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Imaging cortical dopamine D_1 receptors using [^{11}C]NNC112 and ketanserin blockade of the 5-HT_{2A} receptors

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[^{11}C]NNC112 (8-chloro-7-hydroxy-3-methyl-5-(7-benzofuranyl)-2,3,4,5-tetrahydro-IH-3-benzazepine), a selective positron-emission tomography (PET) ligand for the D_1 receptor (R) over the 5-HT_{2A} R *in vitro*, has shown lower selectivity *in vivo*, hampering measurement of D_1 R in the cortex. [^{11}C]NNC112 PET and intravenous (i.v) ketanserin challenge were used to (1) confirm the previous findings of [^{11}C]NNC112 *in vivo* D_1 R selectivity, and (2) develop a feasible methodology for imaging cortical D_1 R without contamination by 5-HT_{2A} R. Seven healthy volunteers underwent [^{11}C]NNC112 PET scans at baseline and after a 5-HT_{2A} R-blocking dose of ketanserin (0.15 mg/kg, i.v.). Percent BP_{ND} change between the post-ketanserin and baseline scans was calculated. Irrespective of the quantification method used, ketanserin pretreatment led to significant decrease of BP_{ND} in the cortical (~30%) and limbic regions (~20%) but not in the striatum, which contains a much lower amount of 5-HT_{2A} R. Therefore, ketanserin allows D_1 R signal to be detected by [^{11}C]NNC112 PET without significant 5-HT_{2A} R contamination. These data confirm the presence of a significant 5-HT_{2A} R contribution to cortical [^{11}C]NNC112 signal, and call for caution in the interpretation of published [^{11}C]NNC112 PET findings on cortical D_1 R in humans. In the absence of more selective ligands, [^{11}C]NNC112 PET with ketanserin can be used for cortical D_1 R imaging *in vivo*.
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Introduction

[^{11}C]NNC112 (8-chloro-7-hydroxy-3-methyl-5-(7-benzofuranyl)-2,3,4,5-tetrahydro-IH-3-benzazepine) has been used as a positron-emission tomography (PET) ligand for *in vivo* study of dopamine D_1 receptors (R). In preclinical studies, a relationship between [^{11}C]NNC112-binding potential (BP_{ND}) and prefrontal cortex dopamine concentrations has been shown both in rats (Guo *et al*, 2003) and monkeys (Tsukada *et al*, 2005). In clinical studies, increased D_1 R availability by means of [^{11}C]NNC112 PET has been reported both in schizophrenic patients with working memory deficits (Abi-Dargham *et al*, 2002) and in chronic ketamine abusers (Narendran *et al*, 2005), thus linking low prefrontal dopamine function to both cognitive impairment in schizophrenia and chronic glutamatergic hypofunction. All these studies were relying on the 100-fold *in vitro* selectivity of this ligand for D_1 R ($K_D = 0.18$) over 5-HT_{2A} R ($K_D = 18$) (Andersen *et al*, 1992). However, more recent *in vivo* studies using 5-HT_{2A} R antagonist challenges both in baboons and in humans, with saturating doses of MDL100907 (0.1 to 1 mg/kg, intravenous (i.v.)) (Ekelund *et al*, 2007) and risperidone (2 mg, per os (p.o.)) (Slifstein *et al*, 2007), have shown a 25% to 30% reduction of [^{11}C]NNC112 BP_{ND} in the cortical regions, suggesting that approximately

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one quarter of the cortical signal was due to binding to 5-HT_{2A} R. In the absence of a selective ligand for *in vivo* D_1 R imaging, pretreatment with a 5-HT_{2A} R blocker could be a practical method to enable accurate quantification of D_1 R *in vivo*. Unfortunately, none of the already used challenges are optimal for human studies. MDL100907 is not approved for human use, and risperidone is not a selective 5-HT_{2A} antagonist. Moreover, the i.v. route of administration may be preferable for PET study challenges, as it should lead to more consistent plasma levels than oral administration. Ketanserin provides an acceptable alternative, as it is a selective 5-HT_{2A} blocker ($K_i = 0.38$ nmol/L) (Israilova *et al*, 2002) and can be administered intravenously to humans. I.v. ketanserin has been used as a challenge to study the 5-HT_{2A} selectivity of PET and SPECT radioligands, both in baboons (Sadzot *et al*, 1995) and healthy volunteers (Travis *et al*, 2000; Pinborg *et al*, 2003; Catafau *et al*, 2006*b*), showing ~90% blockade of 5-HT_{2A} R with a 0.1-mg/kg dose. Although ketanserin is not completely devoid of D_1 R affinity ($K_i = 190$ to 464 nmol/L in cloned human D_1 R using [3H]SCH23390; Sunahara *et al*, 1991; Toll *et al*, 1998), pretreatment with ketanserin at doses of 1 mg/kg (Kassiou *et al*, 1995*a*, *b*, 2001, 2002) and 200 mg/kg (Duffy *et al*, 2000) has shown to be unable to displace other D1 R PET ligands in *in vivo* preclinical studies. In this study, we examined the utility of a ketanserin challenge in conjunction with [11C]NNC112 to quantify D_1 R binding without contamination of 5-HT_{2A} R binding in healthy volunteers. We hypothesized that ketanserin would induce a decrease of [11C]NNC112 binding in the cortical areas, supporting previous evidence of the 5-HT_{2A} R component. Given the low affinity of ketanserin for D_1 R, we also expected no significant change in [11C]NNC112 binding in the striatum, where the density of 5-HT_{2A} R is much lower than that of D1 R (Pazos *et al*, 1985).

Materials and methods

Subjects

The study was approved by the local Ethics Committees and the Spanish Ministry of Health. All subjects provided written informed consent before inclusion in the study.

Seven male human volunteers aged 25 ± 2 years (range 22 to 28 years) were enrolled in this study. None had past or present neurological or psychiatric disorders as assessed by the Structured Clinical Interview for DSM IV (SCID II) questionnaire (First *et al*, 1997). None were taking psychotropic drugs or other relevant medication. All had normal findings on physical examination, 12-lead electrocardiography, clinical chemistry, and hematology. Absence of illegal drug abuse was assessed by interviews with independent physicians in two different occasions, and supported by urine screening using a commercial on-site rapid test (MultiClin Cassette for Urine; SureScreen Diagnostic Ltd, Derby, UK) at screening and before PET scanning sessions. The screening procedure included determination of amphetamines, barbiturates, benzodiazepines, cocaine, tetrahydrocannabinol, methylenedioxymethamphetamine, opiates, oxycodone, phencyclidine, propoxyphene, and tricyclic antidepressants. Subjects had to withdraw from caffeine and alcohol 24 h before the study day and were not allowed to take any medications during the 14 days before the study day.

Study design

All subjects underwent two [11C]NNC112 PET scans, before (baseline scan) and 15 mins (min) after ketanserin challenge (0.15 mg/kg, i.v.), as well as a structural magnetic resonance imaging (MRI) to allow co-registration and region of interest (ROI) drawing. Five subjects underwent both PET scans on the same day, 4 to 6 h apart. For the remaining two subjects, post-ketanserin scan was performed 5 and 11 days after the baseline scan. For safety reasons, the subjects rested lying on the PET bed with continuous monitoring of electrocardiography, blood pressure, and heart rate from 15 mins before ketanserin injection until the end of the post-ketanserin scan. A follow-up phone call was performed 3 to 5 days after the ketanserin PET scan.

Radiochemistry

[11C]NNC112 was synthesized according to the following procedure: [11C]CO_2 was generated in an IBA Cyclone 18/9 cyclotron by bombardment of an N_2/O_2 (95%/5%) gas mixture with high-energy (18 MeV) protons. [11C]CH_3I was formed by reduction of [11C]CO_2 in the presence of lithium aluminium hydride (300 μL, 0.1 M solution in dry tetrahydrofuran (THF); ABX, Radeberg, Germany) and distillation after solvent evaporation and addition of hydriodic acid (300 μL, 57% aqueous solution; ABX). [11C]CH_3I was trapped in a 2-mL stainless steel high-performance liquid chromatography (HPLC) loop pre-charged with a solution of desmethyl-NNC112 (free base, 1 mg; ABX) in dimethylsulfoxide (80 μL). After complete trapping of [11C]CH_3I, the reaction was allowed to occur for 4 mins at room temperature. The reaction mixture was purified by means of HPLC using an RP-C18 column (Mediterranea Sea18, 9.6 × 250 mm, 5 μm particle size) and using sodium phosphate buffer (pH 8)/acetonitrile (35/65) mixture as the mobile phase at 5 mL/minute, using UV ($\lambda = 254$ nm) and isotopic detectors connected in series. The purified fraction (retention time = 8 to 10 mins) was collected in a 40-mL vial pre-charged with 20 mL of physiological saline solution. The resulting mixture was passed through a C-18 cartridge (Sep-Pak plus; Waters, Milford, MA, USA) to retain [11C]NNC112. The radiotracer was eluted with ethanol (2 mL) and collected in a sterile 10-mL vial. The solution was evaporated at 70°C under continuous nitrogen flow until a final volume of approximately 100 μL was obtained. The residue was reconstituted with 7 mL of physiological saline solution. After double filtration through 0.22-μm sterile filters, a fraction of the final solution was subjected to quality control. Residual solvents

were determined by means of gas chromatography; radiochemical purity was measured by means of HPLC and product identification was performed by HPLC and co-elution with standard reference solution. In both cases, chromatography was performed using an RP-C18 column (Mediterranea Sea18, 4.6 × 150 mm, 5 μm particle size) and using sodium phosphate buffer (pH 8)/acetonitrile (35%/65%) mixture as the mobile phase at 1 mL/minute, using UV ($\lambda = 254$ nm) and isotopic detectors connected in series. Specific activity average was 41.8 (± 19.9) GBq/μmol, with radiochemical purity higher than 95% in all cases.

PET and MRI Protocol

PET was performed using a Siemens ECAT-EXACT HR+ camera equipped with transmission sources of ^{68}Ge. Prior to [^{11}C]NNC112 injection, a 10-minute transmission scan was obtained for attenuation correction. After injection of a [^{11}C]NNC112 dose (baseline scan: 520.7 ± 88.3 MBq, post-ketanserin scan: 487.3 ± 54.1 MBq), 100 mins of emission data were acquired as 27 dynamic frames (8 × 15 secs, 3 × 60 secs, 5 × 120 secs, 5 × 300 secs, 6 × 600 secs). The PET data were reconstructed using filtered back projection with a Hanning filter (kernel FWHM of 4.9 mm). Corrections were applied for attenuation (through transmission data), scatter, randoms, and dead time. The volumes produced consisted of 128 × 128 × 63 voxels; each voxel being 2.57 × 2.57 × 2.43 mm.

A structural T1-weighted MRI scan was performed for all subjects and co-registered with PET images for definition of striatal ROIs. An axial three-dimensional spoiled gradient-echo slab was positioned to include the entire head and the images were acquired with the following parameters: repetition time, 25 ms; echo time, 6 ms; flip angle, 28 degrees; field of view, 25 × 25 cm; matrix size, 256 × 256; section thickness, 2 mm, with no interslice gap; and number of excitations, 1.

Input Function Measurement

Arterial blood activity was measured every second for the first 15 mins after injection using a continuous arterial blood sampling system (ABSS Allogg, Mariefred, Sweden). Thereafter, 14 manual samples were collected at the following time points: 5, 10, 14, 17, 22.5, 27.5, 32.5, 37.5, 45, 55, 65, 75, 85, 95 mins. The plasma was centrifuged over 10 mins at 1800*g* and collected in 200-mL aliquots. Each aliquot's activity was counted with a gamma counter (Perkin Elmer Wallac, Waltham, MA, USA; Wizard 1470 Automatic Gamma Counter). Three samples collected at 5, 10, and 14 mins after injection, which overlapped the continuous blood sampling period, were used to calibrate continuous blood samples to match discrete samples. The discrete and continuous whole-blood data were thus combined into a single whole-blood activity curve. The sampled plasma data were divided by the corresponding whole-blood measurements and the average of these values was taken as the plasma-over-blood ratio. The whole blood curve was multiplied by this value to obtain a total plasma activity curve spanning the duration of the scan.

The fraction of plasma activity representing unmetabolized (parent) [^{11}C]NNC112 was measured by HPLC for seven plasma samples (5, 10, 17.5, 27.5, 45, 65.5, and 95 mins) using the method described by Abi-Dargham *et al* (2000). These parent fraction data were fitted to a single exponential decay plus a constant term. The fitted parent fraction data were multiplied by the total plasma activity data to produce a sampled parent plasma activity curve. This curve was smoothed post-peak by fitting to a tri-exponential function, and thus an arterial parent plasma input function was obtained for use in kinetic modeling. The plasma clearance of unmetabolized [^{11}C]NNC112 was calculated as the ratio of injected activity to the area under the fitted parent plasma input function. The plasma free fraction was also measured, using the method described by Abi-Dargham *et al* (2000).

Image Analysis

Dynamic PET images were registered to the subject's MRI and corrected for motion by realigning each time frame to the averaged frame using a mutual information-based algorithm as implemented in the SPM5b software (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm). ROIs were defined on each subject's MRI using a combination of manual and automated methods. The cortical (orbitofrontal cortex; medial and dorsolateral prefrontal cortices; anterior cingulate gyrus; and parietal, temporal, and occipital cortices), amygdala, and cerebellar regions were defined through nonlinear registration (using SPM5b) of a template MRI and the corresponding atlas (developed in-house on the basis of the Harvard–Oxford atlas included with FSL 4.1; http://www.fmrib.ox.ac.uk/fsl). The striatal regions (precommissural dorsal caudate and putamen, ventral striatum, and postcommissural caudate and putamen) were defined manually as described by Martinez *et al* (2003). A gray matter segmentation was applied to each of the cortical ROIs. Each ROI was then applied to the dynamic PET data to derive regional time–activity curves.

Receptor Parameter Estimation

A two-tissue-compartment (2TC) model was fitted to each ROI and the total volume of distribution (V_T) was estimated. BP_{ND} was derived using the indirect method as $BP_{ND} = (V_{T,ROI} - V_{T,CER})/V_{T,CER}$, where $V_{T,ROI}$ and $V_{T,CER}$ are the total volumes of distribution in a region with specific uptake and in the reference region (cerebellum), respectively. The binding potential was also estimated by the simplified reference tissue method (SRTM) using the cerebellum as reference region (Lammertsma and Hume, 1996). The percentage of ketanserin-induced displacement (%Disp) was computed as $\%Disp = 100\% \cdot (BP_{ND,baseline} - BP_{ND,post\text{-}ketanserine})/BP_{ND,baseline}$.

Parametric Maps

BP_{ND} maps obtained from the basis function implementation of SRTM (Gunn *et al*, 1997) were first co-registered with each individual' structural MRI using the SPM5b

software. The BP_{ND} maps were then warped into stereotaxic space using deformation parameters derived from non-linear registration of the individual MRI to an MRI template in stereotaxic space and then smoothed with an 8-mm FWHM kernel. Paired *t*-tests with no proportional scaling were performed to compare baseline to post-ketanserin BP_{ND} maps. T maps were thresholded at $P<0.001$, as this level has been considered a significant threshold for statistical difference maps when there are *a priori* hypotheses regarding the location of findings (Friston *et al*, 1991).

Statistics

Differences in non-receptor-related scan parameters (e.g. injected dose) in baseline versus post-ketanserin scans were tested by two-tailed paired *t*-tests. Whether the percentage BP_{ND} change in each separate region was significantly different from 0, was also tested by two-tailed paired *t*-tests, correcting for multiple comparisons by means of the false discovery rate. The statistical significance of different kinetic analyses (2TC versus SRTM) and different regions in BP_{ND} percentage change from baseline to post-ketanserin were tested by means of repeated-measures analysis of variance in SPSS (Chicago, IL, USA). Three *post hoc* contrasts comparing whole cortex with amygdala, whole cortex with whole striatum, and amygdala with whole striatum, with Dunnet T3 correction were then performed to investigate the differences in the regional selectivity of [11C]NNC112.

Results

Safety data recorded at the beginning and end of the scanning session, including heart rate, blood pressure, and QTc intervals, showed that these parameters remained within reference ranges during the entire scanning period. At baseline, the ranges were 101 to 131 mm Hg (systolic)/49 to 81 mm Hg (diastolic) for blood pressure; 388 to 426 ms for QTc; and 58 to 77 b.p.m. for heart rate. At the end of the experiment, the respective ranges were 98 to 141 mm Hg (systolic)/41 to 82 mm Hg (diastolic); 390 to 418 ms; and 50 to 84 b.p.m.

Out of the seven subjects who completed the study, one showed excessive motion during scanning, which precluded reliable motion correction and subsequent image analysis. Therefore, the PET results reported below correspond to the sample of six subjects with evaluable PET scans. No differences were found for injected dose, specific activity, injected mass, plasma free fraction, $V_{T,CER}$, or clearance between conditions (Table 1).

Individual pre and post-ketanserin BP_{ND} values for cortical regions, amygdala, and striatum, as well as the percentage of change between conditions using both 2TC and SRTM methods both in this study and that in the study of Slifstein *et al* (2007), are presented in Tables 2 and 3. BP_{ND} parametric images at baseline and after ketanserin challenge are shown in Figure 1. Irrespective of the quantification method used, ketanserin pretreatment led to greater BP_{ND} decrease in the cortical regions (25% to 35%, all statistically significant) than that in the amygdala (19% to 20%, statistically significant in SRTM analysis) and striatum (5% to 13%, not statistically significant with the exception of the postcommissural putamen in SRTM analysis). Repeated-measures analysis of variance showed a significant effect of brain region ($F=10.228$, $P<0.001$) but not method (2TC versus SRTM analysis, $F=0.004$ $P=0.950$), with significantly larger BP_{ND} percent decreases in the cortical regions (taken together: $\sim$30%) than in the striatal regions (taken together, $\sim$10%; Dunnet T3 $P<0.001$). The limbic region (amygdala) did not show statistically significant differences with either cortical regions or striatum. Accordingly, T maps of BP_{ND} change between baseline and post-ketanserin conditions showed significant decreases in the cortical regions, with no significant changes in the striatal regions (Figure 2).

Discussion

This study contributes to the previous evidence of limited *in vivo* selectivity of [11C]NNC112 for D_1 R compared to 5-HT_{2A} R (Ekelund *et al*, 2007; Slifstein *et al*, 2007). This is not a problem in the striatum, given the poor concentration of 5-HT_{2A} R as shown by PET and SPECT studies with selective ligands for 5-HT_{2A} R (Travis *et al*, 2000; Pinborg *et al*, 2003; Catafau *et al*, 2006*a*), and the fourfold higher density of D_1 R over 5-HT_{2A} R in this region (List and Seeman, 1981; McBride *et al*, 1997). However, the reverse is true in the cortex, where the density of

Table 1 Scan parameters

	ID (MBq)	*SA (MBq/mmol)*	*IM (μg)*	f_P *(unit-less)*	$V_{T,CER}$ *(ml/cm³)*	*Clearance (L/hour)*
Baseline	513 ± 94	4.54E+7 ± 2.67E+7	4.7 ± 2.2	2.65 ± 2.22%	2.11 ± 0.56	68 ± 31
Post-ketanserin	504 ± 36	4.17E+7 ± 1.94E+7	4.5 ± 2.0	2.80 ± 1.84%	2.01 ± 0.56	62 ± 17
P	0.77	0.56	0.66	0.85	0.42	0.63

Abbreviations: f_P free fraction in plasma; ID, injected dose; IM, injected mass; SA, specific activity; $V_{T,CER}$, volume of distribution of the non-displaceable tissue uptake (cerebellum).
Parameters from quantifiable subjects ($n=6$). The *P*-values are from two-tailed, paired *t*-tests.

Table 2 BP_{ND} results from 2TC modeling

	[11C]NNC112+ketanserin, i.v. (this study)				*[11C]NNC112+risperidone, p.o.* (Slifstein *et al*, 2007)	
	Baseline	*Post-ketanserin*	*% Change*	P	*% Change*	P
Cortical						
ANT CING	0.55 ± 0.06	0.41 ± 0.07	−25 ± 13	0.012	−21 ± 11	0.003
DLPFC	0.41 ± 0.05	0.27 ± 0.06	−35 ± 12	0.007	−30 ± 20	0.011
MPFC	0.46 ± 0.05	0.31 ± 0.07	−32 ± 13	0.005	−28 ± 13	0.002
ORB FC	0.47 ± 0.08	0.32 ± 0.08	−29 ± 19	0.022	−26 ± 13	0.004
TEM CTX	0.47 ± 0.05	0.31 ± 0.04	−33 ± 13	0.006	−27 ± 08	0.000
PAR CTX	0.42 ± 0.02	0.28 ± 0.04	−33 ± 12	0.012	−27 ± 13	0.002
OCC CTX	0.39 ± 0.03	0.27 ± 0.03	−30 ± 12	0.006	−23 ± 12	0.004
Limbic						
AMYG	0.46 ± 0.08	0.37 ± 0.12	−20 ± 24	0.158	−10 ± 11	0.231
Striatal						
Pre-DCA	2.48 ± 0.34	2.33 ± 0.27	−5 ± 13	0.395	−9 ± 21	0.302
Pre-DPU	2.69 ± 0.29	2.50 ± 0.33	−7 ± 13	0.323	0 ± 13	0.992
Post-CA	2.42 ± 0.65	2.03 ± 0.35	−13 ± 19	0.227	−3 ± 26	0.791
Post-PU	2.64 ± 0.32	2.45 ± 0.25	−7 ± 11	0.254	4 ± 11	0.355
VST	2.34 ± 0.32	2.01 ± 0.22	−12 ± 23	0.297	5 ± 14	0.635

Abbreviations: AMYG, amygdala; ANT CING, anterior cingulate gyrus; BP, binding potential; DLPFC, dorsolateral prefrontal cortex; i.v., intravenous; MPFC, medial prefrontal cortex; OCC CTX, occipital cortex; ORB FC, orbitofrontal cortex; PAR CTX, parietal cortex; Pre-DCA, precommissural dorsal caudate; Pre-DPU, precommissural dorsal putamen; Post-CA, postcommissural caudate; Post-PU, postcommissural putamen; 2TC, two-tissue-compartment; TEM CTX, temporal cortex; VST, ventral striatum.
Results from 2TC modeling analysis with arterial input function for six subjects. The *P*-values are from two-tailed paired *t*-tests, corrected by multiple comparisons by FDR.

Table 3 BP_{ND} results from SRTM analysis

	[11C]NNC112+ketanserin, i.v. (this study)				*[11C]NNC112+risperidone, p.o.* (Slifstein *et al*, 2007)	
	Baseline	*Post-ketanserin*	*% Change*	P	*% Change*	P
Cortical						
ANT CIN	0.54 ± 0.04	0.40 ± 0.06	−26 ± 09	0.001	−22 ± 18	0.012
DLPFC	0.42 ± 0.04	0.27 ± 0.05	−35 ± 07	0.000	−35 ± 18	0.002
MPFC	0.46 ± 0.04	0.32 ± 0.06	−31 ± 08	0.000	−31 ± 15	0.001
ORB FC	0.47 ± 0.05	0.32 ± 0.06	−31 ± 08	0.001	−33 ± 15	0.002
TEM CTX	0.45 ± 0.04	0.31 ± 0.03	−32 ± 05	0.000	−29 ± 10	0.000
PAR CTX	0.42 ± 0.03	0.28 ± 0.03	−33 ± 05	0.000	−32 ± 17	0.002
OCC CTX	0.40 ± 0.02	0.28 ± 0.02	−30 ± 02	0.000	−27 ± 11	0.001
Limbic						
AMYG	0.41 ± 0.06	0.34 ± 0.06	−19 ± 11	0.012	−17 ± 21	0.145
Striatal						
Pre-DCA	1.98 ± 0.25	1.85 ± 0.18	−7 ± 07	0.078	−4 ± 24	0.632
Pre-DPU	2.21 ± 0.25	2.05 ± 0.23	−7 ± 08	0.076	2 ± 18	0.792
Post-CA	1.75 ± 0.19	1.60 ± 0.22	−9 ± 08	0.068	4 ± 38	0.765
Post-PU	2.17 ± 0.27	2.02 ± 0.22	−6 ± 05	0.043	5 ± 01	0.336
VST	1.89 ± 0.20	1.65 ± 0.14	−12 ± 14	0.088	9 ± 19	0.388

Abbreviations: AMYG, amygdala; ANT CING, anterior cingulate gyrus; BP, binding potential; DLPFC, dorsolateral prefrontal cortex; i.v., intravenous; MPFC, medial prefrontal cortex; OCC CTX, occipital cortex; ORB FC, orbitofrontal cortex; PAR CTX, parietal cortex; Pre-DCA, precommissural dorsal caudate; Pre-DPU, precommissural dorsal putamen; Post-CA, postcommissural caudate; Post-PU, postcommissural putamen; SRTM, simplified reference tissue method; TEM CTX, temporal cortex; VST, ventral striatum.
Results from SRTM analysis of six subjects. The *P*-values are from two-tailed paired *t*-test, corrected by multiple comparisons by FDR.

5-HT_{2A} R is four- to five-fold that in the striatum (Pazos *et al*, 1985), and therefore the [11C]NNC112 signal is significantly contaminated by 5-HT_{2A} R. This study also provides a method for imaging cortical D_1 R without significant contamination from 5-HT_{2A} R.

The ketanserin dose was chosen on the basis of previous experience of ketanserin displacement of

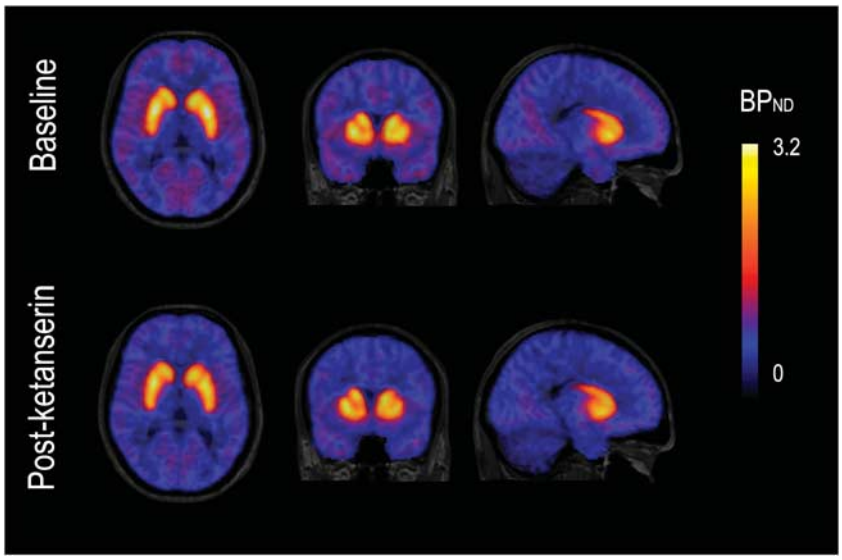

Figure 1 Co-registered MRI: [11C]NNC112 PET BP_{ND} parametric images (mean from all subjects, $n = 6$), at baseline (top) and after ketanserin 0.15 mg/kg, i.v. (bottom). The decrease of [11C]NNC112 signal in the cortical regions, without modification in the striatum, is appreciated.

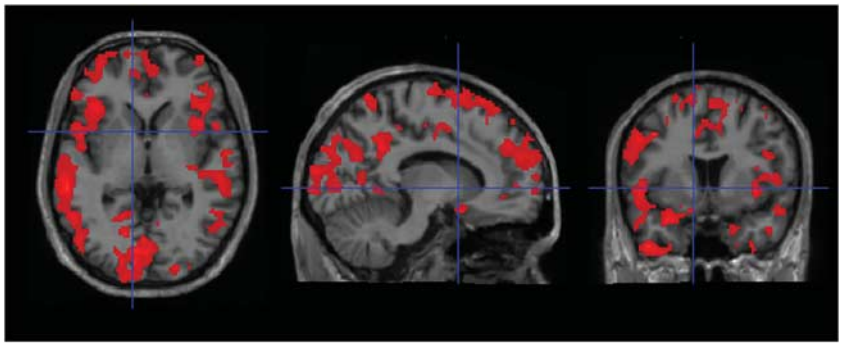

Figure 2 T maps of BP_{ND} change between baseline and post-ketanserin conditions. Threshold: $P < 0.001$. A significant BP_{ND} decrease was shown in the cortical areas, demonstrating [11C]NNC112 displacement from 5-HT_{2A} R, whereas no significant changes are seen in the striatum (area devoid of 5-HT_{2A} R).

selective 5-HT_{2A} R PET and SPECT ligands. Using a 0.1-mg/kg i.v. ketanserin dose, more than 90% displacement has been reported in monkey using [18F]altanserin (Sadzot *et al*, 1995). In humans, the ketanserin-induced displacement of the SPECT ligand [123I]R91150 was reported to be dose-dependent following an E_{max} model relationship where doses beyond 0.1 mg/kg, i.v., would not induce significantly higher ligand displacements (Catafau *et al*, 2006*b*). This is supported by the lack of significant differences in [11C]NNC112 displacement found using a 0.1-mg/kg dose and that using a 10-fold higher dose (1 mg/kg) of MDL100907 in baboons (Ekelund *et al*, 2007). These data were used as the rationale for the 0.15-mg/kg ketanserin dose as a blocking dose in this study, since it lies in the plateau of the described E_{max} model.

It must be noted that ketanserin is already used for patients with hypertension to lower their blood pressure, likely because of its α_1-adrenergic antagonism. The compound has been shown to be well-tolerated and severe side effects after treatment have not been reported (Distler, 1990). The clinical oral dose for hypertension is 20 to 80 mg/day, and an intravenous dose of 10 mg has been reported effective in the treatment of post-anesthetic shivering in humans (Joris *et al*, 1993). In our study, the maximum ketanserin dose administered was 11.96 mg, and no safety issues were found with any of the doses tested. Blood pressure, heart rate, and electrocardiogram, including the QTc interval, were maintained within the reference ranges for the entire scanning period, thus suggesting that ketanserin 0.15 mg/kg is a safe dose that could be administered to healthy volunteers for diagnosis or research

procedures. To generalize the use of i.v. ketanserin as a PET challenge for D_1 R measurement, it has to be taken into account that it is only marketed in some countries. However, it may be imported for its use in other countries, with appropriate regulatory permissions. The latter was the procedure followed in this study. Another limitation of the use of ketanserin as a PET challenge is that it should not be administered to subjects who are at risk of suffering side effects of ketanserin (i.e., cardiovascular diseases, diabetes, renal insufficiency, hypotension, vascular headache, cerebrovascular disease, carcinoid syndrome, and hepatic diseases) or those for whom ketanserin is contraindicated, as indicated in the ketanserin data sheet.

The ketanserin-induced [11C]NNC112 displacement in the cortical regions found in this study supports previous evidence of a 5-HT_{2A} R component in the *in vivo* signal of [11C]NNC112. The magnitude of ligand displacement in the cortical areas and amygdala are in close agreement with that in the study by Slifstein *et al* (2007), who used a 5-HT_{2A} R-blocking dose of risperidone 2 mg p.o. (see Tables 1 and 2). Also in agreement with that study, no significant displacement was found in the striatum. However, the effect of ketanserin seemed to be larger than that of risperidone in this region (Tables 2 and 3). Ketanserine affinity for D_1 R could be a potential explanation. Assuming that 0.15 mg/kg i.v. ketanserin induces 90% of 5-HT_{2A} R occupancy, the corresponding D_1 R occupancy would be 1.77% to 0.85% for the reported *in vitro*, $K_i = 190$ to 464 nmol/L (Sunahara *et al*, 1991; Toll *et al*, 1998), which would have a negligible contribution to the D_1 R measurement. However, limitations of the *in vitro*-based calculations have to be taken into account before extrapolating to the *in vivo* effects of ketanserin on D_1 striatal binding. An alternative explanation could be that i.v. ketanserin was able to displace [11C]NNC112 even from the low amount of 5-HT_{2A} R present in the striatum.

The results of this study were similar, irrespective of the quantification method used (2TC and SRTM), to those reported by Slifstein *et al* (2007). The similarity of results replicated by two different groups may support the use of SRTM as a simpler method for within-subject comparison studies, avoiding the need for arterial blood sampling and thus increasing the availability of this methodology. The cerebellum was used as a reference region for both methods, given the known lower density of D_1 R in this region and its previous use as a reference region for D_1 R PET measurements (Ekelund *et al*, 2007; Slifstein *et al*, 2007). Although some *in vitro* studies have reported a small presence of 5-HT_{2A} R in the cerebellum (Pazos *et al*, 1987; Eastwood *et al*, 2001), data from 5-HT_{2A} R PET and SPECT studies support the use of the cerebellum as a reference region for 5-HT_{2A} R measurement (Pinborg *et al*, 2003; Catafau *et al*, 2006*b*). In this study, there was no significant change in $V_{T,CER}$ between the pre and post-ketanserin scans, further supporting this region as a suitable reference region for [11C]NNC112 PET scanning with ketanserin challenge.

These data contribute to the growing evidence of discrepancies between *in vitro* and *in vivo* studies (Slifstein *et al*, 2007) and support the importance of the *in vivo* studies to assess PET ligand's selectivity. Moreover, the finding of [11C]NNC112's lack of D_1 R selectivity calls for caution in the interpretation of previously published results on D_1 R availability assessed by [11C]NNC112 PET without any drug challenge to block 5-HT_{2A} R (Abi-Dargham *et al*, 2002; Guo *et al*, 2003; Narendran *et al*, 2005; Tsukada *et al*, 2005). Research on D_1 R availability in patients with schizophrenia using PET has reported inconsistent results, with some authors finding increased D_1 R availability (Abi-Dargham *et al*, 2002), whereas other finding it decreased (Okubo *et al*, 1997), and still others finding it to be unchanged in comparison with a control group (Karlsson *et al*, 2002). Since different PET ligands for D_1 R ([11C]NNC112 or [11C]SCH23390) were used in those studies, a ligand selectivity issue was discussed as a potential explanation for these discrepancies. However, this seems unlikely given that although a 200-fold selectivity for D_1 R ($K_D = 0.14$ nmol/L) over 5-HT_{2A} R ($K_D = 37$ nmol/L) has been reported *in vitro* for [11C]SCH23390 (Andersen, 1988), the *in vivo* selectivity of this ligand has been found to be similar to that of [11C]NNC112 (Ekelund *et al*, 2007).

The lack of ketanserin displacement in striatal [11C]NNC112 binding supports the D_1 R selectivity of the ligand in this region. However, a higher-affinity and more selective D_1 R ligand is still warranted, given the low BP_{ND} values obtained with this ligand in the cortex both at baseline (~ 0.5) and after ketanserin (~ 0.3). In the absence of a better ligand, a suitable 5-HT_{2A} R blocker such as ketanserin (0.15 mg, i.v.) would be required to image pure D_1 R signal in the cortical regions.

In conclusion, data from this study support previous evidence of significant contribution from 5-HT_{2A} R in the [11C]NNC112 signal in the cortex, calling for caution in the interpretation of previously published [11C]NNC112 PET findings on D_1 R from humans in this cerebral region. Ketanserin pretreatment may be used to pre-block 5-HT_{2A} R contribution to the [11C]NNC112 PET signal, without affecting the D_1 R signal. Therefore, in the absence of a purely selective ligand for D_1 R, i.v. ketanserin challenge provides a suitable method for D_1 R imaging using [11C]NNC112 PET.

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Conflict of interest

Ana M Catafau, Graham E Searle, Roger N Gunn, Eugenii A Rabiner, and Marc Laruelle are GlaxoSmithKline employees.

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