Published in final edited form as:

Neuroimage. 2012 January 2; 59(1): 271–285. doi:10.1016/j.neuroimage.2011.07.001.

Extended characterisation of the serotonin 2A (5-HT_{2A}) receptor-selective PET radiotracer ¹¹C-MDL100907 in humans: quantitative analysis, test-retest reproducibility, and vulnerability to endogenous 5-HT tone

Peter S. Talbot, MD MRCPsych^{1,3}, Mark Slifstein, PhD¹, Dah-Ren Hwang, PhD¹, Yiyun Huang, PhD^{1,2}, Erica Scher, MPH¹, Anissa Abi-Dargham, MD¹, and Marc Laruelle, MD^{1,2}

¹Department of Psychiatry, Columbia University College of Physicians and Surgeons and the New York State Psychiatric Institute, New York, NY 10032

²Department of Radiology, Columbia University College of Physicians and Surgeons and the New York State Psychiatric Institute, New York, NY 10032

Abstract

Introduction—scanning properties and analytic methodology of the 5-HT_{2A} receptor-selective positron emission tomography (PET) tracer ¹¹C-MDL100907 have been partially characterised in previous reports. We present an extended characterisation in healthy human subjects.

Methods—64 ¹¹C-MDL100907 PET scans with metabolite-corrected arterial input function were performed in 39 healthy adults (18–55 yr). 12 subjects were scanned twice (duration 150 min) to provide data on plasma analysis, model order estimation, and stability and test-retest characteristics of outcome measures. All other scans were 90 min duration. 3 subjects completed scanning at baseline and following 5-HT_{2A} receptor antagonist medication (risperidone or ciproheptadine) to provide definitive data on the suitability of the cerebellum as reference region. 10 subjects were scanned under reduced 5-HT and control conditions using rapid tryptophan depletion to investigate vulnerability to competition with endogenous 5-HT. 13 subjects were scanned as controls in clinical protocols. Pooled data were used to analyze the relationship between tracer injected mass and receptor occupancy, and age-related decline in 5-HT_{2A} receptors.

Results—optimum analytic method was a 2-tissue compartment model with arterial input function. However, basis function implementation of SRTM may be suitable for measuring between-group differences non-invasively and warrants further investigation. Scan duration of 90 minutes achieved stable outcome measures in all cortical regions except orbitofrontal which required 120 minutes. Binding potential (BP_P and BP_{ND}) test-retest variability was very good (7–11%) in neocortical regions other than orbitofrontal, and moderately good (14–20%) in orbitofrontal cortex and medial temporal lobe. Saturation occupancy of 5-HT_{2A} receptors by risperidone validates the use of the cerebellum as a region devoid of specific binding for the

© 2011 Elsevier Inc. All rights reserved.

Author and address for correspondence: Peter S. Talbot MD, MRCPsych, Senior Lecturer in Molecular Neuroimaging, The University of Manchester, Wolfson Molecular Imaging Centre, 27 Palatine Road, Withington, Manchester M20 3LJ, United Kingdom, Tel: +44 (0)161 275 0015, Fax: +44 (0)161 275 0003, peter.talbot@manchester.ac.uk.

³Present address: Wolfson Molecular Imaging Centre, The University of Manchester, Manchester M20 3LJ, UK.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

purposes of PET. We advocate a mass limit of 4.6 μg to remain below 5% receptor occupancy. ^{11}C -MDL100907 specific binding is not vulnerable to competition with endogenous 5-HT in humans. Paradoxical decreases in BP_{ND} were found in right prefrontal cortex following reduced 5-HT, possibly representing receptor internalization. Mean age-related decline in brain 5-HT_{2A} receptors was $14.0 \pm 5.0\%$ per decade, and higher in prefrontal regions.

Conclusions—our data confirm and extend support for ^{11}C -MDL100907 as a PET tracer with very favourable properties for quantifying 5-HT_{2A} receptors in the human brain.

Keywords

^{11}C -MDL100907; 5-HT_{2A} receptors; positron emission tomography (PET); test-retest; endogenous competition; rapid tryptophan depletion

1. INTRODUCTION

^{11}C -MDL100907 (also known as ^{11}C -M100907) is the most selective of the positron emission tomography (PET) radiotracers available to quantify 5-HT_{2A} receptors in the living brain. It is a competitive antagonist with subnanomolar affinity for the 5-HT_{2A} receptor in vivo (K_D 0.1 nM; this report), with at least 300-fold lower affinity for 5-HT_{2C}, 5-HT₆, 5-HT₇ and adrenergic α_1 receptors, and negligible affinity for 5-HT_{1A}, dopamine D₁₋₅, α_2 , muscarinic and benzodiazepine receptors in vitro (Johnson et al., 1996; Kehne et al., 1996; Palfreyman et al., 1993; Sorensen et al., 1993). However, since initial evaluations as a PET tracer in non-human primates were reported (Lundkvist et al., 1996; Mathis et al., 1996), there have been relatively few publications of its use in humans (Bhagwagar et al., 2006; Franceschi et al., 2005; Hinz et al., 2007; Ito et al., 1998; Meyer et al., 2010; Perani et al., 2008; Rosell et al., 2010; Talvik-Lotfi et al., 2000). Characteristics in plasma and optimum kinetic model using metabolite-corrected arterial input function have been established, along with increasing but still qualified support for the use of the cerebellum as reference region (Hinz et al., 2007; Ito et al., 1998). However, definitive evidence for lack of displaceable binding in the cerebellum has remained to be established. Moreover, there have been no publications of test-retest variability and reliability of outcome measures, the scan duration necessary to establish stable outcome measures, the relationship between injected mass of tracer and 5-HT_{2A} receptor occupancy (necessary to ensure tracer occupancy conditions), and its vulnerability to competition with endogenous 5-HT in humans. In addition, although negative correlations between age and binding potential in cortical regions have been reported for ^{11}C -MDL100907 (Bhagwagar et al., 2006; Rosell et al., 2010), the sensitivity of the tracer to quantify age-related reductions in 5-HT_{2A} receptor availability remains to be established.

Here, we report the results of an extensive characterisation of ^{11}C -MDL100907 as a PET tracer in healthy adult human subjects, including the outstanding issues above. Plasma analysis, regional brain uptake, derivation of outcome measures, optimum kinetic modelling strategy, and a limited comparison with a non-invasive reference tissue method are also reported with the aim of comparing with and validating previous reports. To the best of our knowledge, this is largest report of human ^{11}C -MDL100907 PET scans to date, and presents the most extensive characterisation.

Previous preliminary and partial presentations in abstract have been made of data on plasma, test-retest characteristics, time stability and optimum kinetic modelling strategy (Mawlawi et al., 2001) and susceptibility of the tracer to competition with endogenous 5-HT (Talbot et al., 2004).

2. MATERIALS AND METHODS

2.1. Human subjects

We report on 64 PET scans using ^{11}C -MDL100907 in 39 healthy subjects. Twenty six (26) of the healthy subjects were recruited to studies specifically designed to characterize PET with ^{11}C -MDL100907: i) plasma analysis; model order estimation; variability, reliability and stability of outcome measures ($n=12$); ii) cerebellum as reference region ($n=4$); and iii) effects of reduced endogenous 5-HT ($n=10$). Additional data from a further 13 healthy subjects, recruited in a number of clinical protocols investigating 5-HT_{2A} receptor availability, were selected from our database and added to provide a larger pool with which to investigate the mass-occupancy relationship for ^{11}C -MDL100907 (data from 17 healthy subjects used) and effects of age on outcome measures (data from 25 healthy subjects used). Further details are included in the individual sections below.

For all subjects, the absence of pregnancy, medical, neurological, and psychiatric history (including alcohol and drug abuse) were assessed by history, Structured Clinical Interview for DSM-IV Axis I Disorders, Non-patient Edition (First et al., 1996), review of systems, physical examination, routine blood tests including pregnancy test, urine toxicology, and EKG. All studies were approved by the Institutional Review Boards of the New York State Psychiatric Institute and the Columbia Presbyterian Medical Center. All subjects provided written informed consent after receiving an explanation of the relevant study.

2.1.1. Plasma, test-retest characteristics, time stability and optimum modelling strategy

—To provide data for analyses of plasma parameters, test-retest characteristics, stability and optimum modelling strategy for ^{11}C -MDL100907 outcome measures, 12 consecutively recruited healthy subjects were scanned twice on the same day (24 scans). Data for 3 of the subjects were excluded from analysis due to camera malfunction ($n = 1$) and excessive head movement ($n = 2$) in one of their scan pairs. Thus, analyses were based on 18 scans in 9 healthy subjects (5M, 4F; mean $\pm$ SD age: 30 ± 7 yr, range 19–40 yr). Each scan was 150 min duration, with a 60 min gap between the end of the first and the start of the second scan.

2.1.2. Validation of cerebellum as region of reference

—The suitability of the cerebellum as region of reference (ROR) was investigated by scanning with ^{11}C -MDL100907 (scan duration 90 min) twice on the same day: before, and 3 hr after, oral administration of a medication with 5-HT_{2A} receptor antagonist properties. Seven (7) scans were performed in 4 consecutively recruited healthy subjects. Three (3) of the subjects (2M, 1F; 35 ± 9 yr, range 28–44 yr) completed the protocol, receiving ciproheptadine 8 mg ($n = 1$) or risperidone 4 mg ($n = 2$). The remaining subject withdrew from the study after the baseline scan. After their post-medication scan, the 3 subjects who received medication were observed overnight in a clinical research facility as a precaution against adverse effects.

2.1.3. Acute reduction of endogenous 5-HT by rapid tryptophan depletion

(RTD)—Ten (10) consecutively recruited healthy subjects (7M, 3F; 29 ± 6 yr, range 19–38 yr) were scanned (duration 90 min) twice with ^{11}C -MDL100907 on separate days: during acutely reduced endogenous 5-HT, and a control condition. All subjects completed the protocol (20 scans). For this study, additional exclusion criteria included a history of affective disorder or schizophrenia in a first degree relative; current evidence of depressed mood as indicated by a score of 14 or greater on the Beck Depression Inventory (BDI) (Beck et al., 1961); and hormonal contraception. Other criteria, details of screening, and the results of ^{11}C -DASB scans also performed on 8 of the subjects as part of the same protocol are as previously described (Talbot et al., 2005).

2.2. Radiochemistry

3-O-Desmethyl-MDL100907 was prepared according to literature procedure (Huang et al., 1999). ^{11}C -MDL100907 was prepared by reacting 3-O-desmethyl-MDL100907 (0.7 mg in 0.5 mL of acetone and 5 μL of 5 M aqueous sodium hydroxide) with ^{11}C -methyl triflate at 70°C for 5 min. The crude product was purified by HPLC (Prodigy ODS-Prep, 10 m, 10×250 mm, eluted with a solvent mixture of 25% acetonitrile and 75% 0.1 M ammonium formate with 0.5% acetic acid, pH 4.2, flow rate 10 mL/min). The tracer fraction was diluted with 100 mL of water and passed through a C-18 Sep-Pak. After eluting the Sep-Pak with water, the tracer was recovered from the Sep-Pak using 1 mL of 200 proof ethanol. The ethanol solution was mixed with 9 mL of sterile saline and filtered through a sterile membrane filter into a sterile vial. The synthesis time was about 45 min and the averaged specific activity of ^{11}C -MDL100907 was about 55.5 GBq/ μmol (1500 mCi/ μmol) at EOS.

2.3. PET protocol

An arterial catheter was inserted into the radial artery after completion of the Allen test and infiltration of the skin with 1% lidocaine. A venous catheter was inserted into a forearm vein on the opposite side. Head movement was minimized with a polyurethane head immobilization system (Soule Medical) (Mawlawi et al., 1999). PET was performed with the ECAT EXACT HR+ (Siemens/CTI). Following a 10 min transmission scan, ^{11}C -MDL100907 was injected intravenously over 30 s by infusion pump. Depending on the protocol, scan duration was either 150 min (24 frames of increasing duration: 3×20 s, 3×1 min, 3×2 min, 2×5 min, 13×10 min) or 90 min (18 frames: 3×20 s, 3×1 min, 3×2 min, 2×5 min, 7×10 min).

2.4. Input function measurement

After radiotracer injection, arterial samples were collected every 10 s with an automated sampling system for the first 2 min and manually thereafter at longer intervals. A total of either 35 (for the 150 min scans) or 29 (90 min scans) samples were obtained per scan. Samples were processed as previously described (Abi-Dargham et al., 2002). Five selected samples (collected at 2, 16, 30, 50, and 70 min) were further processed to measure the fraction of plasma activity representing unmetabolized parent compound, as previously described (Abi-Dargham et al., 2000). A biexponential function was fitted to the 5 measured unmetabolized fractions and used to interpolate values between and after the measurements. The smallest exponential of the unmetabolized fraction curve, λ_{par} , was constrained to the difference between λ_{cer} , the terminal rate of washout of cerebellar activity, and λ_{tot} , the smallest elimination rate constant of the total plasma activity (Abi-Dargham et al., 1999). The input function, the initial distribution volume (V_{bol} , L), and the clearance of the parent compound (C_L , L/hr) were calculated following published methodology (Abi-Dargham et al., 1994). The plasma free fraction (f_p) was determined as previously described (Gandelman et al., 1994; Talbot et al., 2005).

2.5. MRI acquisition and segmentation procedures

T1-weighted anatomical MR images for region drawing and coregistration were acquired and segmentation was performed following previously published methods (Abi-Dargham et al., 2000; Talbot et al., 2005).

2.6. Image analysis

Images were reconstructed to a $128 \times 128 \times 63$ matrix (voxel size of $1.7 \times 1.7 \times 2.4$ mm) with attenuation correction using the transmission data and a Shepp 0.5 filter (cutoff 0.5 cycles/projection ray). Reconstructed image files were then processed in the image analysis software MEDx (Sensor Systems, Inc.). Following initial investigations demonstrating the

benefits of correction of the regional time-activity curves for small between-frame head movements (unpublished data), later frames (13 through last frame) were routinely realigned to a prior reference frame (frame 12) using Automated Image Registration (AIR) (Woods et al., 1998a; Woods et al., 1998b). All frames were then summed to one data set, which was coregistered to the MRI using AIR. The spatial transformation derived from the summed PET registration procedure was then applied to each individual frame. Regions of interest (ROIs) and region of reference (cerebellum; CER) boundaries were drawn on the MRI according to criteria derived from brain atlases and published reports (see Talbot et al., 2005 for original references). A segmentation-based method was used for neocortical regions, and a direct identification method was used for subcortical regions (Abi-Dargham et al., 2002). For bilateral regions, right and left regions were averaged. Data were corrected for blood activity contribution assuming a 5% blood volume fraction (Mintun et al., 1984).

Following initial evaluation, ROIs with negligible or low 5-HT_{2A} specific binding or poor test-retest characteristics were excluded. Therefore, and to anticipate the results, further analysis was restricted to: anterior cingulate cortex, ACC; medial prefrontal cortex, MPFC; temporal cortex, TEM; occipital cortex, OCC; dorsolateral prefrontal cortex, DLPFC; orbitofrontal cortex, OFC; parietal cortex, PAR; insular cortex, INS; entorhinal cortex, ENT; parahippocampal gyrus, PHG; and medial temporal lobe, MTL (a spatially weighted average of 5 limbic structures: uncus, amygdala, ENT, PHG and hippocampus).

2.7. Derivation of Distribution Volumes

2.7.1. Kinetic analysis—¹¹C-MDL100907 regional tissue total distribution volumes (V_T , mL·cm⁻³) were derived with kinetic modelling using the arterial input function and both a two tissue-compartment model (2-TCM) and a one tissue-compartment model (1-TCM) for comparison. The 2-TCM included the arterial plasma compartment (C_p), the intracerebral free plus nonspecifically bound compartment (nondisplaceable compartment, C_{ND}), and the specifically bound compartment (C_S). The 1-TCM included the arterial plasma compartment (C_p) and 1 tissue compartment which comprises C_{ND} and C_S . V_T was defined as the ratio at equilibrium of the total concentration of radioligand in the region (C_T) to the parent radioligand (C_p) in plasma, separated from radiolabelled metabolites (Innis et al., 2007).

Data were fitted to the solutions of differential equations (Slifstein and Laruelle, 2001), and V_T was derived via a nonlinear regression using a Levenberg-Marquart least-squares minimization procedure implemented in MATLAB (The Math Works, Inc.), and was used for the 1-TCM. For the 2-TCM, a constrained (non-negativity) sequential quadratic programming algorithm (also implemented in MATLAB) was used. Given the unequal sampling over time (increasing frame duration during the scan), the least-squares minimization procedures were weighted by frame duration.

2.7.2. Model Order and Goodness of Fit—For the 1-TCM and 2-TCM, goodness of fit of models with different levels of complexity were compared using the Akaike Information Criterion (AIC) (Akaike, 1974) and the F test (Carson, 1986; Landaw and DiStefano, 1984).

2.8. Derivation of 5-HT_{2A} receptor parameters

Derivation of 5-HT_{2A} receptor parameters was based on the following assumptions: i) because the human cerebellum is virtually devoid of ³H- and ¹¹C-labeled MDL100907 specific binding (Hall et al., 2000), cerebellum V_T ($V_{T\text{ CER}}$) was assumed to be representative of equilibrium nondisplaceable binding (V_{ND}); ii) the nondisplaceable binding did not vary significantly between regions. Two measures of ¹¹C-MDL100907 equilibrium specific binding (binding potential) were calculated:

- i. BP_P (the ratio at equilibrium of specifically bound radioligand to that of parent radioligand in plasma) was derived as the difference between V_T in the ROI and $V_{T\text{ CER}}$, the region of reference. The relationship between BP_P and 5-HT_{2A} receptor parameters is given by (Innis et al., 2007):

$$BP_P = \frac{f_p B_{\text{avail}}}{K_D} = V_T - V_{ND}, \quad \text{Eq 1}$$

where B_{avail} is the regional density of 5-HT_{2A} receptors available to bind radioligand in vivo (nmol/L) and K_D is the in vivo affinity of the tracer for 5-HT_{2A} receptors.

- ii. BP_{ND} (the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue) was derived as the ratio of BP_P to $V_{T\text{ CER}}$. The relationship between BP_{ND} and 5-HT_{2A} receptor parameters is given by (Innis et al., 2007):

$$BP_{ND} = \frac{f_{ND} B_{\text{avail}}}{K_D} = \frac{V_T - V_{ND}}{V_{ND}}, \quad \text{Eq 2}$$

where f_{ND} is the free fraction in the nondisplaceable compartment ($f_{ND} = f_p / V_{ND}$, estimated as $f_p / V_{T\text{ CER}}$).

2.9. Variability and reliability of outcome measures

Values for V_T , BP_P and BP_{ND} were evaluated according to 2 criteria: variability and reliability. The test-retest variability (VAR) was calculated as the absolute value of the difference between the test and retest values, divided by the mean of both values. To evaluate the within-subject (WS) variability relative to the between-subject (BS) variability, both WS standard deviation (SD) and BS SD were calculated and expressed as a percentage of the mean value (WS %CV and BS %CV), where CV is the coefficient of variation. The reliability of the measurements was assessed by the intraclass correlation coefficient (ICC), calculated as (Kirk, 1982):

$$ICC = \frac{BSMSS - WSMSS}{BSMSS + WSMSS}, \quad \text{Eq 3}$$

where BSMSS is the mean sum of squares between subjects and WSMSS is the mean sum of squares within subjects. This coefficient estimates the reliability of the measurement and ranges between -1 (no reliability, that is, WSMSS >> BSMSS) to 1 (maximum reliability, achieved in the case of identity between test and retest, that is, WSMSS = 0).

2.10. Stability of outcome measures

Experimental data were collected for 150 minutes. The relationship between V_T , BP_P and BP_{ND} derivation and the duration of the scan was evaluated by fitting increasingly shorter duration data sets (reduced by 10 minute increments back to the 40–50 minute frame; brain and blood data of equal duration in each data set) and comparing the results to the reference value obtained with the 150 minute data set. For each region and each scan duration the mean $\pm$ SD ($n = 18$) of the results expressed as a percentage of the reference value were calculated to provide an estimate of the bias (mean) or dispersion (SD) induced in the outcome measure by analyzing shorter data sets. The parameter was considered stable

relative to scan duration after time t if all results derived from time t to 150 minutes had a mean within 5% of the reference value and a SD that did not exceed 15% of the mean.

2.11. Estimated peak occupancy

Estimated peak % occupancy of 5-HT_{2A} receptors achieved by ¹¹C-MDL100907 under tracer dose administration was calculated based on scan data from 17 healthy subjects (mean age 34 ± 11 yr; range 18–55; 12M, 7F) and a previously computed K_D value (0.1 nM) obtained from in vivo saturation experiments in nonhuman primates using PET in our laboratory. The human scan data were selected based on the high quality of the brain and plasma TACs and to ensure a broad range of injected mass of ¹¹C-MDL100907 across the scans (mean 4.8 ± 2.4 μ g; range 2.2–9.4 μ g). Emission data were acquired for 90 min in 15 of the subjects and 150 min in the other two. Regions included in the analyses were DLPFC, OFC and ACC. The K_D was set at 0.1 nM. This was estimated from PET data (unpublished) acquired in baboons (*Papio anubis*) in our laboratory using high- and low-specific activity injections of ¹¹C-MDL100907 in which the mean K_D across cortical regions was 0.1 nM. The following additional assumptions were made: 1) under equilibrium conditions, free ¹¹C-MDL100907 concentrations equilibrate on both sides of the blood brain barrier; 2) $V_{T\text{CER}}$ represents V_{ND} . From these, the free fraction in the cerebellum (f_{ND}) is calculated as the ratio of the free fraction of the parent compound in the plasma (f_P) to the cerebellum distribution volume (V_{ND}).

For each region in each scan, the concentration of freely dissolved, unmetabolized ¹¹C-MDL100907 in brain tissue was estimated two ways. In the first method, the TAC of specific binding in the region was determined as the difference between the regional TAC and that in the cerebellum, after correction for blood volume contribution to activity. Free plus nonspecifically bound ligand was estimated as the cerebellum value at the time of the peak of the difference curve. Free concentration was determined as the product of the free plus nonspecifically bound concentration with an estimate of brain free fraction (f_{ND}), determined as the ratio of the plasma free fraction (f_P , directly measured) to the cerebellar distribution volume (V_{ND}). Note that for this method, time resolution is limited by the length of a PET frame (10 min for frames late in the scan). The second approach was based on kinetic modelling: a constrained 2-TCM with arterial input function, a free plus nonspecifically bound compartment (denoted C_{ND}), a specifically bound compartment (denoted C_S) and blood volume correction was fitted to the PET data. The constraint was that the distribution volume of C_{ND} equal that of the cerebellum. Free ligand at the moment of peak equilibrium was determined by multiplication of the value of C_{ND} at the moment of maximum C_S by the same estimate of f_{ND} as above. In this approach time resolution (of the moment of peak binding) is arbitrarily fine.

For each method, peak occupancy was determined from the Michaelis Menten formula, using the estimated free and K_D values as

$$\text{Occupancy (\%)} = 100 * \frac{\text{Free}}{K_D + \text{Free}} \quad \text{Eq 4}$$

For each region and each method, occupancy was regressed onto the injected mass (μ g) of ¹¹C-MDL 100907, with intercept constrained to zero, yielding 2 estimates of the slope in each of 3 regions ($n = 17$). The tracer mass limit was determined as the mass associated with 5% occupancy on the regression line.

2.12. Relationship between age and 5-HT_{2A} receptor availability

Data from 25 healthy subjects (mean age 33±10 yr; range 19–55; 16M, 9F) were selected to investigate the relationship between age and 5-HT_{2A} receptor availability when measured by ¹¹C-MDL100907. Age was regressed against BP_{ND} for all ROIs using both linear and quadratic (second-order polynomial) regressions to measure whether the quadratic was significantly better than the linear fit. Correlations were measured using Pearson product moment (r) and their significance assessed as p values (1-sided). In addition, correlations between age and VT_{CER} (as a measure of free plus nonspecific binding) and injected mass of MDL100907 were measured with the aim of excluding these as potential confounds in the measurement of the relationship between age and BP_{ND}.

2.13. Rapid tryptophan depletion procedure

The RTD paradigm was applied in a double-blind, within-subject, counterbalanced, crossover design, so that over the course of two separate days (3–28 days apart) all subjects were scanned with ¹¹C-MDL100907 under both tryptophan depleted and control conditions. For females, both study days were in the first 10 days (early-mid follicular phase) of the same or consecutive menstrual cycles. RTD was achieved through a Trp-free (T–) amino acid mixture administered 5–8 hours before PET scanning. The T– mixture comprised 15 amino acids, while the control (T+) mixture consisted of the same amino acids plus L-tryptophan 2.2 g. For both mixtures, males received 104.3 g and females received 83.5 g. On both days, blood samples were taken before drinking the amino acid mixture and approximately 6.5 hr later at the time of PET scanning. These were analyzed for plasma total Trp, free Trp, and the ratio of Trp to other large neutral amino acids (Trp/ΣLNAA) using a liquid chromatographic procedure. Details of the RTD protocol, the composition of both amino acid mixtures, and the amino acid assays, have previously been described (Talbot et al., 2005). At the same time points, subjective mood was rated using a 100 mm ‘Happy-Sad’ Visual Analogue Mood Scale (VAMS). Baseline VAMS scores and baseline amino acid concentrations across study days were compared by paired t-test (2-tailed). Changes following treatments were compared by repeated measures (RM) ANOVA, with condition (T+ vs T– drink) as within-subjects factor.

2.14. Region of interest (ROI) analysis

BP_{ND} values in 7 cortical regions (ACC, DLPFC, MPFC, OCC, OFC, PAR and TEM) were derived indirectly from ROI V_T values using a 2-TCM for ROIs and cerebellum and using V_T_{CER} as a measure of V_{ND} as described in Equation 2. In order to compare the results with the voxelwise analysis, right and left ROIs were analyzed separately for each cortical region. In an additional exploratory analysis, BP_{ND} values were also derived using a basis function implementation of the simplified reference tissue method (BF SRTM) (Gunn et al., 1997; Lammertsma and Hume, 1996) applied without blood volume correction, with the aim of investigating whether between-condition differences in BP_{ND} derived using invasive methodology could also be detected using bloodless methodology likely to have greater clinical applicability.

Data were analysed by RM ANOVA in SPSS version 16.0 (SPSS Inc., Chicago IL), with condition, region and side as within-subject factors. Post-hoc comparison of individual ROIs between conditions was by paired t-test. The effect of RTD on regional BP_{ND} was expressed as percent difference between the control and RTD conditions ($\Delta BP_{ND} (\%) = [(RTD \text{ value} - \text{control value}) / \text{control value}] * 100$).

2.15. Voxelwise analysis

Voxelwise BP_{ND} maps were generated for each scan. First, 1-TCM fits using the arterial input function were performed in the original reconstructed PET space using a basis function (Cunningham and Jones, 1993; Gunn et al., 1997) approach. Parametric images of V_T and K₁ were formed. BP_{ND} images were then derived from the V_T images according to Equation 2, using the ROI based V_T CER (2-TCM) of each scan for the V_{ND} value. In order to perform between-condition statistical comparisons at each voxel, the BP_{ND} images were normalized into a common template space. To accomplish this, the K₁ images were mapped into Montréal Neurological Institute (MNI) space using the nonlinear warping algorithm contained in SPM99 (Ashburner and Friston, 1999; Friston et al., 1995). The transformation parameters from the K₁ images were then applied to the BP_{ND} images. Spatially normalized BP_{ND} images were then smoothed to approximately twice the FWHM system resolution using an isotropic (10 mm FWHM) Gaussian filter. A threshold was set at 20% of the maximum voxel value in the volume, to exclude subcortical voxels. Differences between mean BP_{ND} in the RTD and control conditions were compared at each voxel by paired t-test. The threshold for statistically significance voxels and clusters was set at $p \leq 0.05$, corrected for multiple comparisons across the whole cortex.

3. RESULTS

3.1. Injected dose

The injected dose (555 ± 104 MBq [15.0 ± 2.8 mCi], $n = 18$), injected mass (5.8 ± 2.1 μ g, $n = 18$) and specific activity (39.1 ± 12.5 GBq/ μ mol [1058 ± 337 mCi/ μ mol], $n = 18$) did not differ between the test and retest conditions (paired t-test, 2-tailed; $p = 0.50, 0.11$ and 0.16 , respectively).

3.2. Plasma analysis

Following bolus i.v. injection, plasma total activity peaked rapidly (1.25 ± 0.14 min), decreased moderately slowly to a nadir of 2.24 ± 0.57 Bq per ml per MBq of injected dose (Bq/ml*MBq ID) at 43.5 min, then increased linearly at a rate of 23% per hour (0.52 Bq/ml*MBq ID*h) to the end of the scan (Figure 1, Panel A). ¹¹C-MDL100907 underwent significant metabolism over the duration of the study (Figure 1, Panel B). Total activity corresponding to the parent compound was as follows (mean $\pm$ SD, $n=18$): 2 min, $94 \pm 5\%$; 16 min, $80 \pm 5\%$; 30 min, $70 \pm 7\%$; 50 min, $59 \pm 6\%$; 70 min, $48 \pm 9\%$. The estimated parent plasma input function (mean of 18 scans) is shown in Figure 1, Panel C). From approximately 45 min onwards, ¹¹C-MDL100907 mean plasma parent decreased moderately slowly, with a terminal half-life of 94 min. The initial distribution volume (V_{bol}) of ¹¹C-MDL100907 was 16 ± 4 L, and the average parent plasma clearance rate (C_L) was 158 ± 57 L/h. The C_L for the test condition was slightly lower than for the retest condition (138 ± 56 vs. 178 ± 54 L/h, $p = 0.03$). The free fraction of ¹¹C-MDL100907 in plasma (f_p) was $31.4\% \pm 4.0\%$ and did not differ between conditions ($n = 18$, $p = 0.79$); test/retest variability = $7.7\% \pm 5.9\%$, ICC = 0.85.

3.3. Brain analysis

Over time, ¹¹C-MDL100907 activity concentrated in regions with high 5-HT_{2A} receptor density (neocortex and insula; Figure 2, Column A). Intermediate levels of activity were seen in several medial temporal lobe structures. Levels were lowest in other subcortical gray matter regions (caudate, putamen, ventral striatum and thalamus), hippocampus, midbrain and cerebellum. However, tracer uptake was readily visible in these regions of low-to-negligible 5-HT_{2A} receptor density (Figure 2, Column A), suggesting a relatively high non-specific binding for ¹¹C-MDL100907.

In cerebellum, putamen, caudate, thalamus, midbrain, ventral striatum and hippocampus, activity peaked relatively early (16–28 min) and the degree of regional tracer washout, defined as the percentage decrease in regional activity from peak to the end of the scan, was relatively high (32–52%). Activity peaked later in medial temporal lobe structures other than hippocampus (52–70 min), and washout was slower (16–20%). In neocortical regions and insula, peak activity was reached relatively slowly (58–93 min) and washout was low (6–11%).

3.4. 1-TC and 2-TC kinetic analysis

3.4.1. Model order estimation—The models were compared for goodness of fit by the AIC and F test. For the AIC, across all subjects and regions, the 2-TC model provided a significantly better fit (lower AIC values) than the 1-TC model (RM ANOVA effect of model, $p < 0.0001$). Post hoc investigation showed that improved goodness of fit was most pronounced in low binding areas: the 2-TCM was significantly superior ($p \leq 0.05$, paired t-test) in all regions of negligible (cerebellum, midbrain) and low (caudate, putamen, hippocampus, thalamus, ventral striatum) specific binding. For regions of high specific binding (ACC, DLPFC, MPFC, OCC, OFC, PAR, TEM, INS), the 2-TC model was slightly favoured in all except one region (OFC) but reached statistical significance in only 2 of the regions (DLPFC and PAR). For regions of moderate specific binding the favoured model varied by region without a clear pattern: MTL (2-TCM, $p = 0.038$), parahippocampal gyrus (2-TCM, $p = \text{ns}$); entorhinal cortex and uncus (1-TCM, $p < 0.001$); amygdala (1-TCM, $p = \text{ns}$). The general superiority of the 2-TCM across regions and subjects was confirmed by the F-test: mean regional F values were significantly different from unity (i.e. the null hypothesis of no difference in goodness of fit between the 2-TC and 1-TC models; paired t-test, $p = 0.0003$). For all fits examined (20 regions $\times$ 9 subjects $\times$ 2 studies = 360 fits), the F test was significant ($p \leq 0.05$) in 150, indicating the higher order model (2-TC) provided a better fit for 42% of the datasets. In the 7 negligible and low binding regions (126 fits), the F test was significant in 81 (64%). In all cases, when the F-test indicated a better fit using the 2-TCM (150/360), this was confirmed by the AIC.

3.4.2. Outcome parameters and test-retest characteristics—The results from 1-TC and 2-TC analyses for 12 (cerebellum and 11 ROIs) of the 20 regions are shown in Table 1 and Table 2, respectively. Of the remaining 8 regions, 5 had low or negligible specific binding (mean $\pm$ SD regional BP_{ND} values from 2-TC model: ventral striatum, 0.45 ± 0.17 ; putamen, 0.16 ± 0.10 ; thalamus, 0.06 ± 0.09 ; caudate nucleus, 0.01 ± 0.08 ; midbrain, -0.08 ± 0.06), and 3 medial temporal lobe sub-regions were found to be individually unreliable, due to low specific binding and/or high test-retest variability (VAR) in outcome measures (mean $\pm$ SD regional BP_{ND} and VAR values from 2-TC model: amygdala, 0.64 ± 0.12 [VAR = $23.7\% \pm 17.5\%$]; hippocampus, 0.31 ± 0.11 [VAR = $29.5\% \pm 17.6\%$]; uncus, 0.95 ± 0.21 [VAR = $24.9\% \pm 22.2\%$]). However, these 3 regions are included (with entorhinal cortex [ENT] and parahippocampal gyrus [PHG]) in the composite medial temporal lobe ROI (MTL).

3.4.3. Cerebellum distribution volume—In the cerebellum, the 2-TC model provided a significantly better fit (AIC, $p = 0.0002$; F test significant in 15 of 18 fits), and identifiability (V_T %CV: 1-TCM, $1.19\% \pm 0.43\%$; 2-TCM, $0.97\% \pm 0.38\%$; $p = 0.004$). Compared to 2-TC model, V_T values derived by 1-TC model were slightly but significantly lower (by $1.6\% \pm 1.4\%$; $p = 0.001$). Nevertheless, values derived from both models were extremely similar and highly correlated ($r^2 = 0.99$). Test-retest variability was good for both models (1-TCM: 8.5 ± 9.9 ; 2-TCM: 8.8 ± 9.3) and did not significantly differ between models ($p = 0.52$). Reliability for both models was good (ICC: 1-TCM, 0.73; 2-TCM, 0.75).

3.4.4. ROI distribution volumes—Across the 11 tabulated ROIs, V_T values derived by 1-TC model were consistently slightly lower compared to 2-TC model, by $0.7\% \pm 2.3\%$ (RM ANOVA effect of model, $p = 0.002$). Nevertheless, V_T values derived from both models were extremely similar and highly correlated ($r^2 = 0.99$, $p < 0.0001$, $n = 198$). In contrast to the CER, identifiability across regions was slightly but significantly better for the 1-TCM (V_T %CV: 1-TCM, $1.63\% \pm 0.80\%$; 2-TCM, $1.86\% \pm 1.09\%$; RM ANOVA effect of model $p < 0.0001$). Post hoc regional paired t-tests found this reached significance ($p \leq 0.05$) in 7 of the 11 regions: ACC, INS, MPFC, OFC, TEM, ENT and PHG. Test-retest variability across regions was very good for both models (≈ 6 – 10%) and was not significantly different between models in any ROI. For both models, reliability was very good (ICC > 0.8) or excellent (ICC > 0.9) in all regions.

3.4.5. 5-HT_{2A} receptor binding potentials—Across the 11 tabulated ROIs, there was no significant difference in BP_P values derived by 1-TC and 2-TC models (RM ANOVA effect of model, $p = 0.99$). Values derived from both models were extremely similar and highly correlated ($r^2 = 0.99$, $p < 0.0001$, $n = 198$). BP_P test-retest variability (VAR) was in the range of ~ 7 – 11% in the majority of regions, and was not significantly different between models in any ROI. However, in contrast to other neocortical regions, VAR in OFC was only moderately good (~ 15 – 18%). For both models, reliability of BP_P (ICC) was very good or excellent in all regions except OFC. However, in OFC it remained good (> 0.7).

BP_{ND} values ranged between ~ 0.6 – 1.0 in medial temporal lobe regions and ~ 1.9 – 2.4 across neocortex and insula. BP_{ND} derived by 1-TC model was consistently slightly higher compared to 2-TC model by $1.5\% \pm 3.0\%$ (RM ANOVA effect of model, $p < 0.0001$). Post hoc regional analysis (paired t-tests) showed this reached significance ($p \leq 0.05$) in ACC, DLPFC, MPFC, OCC, PAR, TEM and ENT. Nevertheless, values derived from both models were extremely similar and highly correlated ($r^2 = 0.99$, $p < 0.0001$, $n = 198$). BP_{ND} VAR was very good (~ 7 – 10%) in neocortical regions other than OFC, and moderately good (~ 14 – 20%) in OFC and medial temporal lobe regions. It was not significantly different between models in any ROI. For both models, BP_{ND} was less reliably derived (ICC) than V_T or BP_P . However, ICC remained good or very good across neocortical regions, and moderate in medial temporal regions.

3.5. Stability of outcome measures

The minimal scanning time needed to derive stable outcome measures for each region is presented in Table 3. When using a 2-TC model, V_T values in cerebellum and MTL were stable by the earliest time point measured (50 minutes), and stable in all other regions by 90 minutes. For BP_P and BP_{ND} , values were stable by 90 minutes in all regions except OFC and ENT. For OFC, scanning time of 120 minutes was required to achieve stable binding potential values.

Use of a 1-TC model conferred no overall advantage for scan duration. While BP_P values reached stability 10–30 minutes earlier in 10 of the 11 ROIs compared to the same values derived by 2-TCM, this advantage was generally not seen for BP_{ND} , and a minimum scan duration of 120 minutes was still required for BP_{ND} to reach stability in OFC.

3.6. Validation of cerebellum as region of reference

Injected activity (mean $\pm$ SD, 618 ± 40 MBq [16.7 ± 1.08 mCi]), specific activity at time of injection (35.9 ± 12.7 GBq/ μ mol [970 ± 343 mCi/ μ mol]), and injected mass of ^{11}C -MDL100907 (7.0 ± 2.0 μ g) did not significantly differ between pre- and post-drug scans. Nor did the drugs significantly affect Cl (183.4 ± 14.7 L/hr) or f_{ND} ($32.9\% \pm 3.3\%$). In ROIs, cyproheptadine 8 mg resulted in $61.6\% \pm 6.2\%$ occupancy (range $48.3\% - 70.0\%$, $n =$

11 ROIs) of 5-HT_{2A} receptors. Risperidone 4 mg resulted in complete ($99.0\% \pm 1.6\%$; range 95.1% – 100%, $n = 11$ ROIs) occupancy (Figure 2, Column B). Despite this, there was no change in cerebellar V_T (pre- vs post-drug V_T , 21.8 ± 1.7 vs 21.7 ± 3.3 ; $p = 0.94$, $n = 3$). These results suggest that the cerebellum lacks significant ¹¹C-MDL100907 specific binding, and support the use of cerebellum as a suitable ROR for ¹¹C-MDL100907.

3.7. Estimated peak occupancy

Regression of occupancy (%) against injected mass (IM, μg) yielded occupancy = $1.09 \times \text{IM}$. Thus, 1 μg of injected mass results in 1.09% occupancy (Figure 3). On the assumption that receptor occupancy of $\leq 5\%$ is suitable for tracer dose assumptions of the analytical methods and is not expected to produce pharmacological effects, we conclude that in order for 5-HT_{2A} receptor occupancy to remain $\leq 5\%$, the maximum injected mass of ¹¹C-MDL100907 is 4.6 μg (i.e. $5/1.09$).

3.8. Effects of age

Age was not correlated with $V_{T\text{CER}}$ ($r^2 = 0.032$, $df = 23$, $p = 0.39$) or injected mass of MDL100907 ($r^2 = 0.006$, $df = 23$, $p = 0.70$). Within the age range of our group (19 – 55 yr), there was a significant age-related decline in BP_{ND} across regions (ANOVA of all ROIs, main effect of age, $p < 0.0001$) with a mean of $14.0 \pm 5.0\%$ per decade. Post hoc analysis of individual regions suggested an apparent fronto-occipital gradient in the magnitude of this decline (% per decade, computed from the slope of the linear regression): ACC, 18.8%; MPFC, 20.3%; DLPFC, 16.8%; OFC, 18.2%; INS, 13.5%; PAR, 12.6%; TEM, 9.8%; OCC, 10.4%; MTL, 5.3%. Mean decline in prefrontal regions was $18.5 \pm 1.5\%$ per decade. The linear regression of age against BP_{ND} was significant ($p \leq 0.05$, 1-sided) in all prefrontal cortical regions (ACC, MPFC, DLPFC, OFC), INS, PAR and MTL; reached trend significance in OCC ($p = 0.09$) and was not significant in TEM ($p = 0.14$). Figure 5 shows the data for a representative region (MPFC) regressed by linear and quadratic fits. In all regions (except OFC), the quadratic equation did not fit the data significantly better than the linear equation. Only in OFC was the fit improved by the quadratic equation (p -value of the increase in r^2 from the linear to the quadratic fit = 0.041).

3.9. Acute reduction of endogenous 5-HT

3.4.2. Plasma amino acid levels—Subjects' baseline plasma total Trp, free Trp and Trp/ Σ LNAA ratio did not significantly differ between the two study days (Table 4). At the time of PET scanning, plasma total Trp (Condition*Time interaction: $F[1,9] = 15.49$, $p = 0.003$), free Trp ($F[1,6] = 12.96$, $p = 0.011$) and Trp/ Σ LNAA ratio ($F[1,9] = 15.91$, $p = 0.003$) were significantly reduced following the T– drink when compared to the T+ drink. In the T– condition, the mean changes in plasma total Trp, free Trp and Trp/ Σ LNAA ratio were $-77.3 \pm 21.4\%$, $-58.8 \pm 48.9\%$ and $-87.6 \pm 16.4\%$ respectively at the time of PET scanning. The corresponding changes in the T+ condition were $+22.9 \pm 67.1\%$, $+56.2 \pm 73.3\%$ and $-48.8 \pm 13.8\%$ respectively. Although the Trp/ Σ LNAA ratio was also reduced in the control (T+) condition, the reduction in the T– condition was significantly greater ($p = 0.003$).

3.4.3. Mood—Subjects were euthymic on entry to the study (BDI score = 3.5 ± 4.3). After both amino acid mixtures, subjects experienced a range of modest mood changes (Δ VAMS) over time. On the T+ day, mean Δ VAMS was -2.6 ± 15.1 mm (range -23 to $+20$ mm). On the T– day, subjects tended to become less happy (Δ VAMS = $+16.5 \pm 22.5$ mm [range 0 to $+74$ mm]). However, the effect of amino acid mixture on mood did not significantly differ between the T+ and T– conditions (Condition*time interaction: $F[1,8] = 3.14$; $p = 0.12$).

3.4.4. Scan parameters—Across all subjects and both study days, PET scanning started 6.9 ± 1.8 hr post-amino acid mixture in the T+ condition, and 6.8 ± 1.5 hr in the T− condition. Start times did not significantly differ between conditions (paired t-test; $p = 0.46$). Scan parameters, including injected activity, specific activity, injected mass of tracer, plasma free fraction, plasma clearance and nonspecific distribution volume ($V_{T\text{ CER}}$) did not differ between conditions (Table 4).

3.4.5. ^{11}C -MDL100907 BP_{ND}

Voxelwise analysis: no regions showed an increase in BP_{ND} under conditions of reduced 5-HT (T− condition), suggesting that ^{11}C -MDL100907 binding is not susceptible to competition with endogenous 5-HT within the physiological range of altered 5-HT that can safely be investigated in humans. In fact, visual inspection of the mean voxelwise BP_{ND} maps suggested that BP_{ND} may be lower in the T− condition in an extended area of ventral and orbital prefrontal cortex (Figure 4, left and middle columns). Statistical comparison using SPM99 confirmed that the T− condition was associated with significantly reduced BP_{ND} in two large, right-sided, prefrontal clusters extending from right dorsal and pre-genual anterior cingulate areas into right anterior OFC and extending slightly beyond the midline into left anterior OFC (Figure 4, right hand column).

ROI analysis (2-TCM): results of the 2-TCM ROI analysis were in support of the voxelwise findings. Although there was no main effect of Condition ($p=0.20$), Condition*Region interaction ($p=0.27$), or Condition*Side interaction ($p=0.24$), there was a significant Condition*Region*Side interaction ($p=0.05$), suggesting a lateralized effect of RTD on a subset of brain regions. Post hoc comparison of individual ROIs between conditions by paired t-test found significantly lower BP_{ND} ($p \leq 0.05$) in the T− condition in right prefrontal cortical regions: right ACC, right DLPFC, right MPFC and right OFC. BP_{ND} in other right-sided ROIs, and in all the left sided ROIs, was not significantly different between conditions (Table 5). No ROI showed significantly increased BP_{ND} in the T− condition.

In no region was there a significant correlation between the changes in mood ($\Delta\Delta\text{VAMS}$) and BP_{ND} ($\Delta\text{BP}_{\text{ND}}$) attributable to RTD. Nor did any region show a significant correlation between the depth of tryptophan depletion (as measured by $\Delta\Delta\text{Trp}/\Sigma\text{LNAA}$) and the associated $\Delta\text{BP}_{\text{ND}}$.

ROI analysis (BF SRTM): in comparison to 2-TCM-derived values, BP_{ND} derived by BF SRTM was lower (by a mean of 42%), with the ratio SRTM/2-TCM decreasing as BP_{ND} increased (linear regression: $\text{BP}_{\text{ND}} \text{ BF SRTM} = 0.5 * \text{BP}_{\text{ND}} \text{ 2-TCM} + 0.17$; data not shown). Despite this, the ROI comparison between conditions was qualitatively similar to the comparison using 2-TCM-derived BP_{ND} . The Condition*Side interaction was significant ($p=0.03$) and the Condition*Region*Side interaction reached strong trend significance ($p=0.06$). Post-hoc analysis showed significant reductions in right DLPFC and MPFC (as in the 2-TCM-derived comparison), right TEM, and OCC bilaterally, although not in right ACC (see Table 5).

4. DISCUSSION

Our data confirm and extend support for ^{11}C -MDL100907 as a PET tracer with very favourable properties for quantifying 5-HT_{2A} receptors in the human brain. In plasma, ^{11}C -MDL100907 undergoes slow metabolism, with $59 \pm 6\%$ of the activity corresponding to the parent compound at 50 min. Our values for parent fractions at increasing time points are very similar to reports from other centres (Hinz et al., 2007), and we found no evidence for

radiolabeled lipophilic metabolites (Hinz et al., 2007; Scott and Heath, 1998). This would appear to be an advantage of ^{11}C -MDL100907 over ^{18}F -altanserin, which requires compensatory methodology to take account of radiolabelled metabolites that cross the BBB (Pinborg et al., 2003). In brain, activity uptake was high in cortical regions; intermediate in medial temporal structures; low in caudate, putamen, ventral striatum, thalamus and hippocampus; and lowest in midbrain and cerebellum, consistent with previous reports and the known distribution of 5-HT_{2A} receptors in the non-human primate and human brain. Mean cerebellar V_T was $20.8 \pm 4.1 \text{ mL cm}^{-3}$. This is comparable to the value of 18.8 ± 1.8 reported by Hinz et al (2007). In regions of highest 5-HT_{2A} receptor density (neocortical regions and insula), peak activity was reached relatively slowly (58–93 min) and washout was low (6–11%), consistent with the need for a minimum scan duration of at least 90 minutes (and 120 min in OFC) to achieve a stable measure of specific binding (see below). Consistent with previous reports in humans and non-human primates (Hinz et al., 2007; Ito et al., 1998; Watabe et al., 2000), we found that a 2-TCM provided a significantly better fit to the data in areas of negligible (cerebellum, midbrain) and low specific binding. In cortical regions the general superiority of the 2-TCM was also evident but less prominent, and there was no clear superiority of one model over the other in regions of moderate specific binding. For these reasons, and consistent with previous reports (Hinz et al., 2007), we advocate metabolite-corrected arterial input function with a 2-TCM as the model of choice for all ROIs.

Test-retest data for ^{11}C -MDL100907 have not been previously published. For the favoured 2-TCM (see Table 2), we found that BP_P test-retest variability (VAR) was good (~ 7 –11%) in the majority of regions, and moderately good in OFC ($\sim 18\%$). Reliability of BP_P (ICC) was very good or excellent in all regions except OFC. However, in OFC it remained good (> 0.7). BP_{ND} VAR was very good (~ 7 –10%) in neocortical regions other than OFC, and moderately good (~ 15 –20%) in OFC and medial temporal lobe regions. BP_{ND} was less reliably derived (ICC) than V_T or BP_P . However, ICC remained good or very good across neocortical regions, and moderate in medial temporal regions. Reasons for the lower VAR of BP_P and BP_{ND} in OFC compared to other neocortical regions may include the fact that it is a relatively small cortical ROI, towards the periphery of the brain and the field of view, and having a relatively long boundary with cold extracerebral tissue, leaving it particularly vulnerable to the effects of errors in coregistration arising from small between-frame head movement during the PET. Because of the poorer VAR in this region, ^{11}C -MDL100907 can be expected to have lower power and require larger group sizes to reliably measure between-group differences in 5-HT_{2A} receptor availability in OFC compared to other cortical areas.

The scan duration required to reach time-stable outcome measures has not previously been reported for ^{11}C -MDL100907. Based on the scan durations (up to 93 min) in the initial studies in monkey (Lundkvist et al., 1996), baboon (Mathis et al., 1996) and humans (Ito et al., 1998), subsequent published human PET studies have acquired emission data for 90–95 min (Bhagwagar et al., 2006; Hinz et al., 2007; Meyer et al., 2010; Perani et al., 2008; Rosell et al., 2010) or less (Franceschi et al., 2005; Talvik-Lotfi et al., 2000) post-injection. Preliminary evaluation in our laboratory of a subset of the 150 min test-retest data presented here, and previously reported in abstract (Mawlawi et al., 2001), suggested that a 90 min scan duration was adequate to derive stable outcome measures and established the duration (90 min) of the majority of scans reported in this paper. However, subsequent evaluation presented in this paper demonstrated that when using the optimum kinetic model (2-TCM) BP_P and BP_{ND} values are stable by 90 minutes in all regions except OFC and ENT (see Table 3). For OFC, scanning time of 120 minutes was required to achieve stable binding potential values. The requirement for a relatively long scan duration is consistent with the slow peak activity (58–93 min) and low washout (6–11%) found in areas of high 5-HT_{2A} receptor density (neocortical regions and insula). Given the short half-life of ^{11}C (20.4 min),

this necessarily results in very low count rates in the late stages of the scan. In this respect, the longer half-life of ^{18}F -altanserin, which has a similar binding profile to MDL100907 (Kristiansen et al., 2005), can be seen as an advantage over ^{11}C -MDL100907. On the other hand, unlike ^{11}C -MDL100907, ^{18}F -altanserin does not permit repeated scans in the same subject over a short period, and its analysis is complicated by brain-penetrant radiolabelled metabolites (Pinborg et al., 2003). Recent efforts to identify potential radiotracers combining the high selectivity and in vivo stability of MDL100907 with the superior isotopic properties of an ^{18}F -label are welcome (Debus et al., 2010; Herth et al., 2009).

An ideal region of reference (ROR) is devoid of significant specific binding, and the cerebellum has been used as ROR for ^{11}C -MDL100907 PET (Bhagwagar et al., 2006; Franceschi et al., 2005; Hinz et al., 2007; Ito et al., 1998; Meyer et al., 2010; Perani et al., 2008; Rosell et al., 2010; Talvik-Lotfi et al., 2000). However, the validity of this has been questioned since the tracer's first published characterisation in humans (Ito et al., 1998) when the superiority of a 2-TCM over a 1-TCM in the cerebellum was interpreted by the authors to infer the presence of a specific binding component. More direct evidence for 5-HT_{2A} receptor gene products (mRNA, protein, binding sites) in the cerebellum at non-negligible levels has been reported in rat (Cornea-Hebert et al., 1999; Maeshima et al., 1998; Pazos et al., 1985), Rhesus monkey (Watabe et al., 2000) and humans (Eastwood et al., 2001). However, it is now recognised that the superiority of a 2-TCM does not necessarily imply the presence of a significant specific binding compartment as model order determination is not always based strictly on the presence or absence of receptors in a region (Slifstein and Laruelle, 2001). Moreover, human PET studies have found no detectable cerebellar binding to 5-HT_{2A} receptors for ^{18}F -altanserin (Pinborg et al., 2003), and no significant change in ^{11}C -MDL100907 cerebellar V_T following 5-HT_{2A} receptor antagonism by mirtazapine, an antidepressant with subnanomolar affinity for the 5-HT_{2A} receptor (Hinz et al., 2007). However, the mean 5-HT_{2A} receptor occupancy achieved in that study was only 60%, and the authors acknowledged that higher occupancy levels would be required to more definitively validate the cerebellar V_T as a measure of free and nonspecific binding. In our current study, we found no significant change in cerebellar V_T following a dose of risperidone (4 mg) that resulted in complete ($99.0\% \pm 1.6\%$) 5-HT_{2A} receptor blockade across the brain. We would propose that our data provide the most compelling evidence to date for the validity of using the cerebellum as ROR for ^{11}C -MDL100907 in human studies. They also support the finding of Pinborg et al (2003) that human cerebellar TACs were unchanged following complete displacement of ^{18}F -altanserin by infusion of the 5-HT_{2A} antagonist ketanserin. They are also consistent with human post-mortem data (Hall et al., 2000) showing that the cerebellum is virtually devoid of ^3H -MDL100907 binding at all levels (including cerebellar nuclei), that total ^3H -MDL100907 binding is the same as nonspecific binding, and that slight ^{11}C -MDL100907 labelling in the cerebellum is not displaced by 10 μM ketanserin indicating that binding is primarily nonspecific. Similar non-displacement in the cerebellum has also been reported for ^{11}C -MDL100907 in ex vivo rat brain (Mathis et al., 1996) and using PET in the Cynomolgus monkey (Lundkvist et al., 1996). With the addition of the data from our current study, a consensus of the literature would now support the validity of the cerebellum as ROR for human ^{11}C -MDL100907 PET.

Cyproheptadine in a dose of 8 mg orally (n=1) was well tolerated and resulted in $61.6\% \pm 6.2\%$ 5-HT_{2A} receptor occupancy across ROIs. The only reported subjective side effects were transient and mild sedation and dizziness, most likely attributable to the drug's histamine H₁ receptor antagonism. Risperidone in a dose of 4 mg (n=2) resulted in complete ($99.0\% \pm 1.6\%$) occupancy, but gave rise to clinically significant orthostatic hypotension and sedation, and mild and transient akathisia, attributable to antagonism at adrenergic α_1 and α_2 , histamine H₁ and dopamine D₂ receptors. These did not prevent the subjects completing the scanning, and fully resolved overnight. However, they were aversive enough

for us to limit the cohort to 2 completers. Future studies aiming to achieve saturation occupancy of 5-HT_{2A} receptors may wish to consider alternative pharmacological methods.

Using high quality data from 3 cortical regions (DLPFC, OFC and ACC) in 17 healthy subjects, we estimate that 1 µg of injected ¹¹C-MDL100907 results in peak 5-HT_{2A} receptor occupancy of 1.09%. Thus, in order for 5-HT_{2A} receptor occupancy to remain at tracer levels (≤ 5%) we advocate a maximum injected mass of ¹¹C-MDL100907 of 4.6 µg (12.32 nanomoles). Using an average adult weight of 70 kg, this equates to an injected mass of 0.066 µg/kg (0.176 nanomoles/kg). To the best of our knowledge, this is the first report of a mass-occupancy relationship for ¹¹C-MDL100907. Our methodology relies on a number of assumptions, which we would suggest are valid. First, the assumption that cerebellar V_T is an accurate representation of V_{ND} is supported by our validation of the cerebellum as ROR as described above. Second, based on PET saturation experiments in nonhuman primates in our laboratory (unpublished data) we used a K_D value of 0.10 nM for ¹¹C-MDL100907 at the 5-HT_{2A} receptor. This is lower than the K_D of ³H-MDL100907 reported in rat tissue (0.14–0.56 nM) (Johnson et al., 1996; Lam et al., 2001; Lopez-Gimenez et al., 1997), as well as in non-human primate (0.16–0.19 nM) and human (0.14–0.19 nM) post-mortem cortical slices (Lopez-Gimenez et al., 1998). However, as values may vary with species and assay conditions, and in the absence of in vivo human PET measurement, we would propose that our measured value based on ¹¹C-MDL100907 PET studies in nonhuman primates is as accurate as possible. Nevertheless, the accuracy of the occupancy calculation is determined by the accuracy of our K_D assumption. If the true in vivo K_D of ¹¹C-MDL100907 in humans is 0.05, the mass associated with 5% peak occupancy would be 2.47 µg. Conversely, if the true K_D is 0.15, the corresponding mass constraint will be 6.7 µg. Our mass-occupancy data would also suggest that the initial monkey and human PET studies from the Karolinska group (Ito et al., 1998; Lundkvist et al., 1996) achieved significantly higher than tracer receptor occupancy. Based on the injected dose and specific activity at the time of administration quoted in the papers, the injected mass of ¹¹C-MDL100907 was approximately 0.12–0.25 µg/kg in the monkeys (Lundkvist et al., 1996) and 6–12 µg total mass in the humans (Ito et al., 1998). This was also the case in a study from NIH, Bethesda, Maryland which reported PET studies in Rhesus monkeys with the aim of developing a suitable kinetic analysis method for quantification of 5-HT_{2A} receptor parameters with ¹¹C-MDL100907 (Watabe et al., 2000). This paper confirmed the superiority of a 2-TCM, but also concluded that the mass doses of MDL100907 used in their studies resulted in higher than tracer receptor occupancy and that this had a modest effect on the results.

A significant decline of 5-HT₂ or 5-HT_{2A} receptor availability with age has been shown for over three decades (for example, see Gross-Isseroff et al., 1990). This decline has been measured and characterised to various degrees with ¹¹C-N-methylspiperone (Iyo and Yamasaki, 1993; Wong et al., 1984), N1-([¹¹C]-methyl)-2-Br-LSD (Wong et al., 1987), 2-¹²³I-ketanserin (D'Haenen et al., 1992), ¹⁸F-N-methylspiperone (Wang et al., 1995), ¹⁸F-setoperone (Blin et al., 1993; Meyer et al., 1999), ¹⁸F-altanserin (Adams et al., 2004; Kaye et al., 2001; Meltzer et al., 1998; Rosier et al., 1996; Sheline et al., 2002) and ¹²³I-5I-R91150 (Baeken et al., 1998; Versijpt et al., 2003). Using ¹¹C-MDL100907, a prominent age-related decrease has been observed previously in monkey (Kakiuchi et al., 2000), while in humans significant negative correlations between age and BP_{ND} in cortical regions have been reported (Bhagwagar et al., 2006; Rosell et al., 2010) though not fully characterised. We found a mean age-related decline in BP_{ND} of 14.0% per decade across all regions and 18.5% in prefrontal regions in healthy subjects between the ages of 19 and 55 yr. This is higher than earlier reports in a similar age range using less selective PET tracers (Wang et al., 1995; Wong et al., 1987; Wong et al., 1984) and the SPECT tracer ¹²³I-5I-R91150 (Baeken et al., 1998; Versijpt et al., 2003), and much lower than an early report using ¹⁸F-altanserin (Rosier et al., 1996). However, it is in good agreement with an ¹⁸F-setoperone

study (Blin et al., 1993) and more recent ^{18}F -altanserin studies (Adams et al., 2004; Kaye et al., 2001; Sheline et al., 2002) that show declines of ~13–20% per decade between young adulthood and midlife. Our data also support previous reports that age-related decline is greater in frontal than occipital cortex (Adams et al., 2004; Wang et al., 1995), and that the slowest rate of decline is in MTL (Sheline et al., 2002; Versijpt et al., 2003). We found no effect of age on ^{11}C -MDL100907 V_{ND} (as measured by $V_{\text{T CER}}$), confirming a previous report (Bhagwagar et al., 2006). Based on earlier reports that the relationship between age and 5-HT_{2A} receptor availability may be altered in patient groups (D'Haenen et al., 1992; Kaye et al., 2001), we limited our analysis to healthy subjects. Subsequent reports in clinical disorders (Bhagwagar et al., 2006; Kaye et al., 2001; Rosell et al., 2010; Versijpt et al., 2003) endorse this initial decision. Several studies have shown that the age-related decline in healthy subjects is most pronounced in younger people, slows after mid-life, and that 5-HT_{2A} receptor availability stabilizes or even slightly increases in later life (Blin et al., 1993; Gross-Isseroff et al., 1990; Marner et al., 2009; Sheline et al., 2002; Versijpt et al., 2003), such that the lifetime decline is better described by a quadratic (second-order polynomial) than a linear regression in most regions (Blin et al., 1993; Sheline et al., 2002). Our finding that in all regions (except OFC) the quadratic equation did not fit the data significantly better than the linear equation supports previous findings that 5-HT_{2A} receptor availability declines fairly linearly to midlife. It also demonstrates that ^{11}C -MDL100907 has good sensitivity to detect the relationship between age and 5-HT_{2A} receptor availability in human brain and supports the need for close matching or control for age when using ^{11}C -MDL100907 BP_{ND} for group comparisons or correlational analyses in clinical studies (Bhagwagar et al., 2006; Perani et al., 2008; Rosell et al., 2010).

Using the dietary technique of rapid tryptophan depletion to reduce synaptic 5-HT we compared ^{11}C -MDL100907 BP_{ND} under conditions of reduced synaptic 5-HT and a control condition, with the hypothesis that if tracer binding was susceptible to competition with endogenous 5-HT an increase in regional BP_{ND} would be observed during reduced 5-HT. No regions showed an increase in BP_{ND} , either on ROI or voxelwise analysis, suggesting that ^{11}C -MDL100907 binding is not susceptible to competition with endogenous 5-HT within the physiological range of altered 5-HT that can safely be achieved in humans. This is in agreement with in vitro and small animal PET data showing that ^{11}C -MDL100907 binding is only displaceable by unphysiologically high levels of 5-HT (Hirani et al., 2003; Kristiansen et al., 2005), and is similar to ^{18}F -altanserin's insensitivity to physiologically relevant alterations in 5-HT (Kristiansen et al., 2005; Pinborg et al., 2004). It is unlikely that our negative finding is due to a lack of effect of RTD in reducing synaptic 5-HT. The same RTD protocol has previously been shown to have significant effects on brain function (Talbot and Cooper, 2006; Talbot et al., 2006). Rather, the lack of competitive effect on ^{11}C -MDL100907 binding may be due to the very low baseline 5-HT_{2A} receptor occupancy by synaptic 5-HT that may be extrapolated from the literature, combined with the limited reduction in 5-HT expected from RTD. In vitro experiments report that human 5-HT_{2A} receptors in the agonist high-affinity state bind 5-HT with a K_{D} of 1.3 nM (Sleight et al., 1996), that only a minority of receptors (in the range 13–45%, across studies) are configured for high-affinity agonist binding and that 5-HT has up to two orders of magnitude lower affinity for the remaining receptors (Fitzgerald et al., 1999; Gray et al., 2003; Hazelwood and Sanders-Bush, 2004; Sleight et al., 1996). In vivo microdialysis experiments in awake non-human primates report baseline extracellular 5-HT concentrations in cortex of approximately 0.2 nM (Bradberry and Rubino, 2004; Smith et al., 2000). Notwithstanding the uncertainties associated with extrapolating from in vitro and animal data, substitution of the above values into the following equation:

$$\text{Occupancy (\%)} = 100 * \frac{[5-HT]/K_D}{1 + ([5-HT]/K_D)} * R_{\text{high}}$$

Eq 5

where [5-HT] is 5-HT concentration at the receptor, and R_{high} represents the proportion of 5-HT_{2A} receptors available to bind 5-HT in vivo (i.e. configured in the agonist high-affinity state), suggests that baseline 5-HT_{2A} receptor occupancy by 5-HT may only be in the range of approximately 2–6%. Thus, even if RTD were to cause a reduction in 5-HT synthesis of up to 90% (as suggested by Nishizawa et al., 1997), and this were to be reflected in a comparable reduction in 5-HT release, only another ~1–5% of receptors would become available for ¹¹C-MDL100907 binding. Given that ¹¹C-MDL100907, as an antagonist tracer, will bind to 5-HT_{2A} receptors in both high- and low-affinity states, such a change in signal may be difficult to detect with a realistic sample size. Based on these conclusions, the development of agonist radiotracers for the 5-HT_{2A} receptor such as ¹¹C-CIMBI-5 (Ettrup et al., 2010), which bind preferentially to the agonist high-affinity state thereby offering increased sensitivity to endogenous competition by 5-HT, coupled with paradigms producing changes in extracellular 5-HT of greater magnitude such as acute challenge with citalopram (2- to 6-fold increase) or fenfluramine (10- to 25-fold increase), perhaps offer the best chance for measuring changes in synaptic 5-HT levels at the 5-HT_{2A} receptor. Nevertheless, given the degree of extrapolation and assumption inherent in the estimation of baseline 5-HT occupancy above, we would maintain that the RTD experiment was worth doing. Moreover, we can now conclude with confidence that ¹¹C-MDL100907 may be used to measure differences in 5-HT_{2A} receptor availability between populations and conditions without the need to consider concomitant changes in neurotransmitter concentration.

Conversely, we found that RTD induced a paradoxical and lateralized decrease in BP_{ND} in right prefrontal cortex, taking in parts of ACC, DLPFC, MED and OFC. Although this was unexpected, we report it as it was found on both the ROI and voxelwise comparisons, and survived corrections for multiple comparisons across the whole cortex. Moreover, it is consistent with the only other PET study investigating the effects of RTD on brain 5-HT₂ or 5-HT_{2A} receptor availability of which we are aware (Yatham et al., 2001). In that study, using ¹⁸F-setoperone, a significant widespread reduction in 5-HT₂ BP was observed following RTD in healthy females. The authors hypothesised that this was most likely due to a rapid reduction in synaptic receptor density, and that this downregulation under conditions of acutely reduced 5-HT may be a normal mechanism preventing an acute deterioration of mood. In our study we found a similar reduction, but localised to right prefrontal regions, and it may be relevant that these are closely associated with the regulation of affect. Any explanation of mechanism must remain conjectural. However, 5-HT_{2A} antagonists including clozapine and MDL100907 have been reported to induce internalization of 5-HT_{2A} receptors in vitro (Willins et al., 1999), suggesting that our finding may represent internalisation secondary to an acute reduction of receptor agonism by 5-HT or a direct antagonist effect of MDL100907. Our mass-occupancy finding (see above), strongly suggesting that peak 5-HT_{2A} receptor occupancy by ¹¹C-MDL100907 was below 5% during our scans, may make the former explanation more likely. If valid, our finding of a localised rather than generalised reduction in BP_{ND} may suggest that females (all subjects were female in the Yatham study) are more sensitive to this effect of RTD than males (7 of the 10 subjects in our study were male), consistent with a well-established association between serotonergic vulnerability and female gender (Jans et al., 2007). However, the validity of receptor internalisation as an explanation for reduced BP_{ND} would presumably require ¹¹C-MDL100907 to be able to differentially label externalised over internalised receptors, a property that we are not aware has been established.

In relation to other potential explanations, given the unilateral and peripheral location of the findings, we considered the possibility that they may be an artefact of inaccuracies of spatial normalisation or coregistration. By introducing deliberate 1 mm misregistrations in the x, y, and z directions in a subset of our data, and measuring the effect on regional V_T , BP_P and BP_{ND} across cortical regions, we demonstrated that misregistration was a source of noise but not of bias (data not shown). Given the location of the OFC (as described above), we felt this was a potential source of the relatively poorer OFC test-retest variability compared to other cortical regions and the resultant modest loss of power in this region. However, it provided no support for the idea that small errors in normalization or registration are a likely cause of a systematic and localized bias necessary to explain the RTD findings.

Although we advocate 2-TCM with a metabolite-corrected arterial input function as the model of choice, our limited investigation of non-invasive reference tissue methods suggests that BF SRTM, while not in perfect agreement with 2-TCM as previously reported (Meyer et al., 2010), may be both useful and accurate for measuring between-group or between-condition differences in 5-HT_{2A} receptor availability with ¹¹C-MDL100907. Using this method, the pattern of lateralised significant changes in BP_{ND} detected secondary to RTD was similar to that using metabolite-corrected arterial input function and 2-TC kinetic modelling. If anything, the right-sided reduction was somewhat more generalized with BF SRTM (significant Condition*Side interaction, and significant additional findings in OCC and TEM, although reduction was not found in right ACC). As the need for invasive methods generally prevents widespread clinical utility or patient acceptability, further investigation of the sensitivity and accuracy of BF SRTM and other non-invasive methods to detect group differences is certainly warranted, preferably with better defined and reliable differences than available in this exploratory analysis.

4.1. Conclusions

Our data represent the most comprehensive characterisation to date of the most selective 5-HT_{2A} receptor PET tracer, and confirm and extend support for ¹¹C-MDL100907 as a PET tracer with very favourable imaging properties in the human brain. Scan duration of 90 minutes is adequate to achieve stable outcome measures in all cortical regions except OFC, and reproducibility is very good in neocortical regions other than OFC and MTL, where it remains moderately good. The cerebellum may be used as a reference region devoid of specific binding for the purposes of PET, and the tracer has good sensitivity to detect the relationship between age and 5-HT_{2A} receptor availability. Specific binding is not vulnerable to competition with endogenous 5-HT in humans, allowing ¹¹C-MDL100907 to be used to measure differences in 5-HT_{2A} receptor availability between populations and conditions without the need to consider concomitant changes in neurotransmitter concentration. For non-invasive derivation of BP_{ND} , BF SRTM may be suitable for measuring between-group differences in 5-HT_{2A} receptor availability and warrants further investigation.

Acknowledgments

The authors thank Ingrid Gelbard-Stokes, Elizabeth Hackett, Christina Hansson, John Kim, Elizabeth Mitchell, Kim Ngo, Chaka Peters, Nurat Quadri, Celeste Reinking, Lyudmilla Savenkova, Zohar Zephani, and the staff of The Irving Center for Clinical Research for their excellent technical and clinical assistance. The administrative and technical assistance of the late Susan Curry is respectfully acknowledged.

This work was supported in part by the Seaver Foundation, the Lieber Center for Schizophrenia Research, and a grant from the National Institute for Drug Abuse (R01DA015806). The funding bodies had no role in the study design; data collection, analysis and interpretation; manuscript writing; or in the decision to submit the paper for publication.

REFERENCES

- Abi-Dargham A, Laruelle M, Seibyl J, Rattner Z, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Bremner JD, Hyde TM, Charney DS, Hoffer PB, Innis RB. SPECT measurement of benzodiazepine receptors in human brain with iodine-123-iomazenil: kinetic and equilibrium paradigms. *J. Nucl. Med.* 1994; 35:228–238. [PubMed: 8294990]
- Abi-Dargham A, Martinez D, Mawlawi O, Simpson N, Hwang DR, Slifstein M, Anjilvel S, Pidcock J, Guo NN, Lombardo I, Mann JJ, Van Heertum R, Foged C, Halldin C, Laruelle M. Measurement of striatal and extrastriatal dopamine D1 receptor binding potential with [¹¹C]NNC 112 in humans: validation and reproducibility. *J. Cereb. Blood Flow Metab.* 2000; 20:225–243. [PubMed: 10698059]
- Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M. Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J. Neurosci.* 2002; 22:3708–3719. [PubMed: 11978847]
- Abi-Dargham A, Simpson N, Kegeles L, Parsey R, Hwang DR, Anjilvel S, Zea-Ponce Y, Lombardo I, Van Heertum R, Mann JJ, Foged C, Halldin C, Laruelle M. PET studies of binding competition between endogenous dopamine and the D1 radiotracer [¹¹C]NNC 756. *Synapse.* 1999; 32:93–109. [PubMed: 10231129]
- Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, Madsen K, Frokjaer V, Martiny L, Paulson OB, Knudsen GM. A database of [(18)F]-altanserin binding to 5-HT(2A) receptors in normal volunteers: normative data and relationship to physiological and demographic variables. *Neuroimage.* 2004; 21:1105–1113. [PubMed: 15006678]
- Akaike H. A new look at the statistical identification model. *IEEE T. Automat. Contr.* 1974; 19:716–723.
- Ashburner J, Friston KJ. Nonlinear spatial normalization using basis functions. *Hum. Brain Mapp.* 1999; 7:254–266. [PubMed: 10408769]
- Baeken C, D'Haenen H, Flamen P, Mertens J, Terriere D, Chavatte K, Boumon R, Bossuyt A. 123I-5-I-R91150, a new single-photon emission tomography ligand for 5-HT2A receptors: influence of age and gender in healthy subjects. *Eur. J. Nucl. Med.* 1998; 25:1617–1622. [PubMed: 9871092]
- Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch. Gen. Psychiatry.* 1961; 4:561–571. [PubMed: 13688369]
- Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P. Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907. *Am. J. Psychiatry.* 2006; 163:1580–1587. [PubMed: 16946184]
- Blin J, Baron JC, Dubois B, Crouzel C, Fiorelli M, Attar-Levy D, Pillon B, Fournier D, Vidailhet M, Agid Y. Loss of brain 5-HT2 receptors in Alzheimer's disease. In vivo assessment with positron emission tomography and [18F]setoperone. *Brain.* 1993; 116:497–510. [PubMed: 8513389]
- Bradberry CW, Rubino SR. Phasic alterations in dopamine and serotonin release in striatum and prefrontal cortex in response to cocaine predictive cues in behaving rhesus macaques. *Neuropsychopharmacology.* 2004; 29:676–685. [PubMed: 14747825]
- Carson, RE. Parameters estimation in positron emission tomography. In: Phelps, ME.; Mazziotta, JC.; Schelbert, HR., editors. *Positron emission tomography: Principles and applications for the brain and heart.* Raven Press: New York, NY; 1986. p. 347-390.
- Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. *J. Comp. Neurol.* 1999; 409:187–209. [PubMed: 10379914]
- Cunningham VJ, Jones T. Spectral analysis of dynamic PET studies. *J. Cereb. Blood Flow Metab.* 1993; 13:15–23. [PubMed: 8417003]
- D'Haenen H, Bossuyt A, Mertens J, Bossuyt-Piron C, Gijsemans M, Kaufman L. SPECT imaging of serotonin2 receptors in depression. *Psychiatry Res.* 1992; 45:227–237. [PubMed: 1292040]
- Debus F, Herth MM, Piel M, Buchholz H-G, Bausbacher N, Kramer V, Luddens H, Rosch F. 18F-labeling and evaluation of novel MDL 100907 derivatives as potential 5-HT2A antagonists for molecular imaging. *Nucl. Med. Biol.* 2010; 37:487–495. [PubMed: 20447561]

- Eastwood SL, Burnet PW, Gittins R, Baker K, Harrison PJ, Eastwood SL, Burnet PW, Gittins R, Baker K, Harrison PJ. Expression of serotonin 5-HT_{2A} receptors in the human cerebellum and alterations in schizophrenia. *Synapse*. 2001; 42:104–114. [PubMed: 11574947]
- Ettrup A, Palner M, Gillings N, Santini MA, Hansen M, Kornum BR, Rasmussen LK, Nagren K, Madsen J, Begtrup M, Knudsen GM. Radiosynthesis and evaluation of ¹¹C-CIMBI-5 as a 5-HT_{2A} receptor agonist radioligand for PET. *J. Nucl. Med.* 2010; 51:1763–1770. [PubMed: 20956470]
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Biometrics Research Department. New York: New York State Psychiatric Institute; 1996. Structured Clinical Interview for DSM-IV Axis I Disorders - Non-patient Edition (SCID-I/NP, Version 2.0).
- Fitzgerald LW, Conklin DS, Krause CM, Marshall AP, Patterson JP, Tran DP, Iyer G, Kostich WA, Largent BL, Hartig PR. High-affinity agonist binding correlates with efficacy (intrinsic activity) at the human serotonin 5-HT_{2A} and 5-HT_{2C} receptors: evidence favoring the ternary complex and two-state models of agonist action. *J. Neurochem.* 1999; 72:2127–2134. [PubMed: 10217294]
- Franceschi M, Anchisi D, Pelati O, Zuffi M, Matarrese M, Moresco RM, Fazio F, Perani D. Glucose metabolism and serotonin receptors in the frontotemporal lobe degeneration. *Ann. Neurol.* 2005; 57:216–225. [PubMed: 15668960]
- Friston KJ, Holmes CJ, Worsley KJ, Poline J-P, Frith CD, Frakowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. *Hum. Brain Mapp.* 1995; 2:189–210.
- Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. Evaluation of ultrafiltration for the free-fraction determination of single photon emission computed tomography (SPECT) radiotracers: beta-CIT, IBF, and iomazenil. *J. Pharm. Sci.* 1994; 83:1014–1019. [PubMed: 7965658]
- Gray JA, Bhatnagar A, Gurevich VV, Roth BL. The interaction of a constitutively active arrestin with the arrestin-insensitive 5-HT_{2A} receptor induces agonist-independent internalization. *Mol. Pharmacol.* 2003; 63:961–972. [PubMed: 12695524]
- Gross-Isseroff R, Salama D, Israeli M, Biegon A. Autoradiographic analysis of age-dependent changes in serotonin 5-HT₂ receptors of the human brain postmortem. *Brain Res.* 1990; 519:223–227. [PubMed: 2397409]
- Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage.* 1997; 6:279–287. [PubMed: 9417971]
- Hall H, Farde L, Halldin C, Lundkvist C, Sedvall G. Autoradiographic localization of 5-HT_{2A} receptors in the human brain using [(3)H]M100907 and [(11)C]M100907. *Synapse.* 2000; 38:421–431. [PubMed: 11044889]
- Hazelwood LA, Sanders-Bush E. His452Tyr polymorphism in the human 5-HT_{2A} receptor destabilizes the signaling conformation. *Mol. Pharmacol.* 2004; 66:1293–1300. [PubMed: 15496511]
- Herth MM, Kramer V, Piel M, Palner M, Riss PJ, Knudsen GM, Rosch F. Synthesis and in vitro affinities of various MDL 100907 derivatives as potential ¹⁸F-radioligands for 5-HT_{2A} receptor imaging with PET. *Bioorg. Med. Chem.* 2009; 17:2989–3002. [PubMed: 19329329]
- Hinz R, Bhagwagar Z, Cowen PJ, Cunningham VJ, Grasby PM. Validation of a tracer kinetic model for the quantification of 5-HT_{2A} receptors in human brain with [(11)C]MDL 100,907. *J. Cereb. Blood Flow Metab.* 2007; 27:161–172. [PubMed: 16685260]
- Hirani E, Sharp T, Sprakes M, Grasby P, Hume S. Fenfluramine evokes 5-HT_{2A} receptor-mediated responses but does not displace [¹¹C]MDL 100907: small animal PET and gene expression studies. *Synapse.* 2003; 50:251–260. [PubMed: 14515343]
- Huang Y, Mahmood K, Mathis CA. An efficient synthesis of the precursors of [¹¹C]MDL 100907 labeled in two specific positions. *J. Labelled Comp. Radiopharm.* 1999; 42:949–957.
- Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE. Consensus nomenclature for in vivo imaging of

- reversibly binding radioligands. *J. Cereb. Blood Flow Metab.* 2007; 27:1533–1539. [PubMed: 17519979]
- Ito H, Nyberg S, Halldin C, Lundkvist C, Farde L. PET imaging of central 5-HT_{2A} receptors with carbon-11-MDL 100,907. *J. Nucl. Med.* 1998; 39:208–214. [PubMed: 9443763]
- Iyo M, Yamasaki T. The detection of age-related decrease of dopamine D₁, D₂ and serotonin 5-HT₂ receptors in living human brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 1993; 17:415–421. [PubMed: 8475323]
- Jans LAW, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. *Mol. Psychiatry.* 2007; 12:522–543. [PubMed: 17160067]
- Johnson MP, Siegel BW, Carr AA. [3H]MDL 100,907: a novel selective 5-HT_{2A} receptor ligand. *Naunyn. Schmiedeberg Arch. Pharmacol.* 1996; 354:205–209. [PubMed: 8857599]
- Kakiuchi T, Nishiyama S, Sato K, Ohba H, Nakanishi S, Tsukada H. Age-related reduction of [11C]MDL100,907 binding to central 5-HT(2A) receptors: PET study in the conscious monkey brain. *Brain Res.* 2000; 883:135–142. [PubMed: 11063997]
- Kaye WH, Frank GK, Meltzer CC, Price JC, McConaha CW, Crossan PJ, Klump KL, Rhodes L. Altered serotonin 2A receptor activity in women who have recovered from bulimia nervosa. *Am. J. Psychiatry.* 2001; 158:1152–1155. [PubMed: 11431241]
- Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, Frank RA, van Giersbergen PL, McCloskey TC, Johnson MP, McCarty DR, Poirot M, Senyah Y, Siegel BW, Widmaier C. Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT_{2A} antagonist with a favorable CNS safety profile. *J. Pharmacol. Exp. Ther.* 1996; 277:968–981. [PubMed: 8627580]
- Kirk, RE. *Experimental Design: Procedures for the Behavioral Sciences.* Pacific Grove, CA: Brooks/Cole Publishing Co; 1982.
- Kristiansen H, Elfving B, Plenge P, Pinborg LH, Gillings N, Knudsen GM. Binding characteristics of the 5-HT_{2A} receptor antagonists altanserin and MDL 100907. *Synapse.* 2005; 58:249–257. [PubMed: 16206185]
- Lam HR, Plenge P, Jorgensen OS. Effects of white spirits on rat brain 5-HT receptor functions and synaptic remodeling. *Neurotoxicol. Teratol.* 2001; 23:603–608. [PubMed: 11792528]
- Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *Neuroimage.* 1996; 4:153–158. [PubMed: 9345505]
- Landaw EM, DiStefano JJ 3rd. Multiexponential, multicompartmental, and noncompartmental modeling. II. Data analysis and statistical considerations. *Am. J. Physiol.* 1984; 246:R665–R677. [PubMed: 6720989]
- Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT. Selective visualization of rat brain 5-HT_{2A} receptors by autoradiography with [3H]MDL 100,907. *Naunyn. Schmiedeberg Arch. Pharmacol.* 1997; 356:446–454. [PubMed: 9349630]
- Lopez-Gimenez JF, Vilaro MT, Palacios JM, Mengod G. [3H]MDL 100,907 labels 5-HT_{2A} serotonin receptors selectively in primate brain. *Neuropharmacology.* 1998; 37:1147–1158. [PubMed: 9833645]
- Lundkvist C, Halldin C, Ginovart N, Nyberg S, Swahn CG, Carr AA, Brunner F, Farde L. [11C]MDL 100907, a radioligand for selective imaging of 5-HT(2A) receptors with positron emission tomography. *Life Sci.* 1996; 58:PL 187–PL 192.
- Maeshima T, Shutoh F, Hamada S, Senzaki K, Hamaguchi-Hamada K, Ito R, Okado N. Serotonin_{2A} receptor-like immunoreactivity in rat cerebellar Purkinje cells. *Neurosci. Lett.* 1998; 252:72–74. [PubMed: 9756362]
- Marner L, Knudsen GM, Haugbol S, Holm S, Baare W, Hasselbalch SG. Longitudinal assessment of cerebral 5-HT_{2A} receptors in healthy elderly volunteers: an [18F]-altanserin PET study. *Eur. J. Nucl. Med. Mol. Imaging.* 2009; 36:287–293. [PubMed: 18828013]
- Mathis CA, Mahmood K, Huang Y, Simpson N, Gerdes JM, Price JC. Synthesis and preliminary in vivo evaluation of [11C]MDL 100907: a potent and selective radioligand for the 5-HT_{2A} receptor system. *Med. Chem. Res.* 1996; 6:1–10.

- Mawlawi O, Huang Y, Hwang DR, Martinez D, Lombardo I, Slifstein M, Amstel D, Eftychiou N, Gelbard I, Van Heertum R, Abi-Dargham A, Laruelle M. Mapping 5HT_{2A} receptors in human brain with (11)C[MDL100907]: Validation and reproducibility. *J. Nucl. Med.* 2001; 42:106. [PubMed: 11197957]
- Mawlawi OM, Weiss R, Shinn A, Pidcock J, Slifstein M, Laruelle M. Performance characteristics of a head immobilization device for PET imaging. *J. Nucl. Med.* 1999; 40:281.
- Meltzer CC, Smith G, Price JC, Reynolds CF 3rd, Mathis CA, Greer P, Lopresti B, Mintun MA, Pollock BG, Ben-Eliezer D, Cantwell MN, Kaye W, DeKosky ST. Reduced binding of [18F]altanserin to serotonin type 2A receptors in aging: persistence of effect after partial volume correction. *Brain Res.* 1998; 813:167–171. [PubMed: 9824691]
- Meyer JH, Kapur S, Houle S, DaSilva J, Owczarek B, Brown GM, Wilson AA, Kennedy SH. Prefrontal cortex 5-HT₂ receptors in depression: an [18F]setoperone PET imaging study. *Am. J. Psychiatry.* 1999; 156:1029–1034. [PubMed: 10401447]
- Meyer PT, Bhagwagar Z, Cowen PJ, Cunningham VJ, Grasby PM, Hinz R. Simplified quantification of 5-HT_{2A} receptors in the human brain with [11C]MDL 100,907 PET and non-invasive kinetic analyses. *Neuroimage.* 2010; 50:984–993. [PubMed: 20083209]
- Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann. Neurol.* 1984; 15:217–227. [PubMed: 6609679]
- Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, de Montigny C, Blier P, Diksic M. Differences between males and females in rates of serotonin synthesis in human brain. *Proc. Natl. Acad. Sci. U. S. A.* 1997; 94:5308–5313. [PubMed: 9144233]
- Palfreyman MG, Schmidt CJ, Sorensen SM, Dudley MW, Kehne JH, Moser P, Gittos MW, Carr AA. Electrophysiological, biochemical and behavioral evidence for 5-HT₂ and 5-HT₃ mediated control of dopaminergic function. *Psychopharmacology (Berl).* 1993; 112:S60–S67. [PubMed: 7831442]
- Pazos A, Cortes R, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.* 1985; 346:231–249. [PubMed: 4052777]
- Perani D, Garibotto V, Gorini A, Moresco RM, Henin M, Panzacchi A, Matarrese M, Carpinelli A, Bellodi L, Fazio F. In vivo PET study of 5HT(2A) serotonin and D(2) dopamine dysfunction in drug-naïve obsessive-compulsive disorder. *Neuroimage.* 2008; 42:306–314. [PubMed: 18511303]
- Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, Madsen J, Knudsen GM. Quantification of 5-HT_{2A} receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. *J. Cereb. Blood Flow Metab.* 2003; 23:985–996. [PubMed: 12902843]
- Pinborg LH, Adams KH, Yndgaard S, Hasselbalch SG, Holm S, Kristiansen H, Paulson OB, Knudsen GM. [18F]altanserin binding to human 5HT_{2A} receptors is unaltered after citalopram and pindolol challenge. *J. Cereb. Blood Flow Metab.* 2004; 24:1037–1045. [PubMed: 15356424]
- Rosell DR, Thompson JL, Slifstein M, Xu X, Frankle WG, New AS, Goodman M, Weinstein SR, Laruelle M, Abi-Dargham A, Siever LJ. Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. *Biol. Psychiatry.* 2010; 67:1154–1162. [PubMed: 20434136]
- Rosier A, Dupont P, Peuskens J, Bormans G, Vandenberghe R, Maes M, de Groot T, Schiepers C, Verbruggen A, Mortelmans L. Visualisation of loss of 5-HT_{2A} receptors with age in healthy volunteers using [18F]altanserin and positron emission tomographic imaging. *Psychiatry Res.* 1996; 68:11–22. [PubMed: 9027929]
- Scott DO, Heath TG. Investigation of the CNS penetration of a potent 5-HT_{2a} receptor antagonist (MDL 100,907) and an active metabolite (MDL 105,725) using in vivo microdialysis sampling in the rat. *J. Pharm. Biomed. Anal.* 1998; 17:17–25. [PubMed: 9608422]
- Sheline YI, Mintun MA, Moerlein SM, Snyder AZ. Greater loss of 5-HT(2A) receptors in midlife than in late life. *Am. J. Psychiatry.* 2002; 159:430–435. [PubMed: 11870007]
- Sleight AJ, Stam NJ, Mutel V, Vanderheyden PM. Radiolabelling of the human 5-HT_{2A} receptor with an agonist, a partial agonist and an antagonist: effects on apparent agonist affinities. *Biochem. Pharmacol.* 1996; 51:71–76. [PubMed: 8534270]

- Slifstein M, Laruelle M. Models and methods for derivation of in vivo neuroreceptor parameters with PET and SPECT reversible radiotracers. *Nucl. Med. Biol.* 2001; 28:595–608. [PubMed: 11516703]
- Smith TD, Kuczenski R, George-Friedman K, Malley JD, Foote SL. In vivo microdialysis assessment of extracellular serotonin and dopamine levels in awake monkeys during sustained fluoxetine administration. *Synapse.* 2000; 38:460–470. [PubMed: 11044893]
- Sorensen SM, Kehne JH, Fadaye GM, Humphreys TM, Ketteler HJ, Sullivan CK, Taylor VL, Schmidt CJ. Characterization of the 5-HT₂ receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. *J. Pharmacol. Exp. Ther.* 1993; 266:684–691. [PubMed: 8102646]
- Talbot PS, Cooper SJ. Anterior cingulate and subgenual prefrontal blood flow changes following tryptophan depletion in healthy males. *Neuropsychopharmacology.* 2006; 31:1757–1767. [PubMed: 16407892]
- Talbot PS, Frankle WG, Hwang DR, Huang Y, Abi-Dargham A, Laruelle M. Effects of transient reduction of endogenous 5-HT levels on brain 5-HT transporter and 5-HT_{2A} receptor availability: a PET study using [11C]DASB, [11C]MDL100907 and the rapid tryptophan depletion method in healthy humans. *Biol. Psychiatry.* 2004; 55:63S–64S.
- Talbot PS, Frankle WG, Hwang DR, Huang Y, Suckow RF, Slifstein M, Abi-Dargham A, Laruelle M. Effects of reduced endogenous 5-HT on the in vivo binding of the serotonin transporter radioligand 11C-DASB in healthy humans. *Synapse.* 2005; 55:164–175. [PubMed: 15605360]
- Talbot PS, Watson DR, Barrett SL, Cooper SJ. Rapid tryptophan depletion improves decision-making cognition in healthy humans without affecting reversal learning or set shifting. *Neuropsychopharmacology.* 2006; 31:1519–1525. [PubMed: 16319909]
- Talvik-Lotfi M, Nyberg S, Nordstrom AL, Ito H, Halldin C, Brunner F, Farde L. High 5HT_{2A} receptor occupancy in M100907-treated schizophrenic patients. *Psychopharmacology (Berl).* 2000; 148:400–403. [PubMed: 10928313]
- Versijpt J, Van Laere KJ, Dumont F, Decoo D, Vandecapelle M, Santens P, Goethals I, Audenaert K, Slegers G, Dierckx RA, Korf J. Imaging of the 5-HT_{2A} system: age-, gender-, and Alzheimer's disease-related findings. *Neurobiol. Aging.* 2003; 24:553–561. [PubMed: 12714112]
- Wang GJ, Volkow ND, Logan J, Fowler JS, Schlyer D, MacGregor RR, Hitzemann RJ, Gur RC, Wolf AP. Evaluation of age-related changes in serotonin 5-HT₂ and dopamine D₂ receptor availability in healthy human subjects. *Life Sci.* 1995; 56:PL249–PL253. [PubMed: 7475891]
- Watabe H, Channing MA, Der MG, Adams HR, Jagoda E, Herscovitch P, Eckelman WC, Carson RE. Kinetic analysis of the 5-HT_{2A} ligand [11C]MDL 100,907. *J. Cereb. Blood Flow Metab.* 2000; 20:899–909. [PubMed: 10894173]
- Willins DL, Berry SA, Alsayegh L, Backstrom JR, Sanders-Bush E, Friedman L, Roth BL. Clozapine and other 5-hydroxytryptamine-2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine-2A receptors in vitro and in vivo. *Neuroscience.* 1999; 91:599–606. [PubMed: 10366017]
- Wong DF, Lever JR, Hartig PR, Dannals RF, Villemagne V, Hoffman BJ, Wilson AA, Ravert HT, Links JM, Scheffel U, et al. Localization of serotonin 5-HT₂ receptors in living human brain by positron emission tomography using N1-([11C]-methyl)-2-Br-LSD. *Synapse.* 1987; 1:393–398. [PubMed: 2905532]
- Wong DF, Wagner HN Jr, Dannals RF, Links JM, Frost JJ, Ravert HT, Wilson AA, Rosenbaum AE, Gjedde A, Douglass KH, Petronis JD, Folstein MF, Toung JKT, Burns HD, Kuhar MJ. Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science.* 1984; 226:1393–1396. [PubMed: 6334363]
- Woods RP, Grafton ST, Holmes CJ, Cherry SR, Mazziotta JC. Automated image registration: I. General methods and intrasubject, intramodality validation. *J. Comput. Assist. Tomogr.* 1998a; 22:139–152. [PubMed: 9448779]
- Woods RP, Grafton ST, Watson JD, Sicotte NL, Mazziotta JC. Automated image registration: II. Intersubject validation of linear and nonlinear models. *J. Comput. Assist. Tomogr.* 1998b; 22:153–165. [PubMed: 9448780]

Yatham LN, Liddle PF, Shiah IS, Lam RW, Adam MJ, Zis AP, Ruth TJ. Effects of rapid tryptophan depletion on brain 5-HT(2) receptors: a PET study. *Br. J. Psychiatry.* 2001; 178:448–453. [PubMed: 11331561]

Figure 1.

Panel A: plasma total activity; mean of 18 measurements in 9 subjects; error bars are SD.

Panel B: fraction of unmetabolized parent compound in arterial plasma; mean of 18 measurements in 9 subjects; error bars are SD. Panel C: unmetabolized parent concentration in plasma following injection of 518 MBq (14.0 mCi) in a representative human subject. Points are measured values, fitted to a three exponential function.

Figure 2.

Coronal (top), sagittal (middle) and transverse (bottom) coregistered PET images acquired from 50 to 90 min following injection of ^{11}C -MDL100907 in a representative 31-year-old healthy male volunteer. Column A: baseline condition shows widespread ^{11}C -MDL100907 activity concentration throughout cortical regions. In contrast, low levels of activity are seen in subcortical regions, with lowest levels in midbrain and cerebellum. Column B: following 5-HT_{2A} receptor blockade by risperidone 4 mg orally, uptake is homogeneously low throughout the brain, and cortical and cerebellar (region of reference) levels are visually indistinguishable. The PET images in columns A and B are normalized to their respective injected doses of activity (651 and 655 MBq [17.6 and 17.7 mCi], respectively) and

displayed with the same intensity range. Column C: coregistered MRI images of same subject.

Figure 3. Relationship between injected mass of ¹¹C-MDL100907 (IM; x axis, μg) and 5-HT_{2A} receptor occupancy at peak specific binding (y axis, percent) in human volunteers (n = 17 experiments). Linear regression yielded occupancy (%) = 1.09*IM (μg), $r^2 = 0.80$, $p < 0.0001$.

Figure 4.

Voxelwise maps of mean BP_{ND} under control conditions (left) and after 5-HT has been reduced by rapid tryptophan depletion (RTD; middle) in the same healthy subjects ($n=10$), superimposed on structural MRI. RTD is associated with reduced BP_{ND} in an extended area of ventromedial prefrontal cortex (A–C). Statistically significant clusters (right column; SPM99, $p<0.05$, corrected) extend from right dorsal and pregenual anterior cingulate into right anterior orbitofrontal cortex (OFC), with some extension into left anterior OFC.

Medial prefrontal cortex

Figure 5. Decline in $^{11}\text{C-MDL100907 BP}_{\text{ND}}$ with increasing age in a representative cortical region (medial prefrontal cortex). The regression is highly significant with a linear fit (line L), and is not significantly improved by a quadratic fit (curve Q).

Table 1

Reproducibility of ^{11}C -MDL100907 total volume of distribution (V_T , mL/cm^3), binding potential normalised to total parent radioligand concentration in plasma (BP_P , mL/cm^3) and binding potential normalised to nondisplaceable radioligand in tissue (BP_{ND} , unitless) derived via 1-TC kinetic model

Region	V _T (mL/cm ³)						BP _P (mL/cm ³)						BP _{ND} (unitless)								
	BS			WS			VAR ± SD (%)			ICC	BS			WS			VAR ± SD (%)			ICC	
	Mean	SD		%CV	SD	%CV	Mean	SD	%CV		Mean	SD	%CV	Mean	SD	%CV					
CER	20.4	3.9	19.2	1.6	7.7	8.5 ± 9.9	0.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ACC	70.0	17.7	25.3	5.3	7.6	9.8 ± 7.3	0.83	49.6	14.1	28.4	4.0	8.2	11.6 ± 6.5	0.85	2.4	0.4	14.5	0.1	5.6	9.3 ± 5.9	0.74
MPFC	66.9	17.1	25.6	4.4	6.6	8.3 ± 7.2	0.87	46.4	13.6	29.2	3.2	6.9	9.7 ± 7.0	0.89	2.3	0.4	17.1	0.1	6.2	8.7 ± 7.9	0.77
TEM	62.9	16.5	26.2	4.7	7.5	9.6 ± 7.2	0.85	42.5	13.0	30.6	3.5	8.1	10.7 ± 8.1	0.87	2.1	0.4	18.6	0.1	5.5	9.3 ± 6.6	0.84
OCC	62.5	16.2	25.9	4.9	7.8	7.8 ± 9.2	0.83	42.1	12.7	30.2	3.4	8.1	9.2 ± 8.3	0.87	2.0	0.4	18.5	0.1	3.8	7.1 ± 3.3	0.92
DLPFC	61.4	16.3	26.6	3.5	5.7	8.0 ± 5.4	0.91	41.0	13.1	31.9	2.2	5.4	8.7 ± 5.0	0.94	2.0	0.5	22.7	0.1	5.8	9.5 ± 5.4	0.88
OFC	58.3	13.9	23.9	3.8	6.6	8.9 ± 9.1	0.86	37.9	11.5	30.4	3.9	10.4	15.1 ± 12.1	0.79	1.9	0.5	29.1	0.2	12.5	18.4 ± 16.7	0.69
PAR	58.6	13.6	23.1	3.8	6.5	9.3 ± 6.6	0.85	38.2	10.2	26.6	2.5	6.5	10.9 ± 6.2	0.89	1.9	0.3	18.4	0.1	5.2	9.0 ± 6.0	0.85
INS	59.4	13.2	22.2	3.6	6.1	7.0 ± 7.0	0.86	39.0	9.7	24.7	2.6	6.6	7.0 ± 8.1	0.87	1.9	0.3	14.6	0.1	6.6	8.5 ± 9.2	0.67
ENT	41.1	10.1	24.6	2.2	5.3	6.7 ± 6.1	0.91	20.7	6.7	32.4	1.2	5.7	9.0 ± 10.2	0.94	1.0	0.2	20.2	0.1	9.1	13.5 ± 12.6	0.66
PHG	38.7	10.0	25.9	2.1	5.4	8.9 ± 4.4	0.92	18.2	6.6	36.2	1.2	6.8	13.3 ± 5.9	0.93	0.9	0.2	23.9	0.1	10.2	15.0 ± 9.8	0.69
MTL	33.4	7.6	22.6	1.6	4.9	6.4 ± 5.2	0.91	13.0	4.1	31.6	0.6	4.3	9.3 ± 5.5	0.96	0.6	0.1	20.4	0.1	9.3	15.2 ± 8.7	0.66

Values are mean of 9 subjects with each value measured twice; BS=between subjects; WS=within subject; SD=standard deviation; %CV=coefficient of variation; VAR=test/retest variability; CC=intraclass correlation coefficient

Table 2

Reproducibility of ^{11}C -MDL100907 total volume of distribution (V_T , $\text{mL}\cdot\text{cm}^{-3}$), binding potential normalised to total parent radioligand concentration in plasma (BP_P , $\text{mL}\cdot\text{cm}^{-3}$) and binding potential normalised to nondisplaceable radioligand in tissue (BP_{ND} , unitless) derived via 2-TC kinetic model

Region	V _T (mL/cm ³)						BP _P (mL/cm ³)						BP _{ND} (unitless)								
	BS			WS			BS			WS			BS			WS					
	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV			
CER	20.8	4.1	19.9	1.6	7.6	8.8 ± 9.3	0.75	-	-	-	-	-	-	-	-	-	-	-			
ACC	70.2	17.9	25.5	5.0	7.1	8.8 ± 6.9	0.86	49.4	14.1	28.5	3.8	7.6	10.6 ± 6.8	0.87	2.4	0.3	14.7	0.2	6.4	10.2 ± 7.6	0.68
MPFC	67.2	17.0	25.4	4.5	6.8	7.6 ± 8.0	0.87	46.4	13.3	28.6	3.5	7.6	9.5 ± 8.3	0.87	2.2	0.4	16.8	0.2	7.2	9.9 ± 9.4	0.69
TEM	63.1	16.5	26.1	4.3	6.8	8.1 ± 7.0	0.87	42.3	12.8	30.3	3.1	7.3	8.5 ± 8.3	0.89	2.0	0.4	18.6	0.1	5.7	8.6 ± 7.9	0.83
OCC	62.5	16.0	25.6	4.3	6.9	6.5 ± 8.6	0.86	41.8	12.3	29.4	2.9	7.0	7.5 ± 7.7	0.89	2.0	0.4	18.1	0.1	4.3	7.1 ± 4.9	0.89
DLPFC	61.6	16.0	26.0	3.0	4.8	6.8 ± 4.9	0.93	40.9	12.6	30.8	1.9	4.7	7.3 ± 5.1	0.95	2.0	0.4	22.5	0.1	6.6	10.0 ± 7.2	0.84
OFC	59.3	13.3	22.4	4.2	7.2	10.8 ± 8.4	0.82	38.5	11.1	29.0	4.5	11.8	18.0 ± 11.9	0.72	1.9	0.6	31.7	0.3	13.4	20.4 ± 18.4	0.70
PAR	58.9	13.4	22.7	3.5	5.9	8.7 ± 6.0	0.88	38.2	9.9	25.8	2.3	6.0	9.7 ± 7.1	0.90	1.8	0.3	18.6	0.1	6.0	9.9 ± 7.2	0.81
INS	60.0	13.1	21.8	3.4	5.7	6.5 ± 6.7	0.87	39.2	9.4	24.0	2.6	6.7	8.5 ± 6.8	0.86	1.9	0.3	15.1	0.1	7.6	9.4 ± 11.3	0.60
ENT	41.2	9.9	24.1	1.8	4.3	5.6 ± 5.4	0.94	20.4	6.3	31.0	1.2	5.8	8.5 ± 10.3	0.93	1.0	0.2	19.3	0.1	10.5	14.9 ± 14.2	0.54
PHG	39.1	10.2	26.1	2.0	5.2	7.8 ± 5.6	0.92	18.4	6.6	35.8	1.4	7.8	13.5 ± 6.3	0.91	0.9	0.2	23.4	0.1	11.3	15.4 ± 11.8	0.62
MTL	33.9	7.8	23.0	1.5	4.4	5.4 ± 5.2	0.93	13.1	4.1	31.1	0.7	5.6	10.2 ± 6.2	0.94	0.6	0.1	19.4	0.1	10.7	16.4 ± 11.5	0.53

Values are mean of 9 subjects with each value measured twice; BS=within subjects; WS=between subjects; WS=within subject; SD=standard deviation; %CV=coefficient of variation; VAR=test/retest variability; ICC=intra-class correlation coefficient

Table 3
Minimal scanning time (minutes) needed to derive stable estimates of V_T , BP_P and BP_{ND} determined by 1-TC and 2-TC models

Region	Time to stable outcome measures (min) *					
	V_T (mL/cm ³)		BP_P (mL/cm ³)		BP_{ND} (unitless)	
	1-TCM	2-TCM	1-TCM	2-TCM	1-TCM	2-TCM
Cerebellum	80	50	-	-	-	-
ACC	60	70	50	70	70	80
MPFC	60	60	60	70	70	80
TEM	70	80	80	90	90	90
OCC	60	70	70	80	90	90
DLPFC	60	70	50	80	80	90
OFC	100	90	110	120	120	120
PAR	50	60	60	70	80	80
MTL	100	50	110	70	60	70
INS	60	70	60	80	50	80
ENT	60	60	90	110	100	110
PHG	80	50	60	70	70	80

* Values are scan durations (in minutes) after ¹¹C-MDL 100907 injection needed to achieve stable outcome parameter estimates (see Methods section 3.5 for stability criteria)

Table 4

Scan parameters and plasma amino acid concentrations in T+ and T- conditions

Condition	ID MBq	SA GBq/μmol	Mass μg	f _{ND} %	Cl L/hr	V _T CER mL/g	Total Trp nmol/mL		Free Trp nmol/mL		Trp/ΣLNAA %	
							Baseline	PET scan	Baseline	PET scan	Baseline	PET scan
T+	359 ± 148	44.0 ± 16.4	3.1 ± 0.4	33.2 ± 6.4	189 ± 35	21.6 ± 3.0	56.0 ± 10.3	66.1 ± 32.4	6.5 ± 2.7	9.3 ± 4.0	11.0 ± 1.4	5.5 ± 1.0
T-	389 ± 181	48.5 ± 24.4	3.1 ± 0.4	31.8 ± 6.6	181 ± 39	21.1 ± 3.2	53.2 ± 9.2	11.8 ± 11.1	6.6 ± 1.5	2.4 ± 2.5	10.3 ± 1.4	1.3 ± 1.8
<i>p</i> [*]	0.55	0.52	0.92	0.14	0.51	0.67	0.37	0.002	0.93	0.003	0.15	< 0.001

ID: injected dose of radioactivity; SA: decay-corrected specific activity at time of tracer injection; f_{ND} plasma free fraction of tracer; Cl: clearance; V_T CER: total distribution volume in region of reference (cerebellum); Trp: tryptophan; Trp/ΣLNAA: ratio of Trp to other large neutral amino acids;

^{*} significance of difference between means assessed by paired t-test (2-tailed).

Table 5

Regional 5-HT_{2A} receptor availability in control and RTD conditions

ROI	BP _{ND} 2-TCM		Mean change (%)	p	BP _{ND} BF SRTM		Mean change (%)	p
	Control (T+)	RTD (T-)			Control (T+)	RTD (T-)		
ACC	L	2.26 ± 0.25	2.48 ± 0.62	0.29	1.27 ± 0.23	1.44 ± 0.40	14.0	0.14
	R	2.65 ± 0.61	2.31 ± 0.64	0.05 *	1.56 ± 0.45	1.40 ± 0.54	-8.4	0.36
DLPFC	L	2.32 ± 0.52	2.17 ± 0.72	0.24	1.39 ± 0.29	1.24 ± 0.24	-7.7	0.17
	R	2.49 ± 0.64	2.21 ± 0.67	0.02 *	1.58 ± 0.43	1.25 ± 0.19	-17.1	0.04 *
MPFC	L	2.44 ± 0.56	2.47 ± 0.80	0.93	1.44 ± 0.43	1.42 ± 0.40	9.8	0.91
	R	2.67 ± 0.67	2.24 ± 0.64	0.04 *	1.62 ± 0.42	1.25 ± 0.26	-20.0	0.04 *
OCC	L	2.34 ± 0.51	2.24 ± 0.76	0.36	1.36 ± 0.18	1.22 ± 0.16	-9.2	0.04 *
	R	2.30 ± 0.40	2.32 ± 0.83	0.92	1.32 ± 0.20	1.20 ± 0.17	-7.7	0.05 *
OFC	L	2.01 ± 0.51	1.68 ± 0.51	0.11	1.15 ± 0.34	0.98 ± 0.34	1.1	0.35
	R	2.13 ± 0.50	1.78 ± 0.48	0.01 *	1.28 ± 0.31	1.02 ± 0.29	-13.9	0.11
PAR	L	2.18 ± 0.50	2.00 ± 0.66	0.12	1.34 ± 0.36	1.12 ± 0.21	-13.0	0.08
	R	2.10 ± 0.33	2.08 ± 0.71	0.90	1.25 ± 0.20	1.16 ± 0.21	-4.8	0.24
TEM	L	2.40 ± 0.56	2.22 ± 0.69	0.14	1.15 ± 0.21	1.00 ± 0.20	-10.0	0.10
	R	2.53 ± 0.58	2.59 ± 1.18	0.76	1.31 ± 0.26	1.14 ± 0.20	-10.6	0.05 *

2-TCM, BPND derived by 2-tissue compartment kinetic model; BF SRTM, BPND derived by basis function implementation of simplified reference tissue method; RTD, rapid tryptophan depletion;

* significant at $p \leq 0.05$