

REGULAR RESEARCH ARTICLE

Brain PET Imaging of $\alpha 7$ -nAChR with [^{18}F]ASEM: Reproducibility, Occupancy, Receptor Density, and Changes in Schizophrenia

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Abstract

Background: The $\alpha 7$ nicotinic acetylcholine receptor increasingly has been implicated in normal brain physiology, as well as in neuropsychiatric disorders. The highly cortical distribution of $\alpha 7$ nicotinic acetylcholine receptor suggests a role in cognition. **Methods:** We expanded the first-in-human PET imaging of $\alpha 7$ nicotinic acetylcholine receptor with [^{18}F]ASEM from 5 to 21 healthy nonsmoking volunteers and added a feasibility study in 6 male patients with schizophrenia. Study aims included: (1) confirmation of test-retest reproducibility of [^{18}F]ASEM binding, (2) demonstration of specificity by competition with DMXB-A, an $\alpha 7$ nicotinic acetylcholine receptor partial agonist, (3) estimation of [^{18}F]ASEM binding potentials and $\alpha 7$ nicotinic acetylcholine receptor density *in vivo* in humans, and (4) demonstrating the feasibility of studying $\alpha 7$ nicotinic acetylcholine receptor as a target for schizophrenia.

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Results: Test-retest PET confirmed reproducibility (>90%) (variability $\leq 7\%$) of [^{18}F]ASEM volume of distribution (V_T) estimates in healthy volunteers. Repeated sessions of PET in 5 healthy subjects included baseline and effect of inhibition after oral administration of 150 mg DMXB-A. From reduction of binding potentials, we estimated the dose-dependent occupancy of $\alpha 7$ nicotinic acetylcholine receptor by DMXB-A at 17% to 49% for plasma concentrations at 60 to 200 nM DMXB-A. In agreement with evidence postmortem, $\alpha 7$ nicotinic acetylcholine receptor density averaged 0.67 to 0.82 nM and inhibitor affinity constant averaged 170 to 385 nM. Median V_T in a feasibility study of 6 patients with schizophrenia was lower than in healthy volunteers in cingulate cortex, frontal cortex, and hippocampus ($P = 0.02$, corrected for multiple comparisons, Mann–Whitney test).

Conclusions: The current results confirm the reproducibility of [^{18}F]ASEM V_T estimates and the specificity of the tracer for $\alpha 7$ nicotinic acetylcholine receptor. Preliminary findings from our feasibility study of [^{18}F]ASEM binding in patients with schizophrenia are suggestive and provide guidance for future studies with more subjects.

Keywords: PET imaging, nicotinic acetylcholine receptors, schizophrenia

Introduction

As ligand-gated excitatory cation channels, nicotinic acetylcholine receptors (nAChRs) in central nervous system (CNS) are fundamental to physiology (Lukas et al., 1999). The 2 nicotinic receptor subtypes, $\alpha 4\beta 2$ and $\alpha 7$, predominate in CNS among many other nAChR subtypes (Lukas et al., 1999; Gotti and Clementi, 2004). Nicotine binds with high affinity to $\alpha 4\beta 2$ -nAChR ($K_i \sim 1$ nM; Anderson and Arneric, 1994) and with a much lower affinity to $\alpha 7$ -nAChR ($K_i \sim 6000$ – 14000 nM; Davies et al., 1999).

The $\alpha 7$ -nAChR subtype is associated with the pathophysiology of specific CNS diseases, including schizophrenia, drug addiction, depression, anxiety, and multiple neurodegenerative disorders (Verbois et al., 2000, 2002; Woodruff-Pak and Gould, 2002; Albuquerque et al., 2009; D'Hoedt and Bertrand, 2009; Philip et al., 2010; Hoffmeister et al., 2011; Ishikawa and Hashimoto, 2011; Parri et al., 2011) and therefore is an important target of drug discovery. Multiple postmortem studies have shown reduced $\alpha 7$ receptors in brain samples from patients with schizophrenia, suggesting a role of $\alpha 7$ in schizophrenia (see review by Thomsen et al., 2010). In autoradiography of brains from patients with schizophrenia, reductions of α -[^{125}I] BTX binding to $\alpha 7$ were demonstrated in hippocampus, anterior cingulate cortex, and thalamic reticular nucleus (Freedman et al., 1995) but not in orbitofrontal or temporal lobes (Court et al., 1999; Marutle et al., 2001).

The $\alpha 7$ -nAChR has emerged as a potential therapeutic target for treatment of schizophrenia (Wallace et al., 2011; AhnAllen, 2012; Geerts, 2012; Wallace and Bertrand, 2013; Young and Geyer, 2013a; Freedman, 2014b; Beinat et al., 2015). In animals, stimulation of $\alpha 7$ is procognitive (Young and Geyer, 2013a; Potasiewicz et al., 2017). In clinical trials with selective $\alpha 7$ agonists, activation of the receptor improved cognitive performance in patients with schizophrenia. Selected drugs (DMXB-A, also known as GTS-21, TC-5619, and EVP-6124) show some degree of cognitive improvement in trials of patients with schizophrenia, although none has yet proceeded to late phase or FDA approval. A common trend is the improvement of cognition with an inverted U-shaped dose–response curve, when increasing the dose of $\alpha 7$ agonist above a threshold resulted in loss of drug effect (Wallace and Bertrand, 2015; Lewis et al., 2017). The latest research has revealed a new generation of promising $\alpha 7$ -targeting drugs (Beinat et al., 2016).

Despite the importance of the nicotinic receptor system, the physiological and pharmacological roles of specific receptor subtypes in CNS remain poorly understood (Philip et al., 2010; Ishikawa and Hashimoto, 2011; Marrero et al., 2011; Parri et al., 2011). Until recently, the lack of radioligands for quantitative emission tomography imaging of $\alpha 7$ -nAChR in humans

hampered noninvasive research of this receptor system. Many radiotracers of interest to $\alpha 7$ -nAChR (^{11}C -CHIBA-1001; Toyohara et al., 2009), ^{11}C -NS14492 (Ettrup et al., 2011; Magnussen et al., 2015), and others have been tested, but most had suboptimal properties in PET of humans (see Gao et al., 2013 for review). Here, we further tested human subjects with [^{18}F]ASEM, the only $\alpha 7$ -nAChR PET tracer available to humans with promising imaging characteristics (Gao et al., 2013b; Horti et al., 2014b; Horti, 2015; Coughlin et al., 2018; Hillmer et al., 2017; Wong et al., 2017). Objectives included: (1) confirmation of test-retest reproducibility of [^{18}F]ASEM binding in brain of normal volunteers, (2) demonstration for the first time of specific binding and target engagement/occupancy in human brain of the partial agonist DMXB-A (GTS-21), (3) estimation for the first time of [^{18}F]ASEM binding potentials and $\alpha 7$ -nAChR receptor density (B_{max}) and inhibitor affinity constant (K_i) for human brain *in vivo*, and (4) a feasibility study of the target role of $\alpha 7$ -nAChR in psychosis in 6 patients with schizophrenia.

Methods

Subjects

All studies were carried out with the approval of the Johns Hopkins Institutional Review Board, where all subjects provided written informed consent. Participants were recruited via advertisements within the metropolitan Baltimore region.

Twenty-one healthy, nonsmoking control subjects (13 males and 8 females; age range: 18–52 years; means \pm SD: 32.6 ± 12.4 years) volunteered for the study. In addition to baseline PET, 12 subjects had a second baseline PET (test-retest), and 5 other subjects had a second PET after a single dose of DMXB-A. Only 8 of the 12 subjects had successful test-retest PET due to technical problems in either test or retest in 4 subjects. Of the subjects, 5 (including 2 with test-retest from a previous publication by Wong et al., 2014) were included in this study. Healthy volunteers had no psychotropic treatment history, were free of significant medical or neuropsychiatric disorder, and received no current medications.

To examine the feasibility of measuring $\alpha 7$ nAChR in schizophrenia, 6 patients diagnosed with schizophrenia (aged 20–31 years; average 25.0 ± 4.3 [SD] y; all males; 1 smoker only) were recruited. Volunteers with schizophrenia that met DSM-V criteria, with stable antipsychotic medication other than clozapine, were referred by collaborating psychiatrists. Subjects underwent neuropsychiatric characterization including Brief Psychiatric Rating (BPRS) scores. Demographic data

Table 1A. Group Demographics

Diagnosis	Condition	n	Males	Females	Age (mean/SD)	Age (range)
Healthy controls ^a	Baseline	21	13	8	32.0 (12.4)	18–52
	Retest	8	6	2	37.8 (11.7)	20–52
	Blocking	5	3	2	22.1 (4.5)	18–28
Patients with schizophrenia	Baseline	6	6	0	24.8 (3.9)	20–31

^aNo healthy controls reported current or past smoking history.

Table 1B. Patients with Schizophrenia

Subject ID	Age	Sex	Race	Ethnicity	BPRS – Negative (M)	BPRS – Positive (M)	Smoker	Cigarettes/d	Medications
01	27	M	AA	NH	2.25	1.25	No	N/A	Aripiprazole
02	31	M	W	NH	3	1	Yes	20	Haloperidol, benztropine, sertraline
03	22	M	AA	NH	2.75	2.25	No	N/A	Risperidone
04	25	M	AA	NH	2.75	1.75	No	N/A	Quetiapine, haloperidol, benztropine
05	20	M	AA	NH	1.5	1.5	No	N/A	Escitalopram, benztropine, aripiprazole, clonazepam
06 – Scan 1	24	M	W	NH	2.75	3.25	No	N/A	Risperidone, lorazepam
06 – Scan 2	24	M	W	NH	1.0	1.0	No	N/A	Lorazepam

for healthy controls and subjects with schizophrenia are listed in Table 1A, 1B. Participants provided information of history of tobacco use, including number of cigarettes per day and years of tobacco use.

All subjects (controls and patients with schizophrenia) had negative urinary toxicology tests and provided CO breath samples of <4 ppm on the imaging day to confirm abstinence for at least 12 h from tobacco smoking for any subjects (see additional inclusion/exclusion criteria in Supplemental Material).

One patient with schizophrenia was tested with PET [¹⁸F]ASEM while chronically treated with risperidone and then retested with PET without drug for 3 weeks (discontinued for clinical reasons).

To address possible effects of antipsychotics administered to patients, we determined biodistribution in mice treated for 5 d with cohorts of placebo vs risperidone, aripiprazole, or olanzapine, the 3 most common drugs in the present patient population, at doses equivalent to doses used in humans (Reagan-Shaw et al., 2008; Dawe et al., 2010; McOmish et al., 2012; Ning et al., 2017). Mice were i.v. injected with [¹⁸F]ASEM and sacrificed after 60 min (detailed Methods and Figure A in Supplemental Materials).

PET

All subjects underwent PET with the Siemens/CTI High-Resolution Research Tomography (HRRT: resolution <2 mm). The dynamic imaging continued for 90 min with the frame sequences of 4x15 s, 4x30 s, 3x60 s, 2x120 s, 5x240 s, and 12x300 s. Each subject received a radial arterial catheter for collection of approximately 40 arterial blood plasma samples and

subsequent HPLC radiometabolite detection during the 90-min imaging session. Arterial plasma samples were drawn as rapidly as possible for the first 2 min and then every minute until 10 min post injection, every 2 min until 20 min post injection, and at the times 25, 30, 45, 60, 75, and 90 min post injection. Metabolites were measured for the times of 2, 5, 10, 20, 30, 60, and 90 min post injection. (See Supplemental Material for HPLC methods.)

Each subject received 13.8 mCi ± 0.23 (SEM) [¹⁸F]ASEM synthesized by the method of Gao et al. (2013) with average specific activity of 56417 Ci/mmol. There were no significant differences in injected radioactivity or specific activity between the controls and patients with schizophrenia. Tracer-free fraction in plasma across subjects averaged 2.79% ± 0.06 (SD) and was not significantly different in healthy controls and patients with schizophrenia (methods in Supplemental Materials).

Of the healthy volunteers, 12 were retested with [¹⁸F]ASEM imaging approximately 5 weeks (mean 41.2 d) after the first PET session, including 3 reported by Wong et al. (2014). We carefully reviewed the radiometabolite HPLC results and elected to exclude 4 subjects for technical reasons (e.g., defective capture columns during early development of HPLC procedures). To determine the target engagement (occupancy) by a receptor ligand and to obtain evidence of specific binding of [¹⁸F]ASEM to α7-nAChR in humans, 5 healthy subjects additionally underwent a second imaging session after administration of oral 150 mg of the partial agonist DMXB-A, 40 min before the PET tracer injection. Radial arterial samples for detection of concentration DMXB-A were obtained during PET imaging at 70, 100, and 130 min post-oral dose of DMXB-A. Concentrations of DMXB-A in subjects' plasma samples were determined quantitatively by

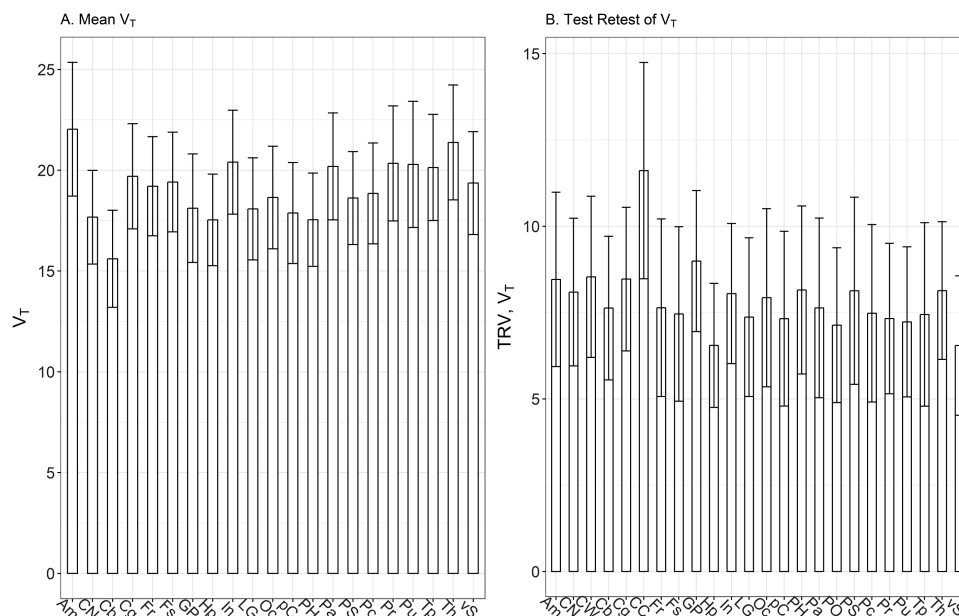


Figure 1. Estimates of V_T and test-retest variability for V_T . (A) Regional estimates of V_T for all healthy control subjects ($n=21$). (B) Test-retest variability of V_T in 8 healthy controls. For both panels, bars show mean \pm SD. Abbreviations: Am, amygdala; CN, caudate nucleus; Cb, cerebellum; Cg, cingulate; Fr, frontal lobe; Fs, fusiform gyrus; GP, globus pallidus; Hp, hippocampus; In, insula; LG, lingular gyrus; Oc, occipital lobe; pC, paracentral; PH, parahippocampus; Pa, parietal lobe; PS, postcentral gyrus; Pc, precentral gyrus; Pr, precuneus; Pu, putamen; Tp, temporal lobe; Th, thalamus; vS, ventral striatum.

HPLC with a modification of the previously reported method (Kem et al., 2004). (Details in Supplemental Material.)

Menstrual phase for the women was determined by a progesterone level using levels exceeding 2 ng/mL, indicating luteal phase. For 2 women (one in luteal phase and one in follicular phase), menstrual phase was determined by self-reported menstrual history, as progesterone samples were not available.

PET Analysis

Volumes of Interest

Volumes of interest (VOI) were selected automatically in individual subject SPGR MRI volumes using FSL (FMRIB Software Library; Jenkinson et al., 2012) FIRST tool (Patenaude et al., 2011) for subcortical regions and Freesurfer tool (Fischl et al., 2004) for cortical regions. Automated VOI were manually edited to suit the structures of interest, using a locally developed VOI tool (VOILand). Refined VOI were transferred from MRI to PET spaces according to MRI to PET coregistration parameters obtained from the co-registration module of SPM12 (Statistical Parametric Mapping; Ashburner and Friston, 2003). The VOI of PET space were applied to PET frames to obtain time-activity curves of regions. Head-motion correction was performed with the image co-registration module of SPM12.

Total volumes of distribution (V_T) and test-retest variability were estimated for all 21 cortical VOI (Figure 1A–B). In addition, the full VOI set was used in the calculation of dose-dependent inhibition (Figure 2) as well as for the averaged estimates of maximum binding capacity (B_{max} ; Figure 3) and inhibitor K_i (Figure 4). For analysis of V_T differences (in patients vs control subjects, Figure 5) and potential correlation with BPRS score (see Supplemental Material), we chose 3 cortical regions implicated in autoradiography with [125 I] α -bungarotoxin ([125 I]BTX) of brain of patients with schizophrenia postmortem, previously reported with significant reductions in hippocampus (~70%, Freedman et al., 1995), frontal cortex (40%, Guan et al., 1999), and cingulate cortex (54%, Marutle et al., 2001).

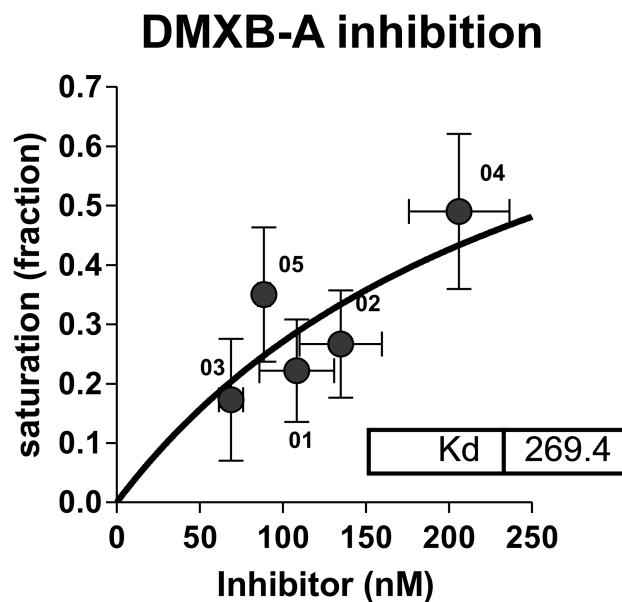


Figure 2. Dose-dependent inhibition with DMXB-A level. The saturation, s , reflects the fraction of receptor occupied by the blocking agent, DMXB-A. Each point on the curve represents individual measurements of the plasma level of DMXB-A, determined by HPLC mass spectroscopy, and the fraction of receptors occupied by DMXB-A, estimated by the Extended Inhibition Plot (EIP). The horizontal error bars represent the SEM from 3 measurements, and the vertical error bars reflect the SEM from the linear analysis of the Extended Inhibition plot. These data were fit to the Michaelis-Menten equation, with O_{max} assigned to a value of 1; (i.e., 100% occupancy, or a saturation fraction, $s=1$). This is typical for receptor occupancy studies with PET. However, further studies at higher plasma concentrations may indicate a lower maximal occupancy, due to the unique nature of the $\alpha 7$ subunit, as stated in the Discussion.

Kinetic Analysis

Quantification of [18 F]ASEM steady-state volumes of distribution (V_T) proceeded as previously published (Wong et al., 2014). Specifically, the plasma reference graphical analysis was used

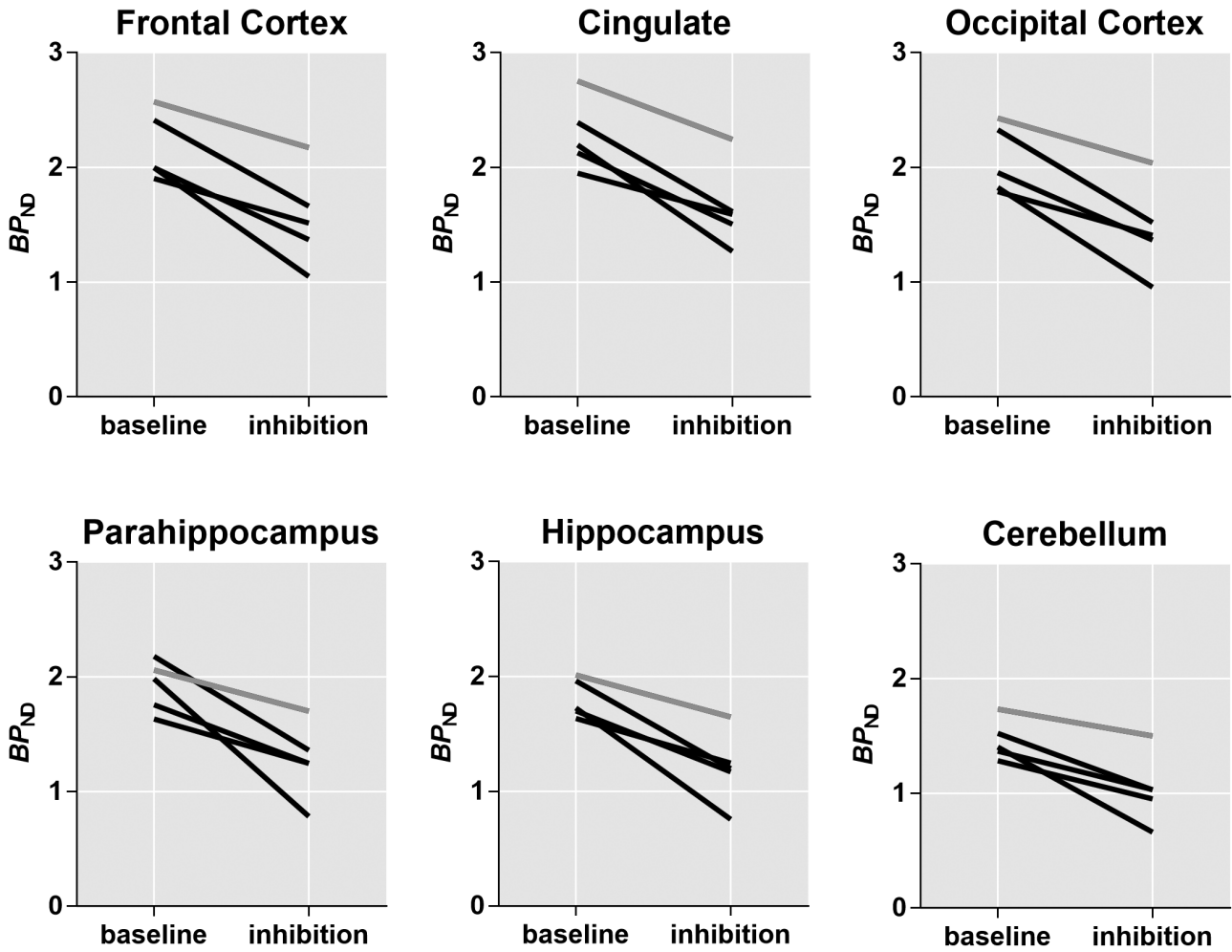


Figure 3. Regional binding potentials (BP_{ND}). The analysis using the Extended Inhibition Plot (EIP) revealed that the BP_{ND} decreased significantly in response to competition with DMXB-A by 31% in average compared with baseline. Each line on the graphs of 6 representative regions of the original 21 regions displays the estimates of BP_{ND} in individual subjects at baseline and inhibition condition.

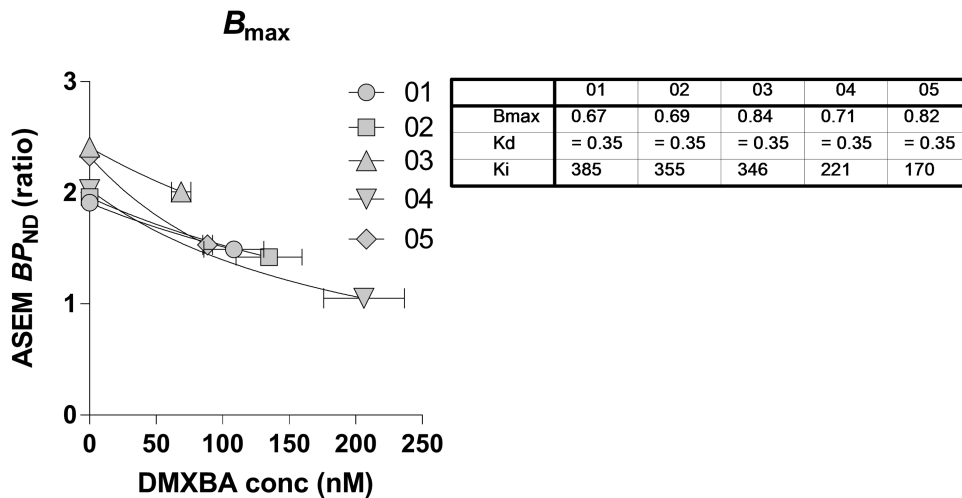


Figure 4. B_{max} estimates. Average B_{max} across all regions (21 VOIs total) were computed from the known BP_{ND} estimates, the plasma concentration of DMXB-A and the receptor affinity, K_D , which was calculated as 0.35 nM based on known values from ^{125}I α -bungarotoxin in vitro studies. The analysis reveals that the average B_{max} across all regions ranged from 0.76 to 0.96 nM in the 5 subjects, and the K_i ranged from 170 to 385 nM.

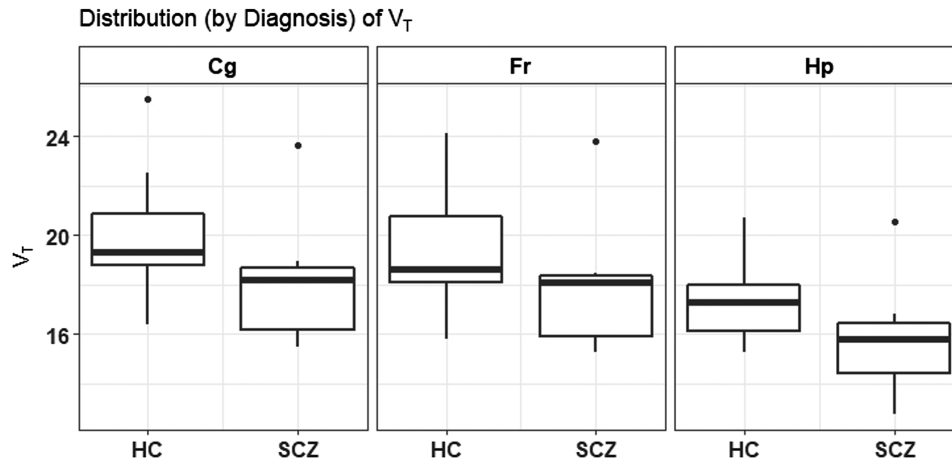


Figure 5. Distribution of V_T in healthy patients and controls. Boxplots of the distribution of V_T in healthy controls (HC) and patients with schizophrenia (SCZ) in cingulate cortex (Cg), hippocampus (Hp), and frontal cortex (Fr). The single points above the limits of the boxplot are outliers.

to obtain regional V_T values that served as primary outcome variable for test-retests and analyses with age, sex, and neuropsychiatric testing as well as serving as secondary outcome variable for estimates of the reference volume of nondisplaceable bound radioligand (V_{ND}), competitor saturation of receptors (s), binding potentials (BP_{ND}), and B_{max} . We completed B_{max} and affinity (IC_{50} , K_i) estimates of $\alpha 7$ -nAChR in 3 steps in 5 subjects by means of DMXB-A inhibition.

We estimated the occupancy of the partial agonist inhibitor by means of estimates of the radioligand's volume of nondisplaceable distributed tracer in the baseline (V_{ND}), obtained from baboon brain *in vivo* (Horti et al., 2014), using the conventional Inhibition Plot (IP) of Gjedde and Wong (2000). We compared the value of V_{ND} obtained from the decline of the magnitude of the total volume of V_T in the presence of 2 different concentrations of SR180711, of which we used the value associated with the lower dose to avoid affecting the value of V_{ND} by changes of protein binding in brain tissue or circulation or both (see supplemental Figure B illustrating the V_{ND} determination in baboon brain).

Applied to regional V_T , the IP yielded reference volumes (V_{ND}) and degrees of saturation (s), according to the equation (Phan et al., 2017),

$$V_{T(i)} = (1-s)V_{T(b)} + sV_{ND}, \quad (1)$$

where $V_{T(b)}$ symbolizes the volume of distribution at baseline and $V_{T(i)}$ the volume at inhibition in presence of DMXB-A that competes with the radiotracer for binding to the binding site. According to Equation (1), when the regional estimates of V_T in the inhibition condition were plotted as functions of the estimates in the baseline condition, the solution yielded the reference volume, V_{ND} , and the fraction of receptors occupied by the competitive drug, s .

The initial analysis revealed V_T estimates before and after DMXB-A inhibition of similar magnitudes, compensating for values of V_{ND} that may be different in the inhibition and baseline conditions. To resolve this issue, we applied the Extended IP equation (EIP; Phan et al., 2017) that relates the radioligand distribution volumes at inhibition and baseline as follows:

$$V_{T(i)} = (1-s) \left[\frac{V_{ND(i)}}{V_{ND(b)}} \right] V_{T(b)} + sV_{ND(i)}, \quad (2)$$

where values of V_{ND} at baseline and inhibition are different, denoted $V_{ND(b)}$ and $V_{ND(i)}$ at baseline and inhibition, respectively. The EIP yielded the value of V_{ND} in the inhibition condition ($V_{ND(i)}$), when the true reference volume at baseline ($V_{ND(b)}$) is known such that estimates yielded by EIP depend on a measure of the magnitude of the reference volume.

Volumes of distribution (V_T and V_{ND}) yielded BP_{ND} . We estimated the BP_{ND} of 5 subjects with DMXB-A inhibition in the baseline and at inhibition, using the values of $V_{T(b)}$ and $V_{ND(b)}$ in the baseline condition and the values of $V_{T(i)}$ and $V_{ND(i)}$ at inhibition obtained by means of Equation (2), in the unblocked and DMXB-A inhibited states, with the equation

$$BP_{ND(b)} = \frac{V_{T(b)}}{V_{ND(b)}} - 1 \text{ and } BP_{ND(i)} = \frac{V_{T(i)}}{V_{ND(i)}} - 1, \quad (3)$$

where the $BP_{ND(b)}$ and $BP_{ND(i)}$ terms symbolize the BP_{ND} at baseline and inhibition, respectively.

The BP_{ND} and inhibitor concentrations (C_i) yielded the B_{max} and K_i (IC_{50}) estimates of the receptor and inhibitor, respectively. We completed B_{max} and affinity of the competitor (IC_{50} , K_i) calculation of $\alpha 7$ -nAChR in 5 subjects by means of DMXB-A inhibition using the values of BP_{ND} of baseline and inhibition that led to the novel (never previously published) *in vivo* estimate of the magnitude of the B_{max} and K_i of nAChR in humans, using the equation

$$BP_{ND} = \frac{B_{max}}{K_d \left(1 + \frac{[DMXB-A]}{K_i} \right)} \quad (4)$$

where the B_{max} is determined relative to the affinity of the radioligand to the receptor, K_d , with known values of the inhibitor concentration [DMXB-A] and the receptor's affinity for inhibitor, K_i . For this calculation, we used a calculated value of ASEM K_d of 0.35 nM (established from prior measure of K_i of 0.4 nM for [^{123}I]bungarotoxin) (Xiao Y et al., 2009).

Schizophrenia

As a feasibility study, we compared 6 patients with schizophrenia to a subset of 15 healthy volunteers matched for age, within 3 specific brain regions: hippocampus and cingulate and frontal cortices. In this subset, the mean age of healthy volunteers was

23±4 (SD) y, range 18 to 30, and for patients with schizophrenia the mean age was 24±4 (SD) y, range 20–31 y.

Results

Regional V_T

Regional estimates of V_T in selected regions are shown in Figure 1A. We tested the test-retest variability of the V_T estimates in 8 healthy subjects, 6 males (average age 41) and 2 females (average age 24 y). Across all cortical and subcortical VOIs, the average test-retest variability was 7.8%±0.6% (SD, see Figure 1B), ranging from 6.5% to 8.9% (repeatability of >90%).

Receptor Kinetics

The reanalysis of inhibition of [18 F]ASEM binding in baboon (Horti et al., 2014) yielded the V_T of nondisplaceable tracer accumulation at baseline ($V_{ND(b)}$). In the baboon, the V_T of [18 F]ASEM declined markedly in response to blocking with the high affinity inhibitor SSR180711. The reference volume V_{ND} assessed by means of the standard IP is presented in Figure 2, with the estimate of $V_{ND(b)}$ of 5.4 ml/cm³ at the lower dose of the SR180711. We note that the magnitude of V_{ND} is a property of the tracer [18 F]ASEM that we assume to be representative of mammalian brains in general.

We used the value of V_{ND} of 5.4 mL/cm³ as the value of $V_{ND(b)}$ in the EIP, required when V_T values failed to yield evidence of inhibition of radioligand binding by DMXB-A in human brain. In 5 subjects, the value of $V_{ND(b)}$ yielded estimates of saturation (s) and inhibition reference volume ($V_{ND(i)}$), as shown in Figure 2 of the individual estimates of s as function of the concentration of DMXB-A in arterial plasma, confirming the dose-dependent inhibition exerted by DMXB-A. Estimates of regional BP_{ND} declined significantly in 6 of the 21 representative regions after blocking with DMXB-A, as shown in Figure 3. The average values for all 21 regions of B_{max} and average values of the receptor's K_1 to DMXB-A ranged from 0.67 to 0.82 nM for B_{max} and from 170 to 385 nM for K_1 , as presented in Figure 4. Regarding the choice of the $V_{ND(b)}$, the standard error of the $V_{ND(b)}$ estimate in baboon of 2.7 mL/cm³ corresponded to ranges of human B_{max} estimates of 0.4 to 1.9 nM and DMXB-A K estimates of 138 to 363 nM.

Menstrual Cycle

The menstrual cycle phase (luteal vs follicular) was determined for the 8 healthy control females. Estimates of V_T did not differ significantly in relation to cycle phase. The lower ages of women

volunteers precluded analysis of different changes of V_T with age in men and women.

Schizophrenia

To evaluate the feasibility of studying patients with schizophrenia, we compared patients with healthy control subjects. In the 3 preselected regions (cingulate and frontal cortices, hippocampus) examined for group differences between patients and the age-matched control subjects, the median V_T values were lower in patients than in age-matched healthy volunteers (Figure 5). Potential outliers, one a control subject and one a patient, were identified (outlier points marked in boxplots of Figure 5). Using the Mann-Whitney U-test, we tested for significant differences in median V_T values between control subjects and patients, before and after excluding outliers. The results, with corrections for multiple comparisons, are given in Table 2. In the cingulate cortex and hippocampus, the difference in V_T was significant after exclusion of outliers and correction for multiple comparisons ($P=.02$ in both regions).

The correlation of estimates of V_T with scores of BPRS did not reach statistical significance, although there were positive trends for values in cingulate and frontal cortices in 5 subjects after outlier analyses (see supplementary Figure C for details).

Antipsychotic Medication

The biodistribution in mice revealed no significant effects on [18 F]ASEM binding of aripiprazole, risperidone, or olanzapine (see Supplemental Materials). As described in Methods, the patient with schizophrenia chronically treated with risperidone had repeated PET after a 3-week drug-free period, imposed for clinical reasons. The V_T estimates did not differ by more than 7% (i.e., within the test-retest variability of control subjects in any brain region for the on- and off-risperidone comparison (Figure 1B) (supplementary Figure D, points labelled “on” and “off”).

Discussion

Subjects

We extended the first-in-human report of Wong et al. (2014) from 5 to 21 healthy subjects (8 test-retest sessions, 5 sessions with inhibition by DMXB-A of [18 F]ASEM binding, and 6 baseline sessions) and added a preliminary study of 6 patients with schizophrenia, with 1 patient receiving test-retest PET in the absence and presence of chronic risperidone medication.

Table 2. Corrected and Uncorrected P Values from V_T in Controls vs Patients Results (uncorrected P values and corrected by different methods) of Mann-Whitney tests for V_T in Healthy Controls vs Patients with Schizophrenia

Region	Uncorrected P value	Adjusted (Horchberg)	Adjusted (Bonferroni)	Adjusted (Simes)
All Data				
Cg	.056	.159	.167	.118
Fr	.171	.172	.515	.172
Hp	.079	.159	.238	.119
With outliers removed				
Cg	.009	.028	.028	.025
Fr	.058	.058	.173	.058
Hp	.017	.033	.05	.025

Adjusted P values were obtained by the STATA package qqvalue using 3 different methods of adjustment (Newson, 2010; specific methods are Horchberg, 1998, standard Bonferroni correction and Simes, 1986).

The results demonstrate excellent test-retest repeatability of [^{18}F]ASEM PET of >90%. In the population of 21 healthy non-smoking volunteers, when we plotted tracer distribution volume V_T against age, we observed an increase with V_T with age. While our smaller sample and lack of a wide age distribution limited our ability to test this relationship formally with regression analysis, the trends in our data match the findings from a larger age range in another set of subjects (Coughlin et al., 2018, which had only 2 subjects in common that we provided to the Coughlin paper previously). The preliminary findings of values of V_T vs age are shown in [supplementary Figure D](#).

We were unable to determine sex differences because of the different age ranges. Of the 8 women, none of them showed a specific effect of menstrual cycle on estimates of V_T .

Receptor BP_{ND}

Estimates of B_{max} are based on determination of BP_{ND} (Gjedde et al., 2005). Importantly, for the first time, we present evidence that competitor occupancy, apparent K_1 , and B_{max} of human $\alpha 7$ -nAChR can be measured, based on the assumptions presented. We determined regional estimates of [^{18}F]ASEM binding as the first step towards complete receptor quantification. In addition to regional values of V_T , the receptor quantification required knowledge of the magnitude of V_T of the tracer in a region of brain tissue with no specific binding. Previously, no such reference volume has been identified for this tracer with prevalent binding in cerebral cortex. As the first PET study with blockade of $\alpha 7$ -nAChR in human brain *in vivo*, this study took advantage of the original development of the well-documented partial agonist DMXB-A at the University of Florida (Kem, 2000; Kem et al., 2004) and the subsequent successful testing in patients with schizophrenia at the University of Colorado (Olincy et al., 2006; Freedman, 2014).

Receptor Occupancy

Recent studies of mouse and baboon brains revealed significant blockade of the $\alpha 7$ -nAChR by the high-affinity partial $\alpha 7$ -nAChR agonists SSR180711 and DMXB-A *in vivo* (Horti et al., 2014a; Wong et al., 2014), but effective inhibition or competition has not yet been confirmed in humans. As a candidate drug, DMXB-A was tested in clinical trials of patients with schizophrenia, Alzheimer's disease, or ADHD (Freedman et al., 2008; Freedman, 2014), and the drug was used here to test target engagement/occupancy *in vivo* at the $\alpha 7$ -nAChR and to demonstrate specificity of [^{18}F]ASEM binding to these receptors.

Previously, we demonstrated dose-dependent tracer inhibition in rodents by DMXB-A with cerebellum as reference region (appropriate in rodents but not human brain) (Wong et al., 2014). Similarly, in the present study, blockade with the highest dose of DMXB-A allowed in humans (150 mg orally) exhibited competition with binding of [^{18}F]ASEM. The occupancy of receptors by DMXB-A displayed a relatively monotonic relationship with plasma levels of DMXB-A.

We used the EIP under the assumption that the apparently absent inhibition of receptor binding would be due to factors such as change of free fractions of the radioligand in circulation or brain tissue or both, with the consequence that the value of V_{ND} would differ between the baseline ($V_{ND(b)}$) and inhibited ($V_{ND(a)}$) states, as reported by Phan et al. (2017). Using EIP and the value of $V_{ND(b)}$ of 5.4 mL/cm³ obtained from moderate blockade of the $\alpha 7$ -nAChR in baboon brain, reported by Horti et al. (2014) as a simple reduction in slope and reanalyzed by the

standard IP here, we obtained DMXB-A occupancies in the human subjects in the range of 17% to 49%. Future studies with new $\alpha 7$ -nAChR drugs will further test the occupancy and the accuracy of the resulting estimates of B_{max} and affinity in human brain *in vivo*.

With the assumptions and translation from autopsy protein and wet tissue-based B_{max} of $\alpha 7$ -nAChR, we find remarkable agreement among the B_{max} estimates that we regard as another innovative aspect of the present study. As 150 mg is the highest single oral dose allowed under the DMXB-A IND based on historical information, we elected not to test lower doses, because of the modest occupancy, compared with occupancy of drugs such as antipsychotics, and more recently glycine transporter (GlyT1) ligands (Martin-Facklam et al., 2013; Wong et al., 2013). However, the dose dependent occupancy with DMXB-A is evidence of specificity of the radiotracer [^{18}F]ASEM in human brain. The occupancy is further evidence of the potential usefulness of [^{18}F]ASEM for therapeutic occupancy trials, as these are somewhat complicated due to the inverted-U-shaped dose effect of $\alpha 7$ -nAChR partial agonists (Young and Geyer, 2013).

The $\alpha 7$ -nAChR receptor has 5 binding sites among the 5 $\alpha 7$ subunits and is maximally activated when at least 2 binding sites are occupied by an agonist. At higher doses, agonists bind at up to all 5 binding sites and progressively desensitize the $\alpha 7$. Moreover, $\alpha 7$ partial agonist drugs such as DMXB-A and others produce their clinical effects at lower concentrations than would be expected from *in vitro* functional experiments where peak currents are recorded (Prickaerts et al., 2012; Papke, 2014; Preskorn et al., 2014). The co-agonistic mechanism of *in vivo* action of $\alpha 7$ partial agonist suggests that DMXB-A, at the clinically effective dose, binds at 1 of 5 binding sites of $\alpha 7$, while brisk activation of the receptor will, however, occur upon exposure to endogenous ACh. Thus, *in vivo*, a single molecule of DMXB-A plus 1 molecule of ACh are sufficient to trigger the opening of the $\alpha 7$ -nAChR channel (Williams et al., 2011; Prickaerts et al., 2012; Papke, 2014; Preskorn et al., 2014). Therefore, in the PET occupancy studies with the DMXB-A-treated humans, the binding of [^{18}F]ASEM is expected to be inhibited by as little as 20% to 40% (one-fifth to two-fifths) instead of 100%. This co-agonistic mechanism agrees reasonably with our PET [^{18}F]ASEM inhibition by DMXB-A in humans at 17% to 49% with a maximum clinical dose of DMXB-A (150 mg). Full blocking of [^{18}F]ASEM with $\alpha 7$ agonists occurs at higher doses, as observed in mice with 20 mg/kg DMXB-A (Horti et al., 2014a; Wong et al., 2014) and in baboons administered 5 mg/kg of the more highly potent SSR180711 that is unavailable in the United States for human use (Horti et al., 2014).

Receptor Density and Tracer Affinity

The use of the EIP with the DMXB-A inhibition and the use of the estimate of V_{ND} from baboon brain (Horti et al., 2014) yielded the binding potentials necessary to estimate the average values across all binding regions of B_{max} (0.67–0.82 nM) and K_1 (range 170–385) in human brain. While the value of V_{ND} in humans currently is unknown, the baboon value of 5.4 mL/cm³ estimated here with SEM of 2.7 mL/cm³ corresponded to ranges of B_{max} estimates of 0.75 (range 0.38–1.83) nM and DMXB-A K_1 estimates of 138 to 363 nM. These values agree favorably with estimates postmortem of 0.28 nM B_{max} for human cerebral cortex with α -[^{125}I]bungarotoxin (Davies and Feisullin, 1981) and cortical B_{max} values of 0.7 nM in patients with schizophrenia compared with 1.5 nM in healthy control subjects (Marutle et al., 2001), assuming 10% mg protein converted to wet weight.

Schizophrenia

In the feasibility study of 6 patients with schizophrenia, we observed reductions of V_T in 5 of the 6 subjects. The finding of significant reduction of regional distribution in 5 of 6 subjects in 2 of 3 regions after outlier exclusion of a single subject agreed with previous findings postmortem. However, in the present study, the difference between controls and patients is lower than observed postmortem. This finding could be due to a number of factors, such as tracer differences ($[^{18}\text{F}]$ -ASEM vs $[^{125}\text{I}]$ -BTX), in vivo vs in vitro tests, and the heterogeneity of the patient population.

Limitations

Antipsychotic Medication

Neuroreceptor studies of patients with schizophrenia in the drug free or naïve state are increasingly logistically challenging, although such studies have been completed in limited populations (Farde et al., 1986; Wong et al., 1986). In the present limited population, patients with schizophrenia were tested while on stable medication for more than 3 weeks with antipsychotics that are held to have only negligible effect on binding to α_7 nAChR and to be nonexclusionary in therapeutic studies of partial agonists such as DMXB-A, e.g., clozapine (which was exclusionary). Several precautions were taken to reduce the possibility of confounding antipsychotic action in the quantification of binding to α_7 nAChR. First, the majority of the patients were treated with risperidone and haloperidol, which unlike clozapine fail to normalize the P50 abnormality (Adler et al., 1998). Second, a rodent biodistribution study found no significant effects on $[^{18}\text{F}]$ ASEM binding. Finally, although only 1 subject was studied off medications, the repeated PET test on and off chronic risperidone was suggestive of the conclusion that this commonly used antipsychotic is unlikely to confound the estimates of the V_T values for α_7 nAChR.

Effects of Smoking

All 21 control subjects and 5 of the 6 patients with schizophrenia were nonsmokers. We considered comparing smokers with nonsmokers but did not recruit a sufficient number of healthy volunteers with clinically verified smoking status. By design therefore we studied only nonsmoking control subjects in the current protocol of test-retest and occupancy studies and comparison with the feasibility study of 6 patients with schizophrenia.

Despite the relatively high prevalence of smoking in patients with schizophrenia, only 1 of the subjects randomly recruited with schizophrenia smoked (20 cigarettes/d). Most subjects were younger (<35 years old), which may be a contributing factor, as it has been reported that younger subjects are less likely to smoke (for an example of this trend, see <https://www.hhs.gov/ash/oah/adolescent-development/substance-use/drugs/tobacco/trends/index.html>). The patient that happened to be a regular smoker also happened to be the oldest of the patients at 31 y (age range of patients 20–31 y, mean 24.9 y). The V_T values did not differ significantly for the smoking subject compared with the other patients with schizophrenia with low value of V_T .

Furthermore, on the day of PET, all control subjects and patients with schizophrenia had CO values <4 PPM, indicating successful abstinence (regardless of their self-reports) and likely longer than 12 h, reducing the acute effects of nicotine, if any, on the PET measure of the tracer V_T .

As a future direction, we specifically will recruit participants for inclusion into cohorts of smokers and nonsmokers of both healthy controls and patients with schizophrenia to further explore smoking effects. The assignments will be verified by smoking history, urine cotinine levels, CO breath measures, and assessments of extent of nicotine use and dependence (e.g., Fagerstrom, MINI nicotine use, Minnesota Nicotine Scale, and Michigan Nicotine Reinforcement Questionnaire). With a larger sample, the schizophrenia group is likely to comprise more smokers, particularly among older patients.

Age and Sex Differences

In the group of 21 subjects in the age range of 18 to 50 y, males and females were not distributed uniformly, ruling out formal determination of sex differences as functions of normal aging. Also, the small number of 5 subjects with DMXB-A occupancy nonetheless yielded a reasonable range of DMXB-A human plasma concentrations, presumably due to individual variability in oral absorption and metabolism of the inhibitor.

Schizophrenia

In this feasibility study, the small number of patients with schizophrenia is a statistical limitation. In 5 of the 6 subjects, estimates of V_T in 2 of 3 regions compared with age-matched control subjects, as well as a formal outlier analysis, suggested that the high values of V_T in one subject may have had other causes leading to the elevation. Thus, expansion of the number of patients with schizophrenia and detailed documentation of the clinical and neuropsychological features of the patients will help expand the findings of factors that influence the heterogeneity of α_7 nAChR findings.

Conclusions

Here, we confirmed the test-retest reliability of tracer binding in healthy human brains, and we demonstrated for the first-time specificity of competition from the partial agonist DMXB-A in normal healthy volunteers, as previously reported in preclinical imaging studies. The novel first time presentation of in vivo B_{max} measures agreed favorably with published human postmortem values. The effects of antipsychotics in our schizophrenia volunteers may not be a major confound based on our preliminary mice and literature and one subject on and off risperidone. The preliminary findings in patients with schizophrenia suggest the feasibility of imaging this important disorder and generally consistent with previous reports of human brains postmortem, but clearly larger samples with a spectrum of clinical severity and comparisons with full cognitive and neuropsychological tests are needed.

Supplementary Material

Supplementary data are available at *International Journal of Neuropsychopharmacology* online.

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Statement of Interest

None.

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