target. As such, *ex vivo* radioligand binding is more often featured on the critical path of drug screening. Despite the relative ease of performing this technique it is still considered resource intensive with low through put and so a more rapid method to predict for receptor occupancy would be of great benefit. Historically, drug companies have used measurement of total brain concentration as a crude filter to demonstrate brain penetration, and as such, assume occupancy of the molecular target. However, it is thought
that only unbound drug in the interstitial spaces in the brain ($C_{u,\text{brain}}$) is available to interact with the majority of CNS receptors (de Lange and Danhof, 2002). Therefore, in an attempt to obtain better correlation of drug exposure with receptor occupancy, and hence pharmacological action, it may be more prudent to determine $C_{u,\text{brain}}$ for brain-targeted compounds.

Considering the free drug hypothesis it may not be unreasonable to consider the unbound drug concentration in blood ($C_{u,\text{blood}}$) as a surrogate for $C_{u,\text{brain}}$ for compounds that passively permeate across the BBB. However, considering the high number of compounds within CNS drug discovery programmes that are substrate for an efflux transporter at the BBB, $C_{u,\text{blood}}$ will not be equal to $C_{u,\text{brain}}$ in many cases. Certainly, clinical PET studies using [$^{11}$C]DASB have shown that plasma levels and serotonin transporter (SERT) occupancy of SSRIs do not correlate when taking into account their affinities for SERT which may, in part, be attributed to efflux transporters (Meyer et al., 2001). There is therefore a need to enhance our understanding of how drug exposures relate to receptor occupancy. To this end, the present study demonstrates that, for a set of marketed $D_2$ receptor antagonists, spanning a wide range of $in vitro$ potencies, total brain and brain free fraction, the fraction of free drug available within the brain extracellular space is a better marker to predict for $D_2$ receptor occupancy and, as such, efficacious drug concentration. In addition, unbound brain concentration is comparable to measured CSF for those compounds with a simple mechanism of entry into brain tissue. Despite this study being limited to a small set of $D_2$ receptor antagonists, it is tempting to speculate that total brain concentrations and $in vitro$ brain tissue binding may represent a useful surrogate for the more technically challenging interstitial fluid values (determined using microdialysis) or CSF values and hence receptor occupancy across a range of molecular targets. Further studies would be required to substantiate this hypothesis but ultimately could provide a high throughput platform to suit CNS drug discovery lead optimization strategies.
Acknowledgements

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Liu et al. 2006 Evaluation of cerebrospinal fluid concentration and plasma free concentration as a surrogate measurement for brain free concentration. Drug Metab Dispos 34: 1443-1447


Legend for figures

Figure 1: [³H]Raclopride autoradiographic binding in rat striatum: Haloperidol significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D₂ receptor occupancy.

Figure 2: [³H]Raclopride autoradiographic binding in rat striatum: Risperidone significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D₂ receptor occupancy.

Figure 3: [³H]Raclopride autoradiographic binding in rat striatum: Quetiapine significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D₂ receptor occupancy.

Figure 4: [³H]Raclopride autoradiographic binding in rat striatum: Ziprasidone significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D₂ receptor occupancy.

Figure 5: [³H]Raclopride autoradiographic binding in rat striatum: Chlorpromazine significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D₂ receptor occupancy.

Figure 6: [³H]Raclopride autoradiographic binding in rat striatum: Clozapine significantly inhibited specific binding in a dose-dependent manner indicative of an increase in D₂ receptor occupancy.
Figure 7: Relationship between receptor occupancy and total brain concentration normalised for the 
*in vitro* affinity for rat striatal D2 receptors. Solid red line represents 50% receptor occupancy.

Figure 8: Relationship between receptor occupancy and unbound brain concentration normalised
for the the *in vitro* affinity for rat striatal D2 receptors. Solid red line represents 50% receptor
occupancy.

Figure 9: Relationship between unbound blood concentration and unbound brain concentration.
Logged data. Solid line represents that of unity.

Figure 10: Relationship between CSF concentration and unbound blood concentration. Logged
data. Solid line represents that of unity.

Figure 11: Relationship between receptor occupancy and unbound blood concentration normalised
for the the *in vitro* affinity for rat striatal D2 receptors. Solid red line represents 50% receptor
occupancy.

Figure 12: Relationship between CSF concentration and unbound brain concentration. Logged
data. Solid line represents that of unity.
<table>
<thead>
<tr>
<th>Compound</th>
<th>pKᵢ</th>
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<tbody>
<tr>
<td>Chlorpromazine</td>
<td>8.4 ± 0.1</td>
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<td>Clozapine</td>
<td>6.8 ± 0.2</td>
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<td>Haloperidol</td>
<td>8.6 ± 0.1</td>
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<td>Quetiapine</td>
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<td>Risperidone</td>
<td>8.4 ± 0.1</td>
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<tr>
<td>Ziprasidone</td>
<td>8.6 ± 0.1</td>
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Table 1: \[^3^H\]FLB-457 binding in rat striatal membranes: Test compounds displaced \[^3^H\]FLB-457 from rat striatal membranes, in a monophasic manner to yield pKᵢ values ranging from 6.7 to 8.6. Data represent mean ± standard error of the mean, from n = 3 experiments.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Fu Brain</th>
<th>Fu Blood</th>
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<td>$0.002 \pm 0.0004$</td>
<td>$0.016 \pm 0.002$</td>
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<td>$0.066 \pm 0.010$</td>
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<td>Haloperidol</td>
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<td>Quetiapine</td>
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<td>Risperidone</td>
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<td>Ziprasidone</td>
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Table 2: Unbound fractions in brain and blood. Data represent mean ± standard deviation, from n = 3 to 5 experiments.
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<th>Compound</th>
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<th>Mean Blood concentration (ng/mL)</th>
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<td>Chlorpromazine 10</td>
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<tr>
<td>Clozapine 0.1</td>
<td>1.6 ± 0.6</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Clozapine 0.3</td>
<td>11 ± 7.3</td>
<td>1.4 ± 1.1</td>
<td>0.1 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Clozapine 1</td>
<td>43 ± 20</td>
<td>6.2 ± 2.9</td>
<td>0.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Clozapine 10</td>
<td>774 ± 148</td>
<td>91 ± 21</td>
<td>9.1 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Clozapine 3</td>
<td>24 ± 8.0</td>
<td>1.2 ± 0.7</td>
<td>0.2 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Clozapine 10</td>
<td>44 ± 39</td>
<td>3.9 ± 2.8</td>
<td>3.3 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Clozapine 30</td>
<td>85 ± 45</td>
<td>9.0 ± 3.2</td>
<td>3.1 ± 2.0</td>
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</tr>
<tr>
<td>Clozapine 100</td>
<td>555 ± 247</td>
<td>67 ± 19</td>
<td>34 ± 9.0</td>
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</tr>
<tr>
<td>Risperidone 0.1</td>
<td>3.4 ± 1.1</td>
<td>2.2 ± 0.9</td>
<td>0.2 ± 0.08</td>
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</tr>
<tr>
<td>Risperidone 0.3</td>
<td>4.8 ± 1.2</td>
<td>4.6 ± 1.7</td>
<td>0.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Risperidone 3</td>
<td>14 ± 2.5</td>
<td>33 ± 5.5</td>
<td>3.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Risperidone 10</td>
<td>39 ± 17</td>
<td>175 ± 115</td>
<td>15 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>Quetiapine 1</td>
<td>48 ± 13</td>
<td>23 ± 9.4</td>
<td>0.3 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Quetiapine 3</td>
<td>117 ± 31</td>
<td>61 ± 23</td>
<td>1.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Quetiapine 30</td>
<td>812 ± 279</td>
<td>462 ± 155</td>
<td>10 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>Ziprasidone 100</td>
<td>1227 ± 299</td>
<td>961 ± 258</td>
<td>23 ± 12</td>
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</tr>
</tbody>
</table>

Table3: Brain, blood and CSF concentrations in male Sprague-Dawley rat at 1 hour following acute oral dosing. Data are reported as mean ± standard deviation, from n = 5 to 7 rats.
*** p< 0.001 vs vehicle, one-way ANOVA followed by Dunnett’s multiple comparison test.
**Figure 2**

*** p< 0.001 vs vehicle, one-way ANOVA followed by Dunnett's multiple comparison test.
Figure 3

% specific binding

- Vehicle
- 3 mg/kg quetiapine
- 10 mg/kg quetiapine
- 30 mg/kg quetiapine
- 100 mg/kg quetiapine

* p < 0.05, *** p < 0.001 vs vehicle, one-way ANOVA followed by Dunnett’s multiple comparison test.
*** p< 0.001 vs vehicle, one-way ANOVA followed by Dunnett’s multiple comparison test.
Figure 5

* p < 0.05, *** p < 0.001 vs vehicle, one-way ANOVA followed by Dunnett’s multiple comparison test.
Figure 6

* p<0.05 , *** p< 0.001 vs vehicle, one-way ANOVA followed by Dunnett’s multiple comparison test.
Figure 7

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Figure 8

- Risperidone
- Haloperidol
- Chlorpromazine
- Ziprasidone
- Quetiapine
- Clozapine

(Brain)u/K

RO (%)
Figure 9

Log(Blood)u (ng/mL) vs Log(Brain)u (ng/g) for various antipsychotics:
- **Risperidone**
- **Haloperidol**
- **Chlorpromazine**
- **Ziprasidone**
- **Quetiapine**
- **Clozapine**
Figure 11

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Figure 12

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