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ORIGINAL INVESTIGATION

5-HT_{2A} receptor density is decreased in the at-risk mental state

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Abstract
Rationale Current perspectives on the pathophysiology of schizophrenia direct attention to serotonergic (serotonin, 5-HT) dysregulation in the prodrome or at-risk mental state (ARMS).
Objective To study the cerebral 5-HT_{2A} receptor (5-HT_{2A}R) in the ARMS with [^{18}F]altanserin positron emission tomography (PET) and a bolus-infusion paradigm.
Materials and methods We quantified the spatial distribution of 5-HT_{2A}R binding potential (BP_1') in never-medicated subjects assigned to early (n=6) and late (n=8) prodromal states of schizophrenia relative to healthy controls (n=21). Five single nucleotide polymorphisms (SNPs) in the 5-HT_{2A}R-encoding gene (HTR2A; 13q14-21) were genotyped to control for a potential bias in BP_1' due to between-group differences in genotype distributions.
Results Group comparisons of partial-volume corrected PET data by statistical parametric mapping and confirmatory volume of interest analysis yielded a dissemination of BP_1' decreases consistent with increasing levels of risk. An additional decrease in caudate BP_1' was present in subjects who subsequently converted to first-episode psychosis (n=5), but absent in non-converters (n=9). Between-group differences were not confounded by a differential distribution of SNP genotypes.
Conclusion These results suggest a progressive reduction of cortical 5-HT_{2A}R density as a surrogate biological measure of increased risk for schizophrenia, irrespective of conversion. Progressive reductions of subcortical 5-HT_{2A}R density could provide an indicator of illness activity and help to predict imminent conversion to schizophrenia. Moreover, our findings substantiate the rationale for establishing a phase-specific psychopharmacological intervention in the ARMS that addresses the serotonergic component of vulnerability to schizophrenia.

Keywords Schizophrenia · Psychosis · Prodrome · At-risk · Serotonin · 5-HT · 5-HT_{2A} Receptor · PET

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Introduction

Current perspectives on the pathophysiology of schizophrenia have directed attention to vulnerability to the illness (Lieberman et al. 2001). The prevailing research paradigms to identify vulnerability to schizophrenia are the genetic and the clinical at-risk strategy (Cannon 2005), with the latter targeting at the identification of prodromal symptoms and behaviors (McGlashan 1996). Operationally, the pro-

drome or at-risk mental state (ARMS) is defined by duration of time, starting with the onset of decline in the baseline level of functioning and ending with the transition to first-episode psychosis (Yung and McGorry 1996). The average duration of the ARMS is about 3 years across studies (McGlashan 1996).

In contrast to studies of established schizophrenia, neurochemical imaging in the ARMS allows crucial insights into the emerging pathophysiology of schizophrenia (Fusar-Poli et al. 2007; Jessen et al. 2006) without the potential confounds of illness chronicity and antipsychotic drug action. The importance of studying neurochemical dysregulation in the ARMS stems from the rationale that early psychopharmacological intervention could prevent or postpone the onset of first-episode psychosis, or at least attenuate illness severity (Cornblatt et al. 2002; McGorry et al. 2002; Woods et al. 2003). If untreated, more than a third of vulnerable individuals convert to first-episode psychosis (McGorry et al. 2002; Yung et al. 2003); the longer the period of untreated illness, the worse the prognosis is (Keshavan et al. 2003; Norman and Malla 2001; Wyatt 1991).

Of the 14 subtypes (seven families) of serotonin (5-HT) receptors expressed in the brain (Barnes and Sharp 1999), the postsynaptic 5-HT_{2A} receptor (5-HT_{2A}R) is suspected to have a prominent role in the pathophysiology of schizophrenia. Psychotogenic agents including *mescaline*, *psylocybine*, and *lysergic acid diethylamide* induce hallucinations by activating the 5-HT_{2A}R (Gonzalez-Maeso et al. 2007), which, in turn, is antagonized by *clozapine* and other atypical antipsychotic drugs that display a preferential 5-HT_{2A}R vs dopamine (DA) D_2 receptor (D_2R) affinity and occupancy (Meltzer et al. 1989, 2003). Moreover, DNA sequence variation in the 5-HT_{2A}R-encoding gene (HTR2A; 13q14-21) has been linked to transmission of susceptibility for schizophrenia (Norton and Owen 2005) and might influence the clinical response to clozapine (Arranz et al. 1995, 2000). In addition, the majority of postmortem autoradiography studies have shown reduced 5-HT_{2A}R binding in the prefrontal cortex of schizophrenic patients (Dean 2003; Matsumoto et al. 2005). However, to date, no consensus has evolved on the 5-HT_{2A}R in vivo status in schizophrenia (Lewis et al. 1999; Ngan et al. 2000; Okubo et al. 2000; Trichard et al. 1998; Verhoeff et al. 2000).

We previously demonstrated that positron emission tomography (PET) of the 5-HT_{2A}R is feasible to study the serotonergic component of vulnerability to schizophrenia and localized reduced 5-HT_{2A}R binding in the prefrontal cortex of six prodromal individuals (Hurlemann et al. 2005a). The question raises whether these abnormalities in serotonergic function are static or progressive, a distinction critical to developing a dynamic model of the pathophysiology of schizophrenia (Keshavan et al. 2005; Weinberger and McClure 2002) and a phase-specific treatment protecting the vulnerable individual from the emergence and/or progression of the illness.

As the ARMS can be considered as a continuum of progressive accrual of morbidity that culminates in first-episode psychosis (Keshavan et al. 2005), we hypothesized that decreases in 5-HT_{2A}R binding would extend gradually, as prodromal symptoms and behaviors accumulate and the level of risk increases. To test this hypothesis within a cross-sectional design, we used [^{18}F]altanserin PET and a bolus-infusion paradigm to investigate 14 never-medicated individuals suspected to be in early (EPS) or late prodromal states (LPS) relative to 21 controls. Five relevant HTR2A single nucleotide polymorphism (SNP) markers were genotyped in all subjects to control for a potential bias in 5-HT_{2A}R binding due to between-group differences in genotype distributions.

Materials and methods

Participants

The present work was conducted as part of the Early Detection and Intervention Programme of the German Research Network on Schizophrenia (GRNS; Hafner et al. 2004). Subjects with symptoms suggestive of either EPS or LPS were recruited as previously described (Hafner et al. 2004). In brief, subjects were screened by general practitioners, counseling services, or secondary health care providers using the 17-item Early Recognition Inventory/Interview for the Retrospective Assessment of the Onset of Schizophrenia (ERIraos) checklist, which was created for this purpose (Hafner et al. 2004). Subjects scoring ≥6 points were referred to the early recognition and intervention center established at the Department of Psychiatry, University of Bonn for detailed assessment with the 110-item ERIraos symptom list (Hafner et al. 2004), which includes items derived from the Bonn Scale for the Assessment of Basic Symptoms (Gross and Huber 1985) and from the Interview for the Retrospective Assessment of the Onset of Schizophrenia (Hafner et al. 1992). EPS diagnostic criteria were based either on the presence of basic symptoms with a positive predictive value of >0.7 and a specificity of >0.85 for the transition to first-episode psychosis (Klosterkotter et al. 2001) and/or a reduction of the global assessment of functioning in conjunction with the presence of a first-degree relative with a psychotic disorder or a history of obstetric complications. While other studies also label individuals to be clinically at ultra-high-risk if they have a genetic risk plus functional decline (McGorry et al. 2002), the additional use of basic symptoms allows prediction of transition to first-episode psychosis already in an earlier

prodromal state (Hafner et al. 2004). In contrast, subjects experiencing attenuated positive symptoms or brief intermittent psychotic symptoms were considered to be in the LPS, which is consistent with conventional ultra-high-risk criteria used in clinical studies (McGorry et al. 2002). A detailed synopsis of inclusion and exclusion criteria is presented in Table 1.

Based on these criteria, we recruited 14 never-medicated ultra-high-risk subjects (four women, eight men; mean age 25.8±3.5 years; age range 22.1–36.2 years), of whom, six subjects (43%; two women, four men) were assigned to the EPS group and eight subjects (57%; two women, six men) to the LPS group. There was no overlap with the at-risk sample (n=6) previously studied with [^{18}F]altanserin PET and a less sophisticated bolus-injection protocol (Hurlemann et al. 2005a). The mean duration of clinical follow-up in the present study was 18±3 months. During this period, five LPS subjects (62.5%), but no EPS subjects, went on to develop schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) diagnostic criteria. This conversion rate is in concordance with the predictive validity of EPS and LPS diagnostic criteria reported in prospective studies. According to these studies, EPS criteria predict psychosis onset within 12 months in 19% of cases and within 64 months in 70% of cases (Klosterkotter et al. 2001), whereas LPS criteria predict psychosis onset within 12 months in 30–54% of cases (Yung et al. 2003).

The healthy control (CTRL) group comprised 21 subjects (8 women, 13 men; mean age 26.8±3.7 years; age range 21.1–33.3 years) carefully matched for age and

Table 1 GRNS diagnostic criteria for early and late prodromal states (Hafner et al. 2004)

Criteria	Parameters
Early prodromal state (EPS)	
One or more of the following basic symptoms appeared in the last 3 months, several times a week	Thought interference *or* thought perseveration *or* thought pressure *or* thought blockage
	Disturbances of receptive language, either heard or read
	Decreased ability to discriminate between ideas and perception, fantasy and true memories
	Unstable ideas of reference (subject-centrism)
	Derealization
	Visual *or* acoustic perception disturbances and/or
Reduction in the global assessment of function score (DSM IV) of at least 30 points within the last year *and* at least one of the following risk factors	First-degree relative with lifetime schizophrenia or schizophrenia-spectrum disorder
	Pre- or perinatal complications
Late prodromal state (LPS)	
Presence of at least one of the following attenuated positive symptoms (APS) within the last 3 months, appearing several times per week for a period of at least 1 week	Ideas of reference
	Odd beliefs or magical thinking
	Unusual perceptual experience
	Odd thinking or speech
	Suspiciousness or paranoid ideation and/or
Brief limited intermitted psychotic symptoms (BLIPS), defined as appearance of one of the following symptoms for less than 1 week (interval between episodes at least 1 week), resolving spontaneously	Hallucinations
	Delusions
	Formal thought disorder
	Gross disorganized or catatonic behavior
Exclusion criteria	
	APS or BLIPS (early prodromal state)
	Present or past diagnosis of schizophrenic, schizophreniform, schizoaffective, delusional or bipolar according to DSM IV
	Present or past diagnosis of brief psychotic disorder according to DSM IV with a duration equal to or more than 1 week or within the last 4 weeks regardless of its duration
	Diagnosis of delirium, dementia, amnestic or other cognitive disorder, mental retardation, psychiatric disorders due to somatic factors or related to psychotropic substances according to DSM IV
	Alcohol or drug abuse within the last three months before inclusion according to DSM IV
	Disease of the central nervous system (inflammatory, traumatic, epileptic etc.)
	above 38 years of age

education and determined to be free of personal as well as family history of DSM-IV axis I and II disorder. Subjects were recruited through local advertisement and excluded from participation if they had any current or previous substance or alcohol abuse or a history of neurological and/or severe somatic disorder. Previous exposure to psychoactive medication was a strict exclusion factor for participation in the present study. The demographical and clinical assessment data are listed in Table 2.

Five relevant HTR2A SNP markers (5-HTR2A-rs6311C/T, 6313C/T, 6314C/T, 1928040C/T, and 7997012A/G) were genotyped in 32 subjects to account for the possibility that between-group differences in 5-HT_{2A}R binding could be due to a confounding bias in genotype distributions. These SNP markers sample the majority of the common variation in HTR2A and have been studied in pharmacogenetic contexts (Arranz et al. 1995, 2000; Erdmann et al. 1996; McMahon et al. 2006). HTR2A consists of three exons and two introns which span ≈63 Kb. Rs6313 resides in exon 1 and rs6314 in exon 3, whereas rs7997012 and rs1928040 reside in intron 2; rs6311 is located in the 5′ untranslated region (NCBI SNP database). Concordance with Hardy–Weinberg equilibrium was tested for all SNP markers using a group (present sample, reference population) × genotype (homozygote A, heterozygote, homozygote B) 2×3 Pearson chi-square test. The association of genotype and assignment to either the CTRL or ARMS sample was tested using a group (CTRL, ARMS) × genotype 2×3 Pearson chi-square test.

Table 2 Clinical characteristics, basic PET parameters, and binding potential (BP_1') values

	CTRL		EPS		LPS	
Clinical characteristics and basic PET parameters						
N	21		6		8	
Female sex	8		2		2	
Age (years)	26.8	(3.7)	25.8	(2.5)	27.5	(4.4)
Injected dose (MBq)	230	(11)	231	(12)	228	(12)
Specific activity (GBq/μmol)	177	(104)	213	(121)	150	(88)
Plasma activity (KBq/mL)	1.32	(0.3)	1.32	(0.4)	1.32	(0.2)
PANSS positive			8.7	(2.0)	14.1**	(5.3)
PANSS negative			12.8	(5.2)	13.9	(4.8)
PANSS total			36.2	(8.5)	37.3	(4.7)
MADRS			22.5	(4.8)	21.8	(5.7)
GAF			53.3	(3.2)	54.0	(8.8)
Binding potential (BP_1') values						
Neocortex (global)	2.31	(0.4)	2.48	(0.4)	2.22	(0.4)
Left orbital prefrontal cortex	2.61	(0.5)	2.91	(0.7)	2.72	(0.5)
Right orbital prefrontal cortex	2.50	(0.4)	2.88	(0.7)	2.68	(0.5)
Left dorsolateral prefrontal cortex	2.61	(0.4)	2.57	(0.6)	2.42	(0.4)
Right dorsolateral prefrontal cortex	2.70	(0.5)	2.64	(0.4)	2.40*	(0.4)
Left anterior cingulate cortex	2.30	(0.5)	2.25	(0.4)	2.09	(0.4)
Right anterior cingulate cortex	2.25	(0.4)	2.37	(0.5)	2.08	(0.3)
Left posterior insular cortex	1.91	(0.5)	1.93	(0.5)	1.76	(0.3)
Right posterior insular cortex	1.88	(0.4)	1.55*	(0.4)	1.63*	(0.3)
Left temporopolar cortex	2.09	(0.4)	2.20	(0.4)	2.03	(0.3)
Right temporopolar cortex	2.01	(0.4)	2.01	(0.4)	2.06	(0.2)
Sub- and archicortex (global)	0.49	(0.1)	0.42	(0.1)	0.37*	(0.1)
Left amygdala	0.51	(0.2)	0.33*	(0.3)	0.32*	(0.2)
Right amygdala	0.45	(0.2)	0.30	(0.4)	0.38	(0.3)
Left hippocampus	0.61	(0.2)	0.62	(0.3)	0.35**	(0.2)
Right hippocampus	0.65	(0.2)	0.52	(0.4)	0.45*	(0.3)
Left caudate nucleus	0.43	(0.2)	0.37	(0.1)	0.41	(0.2)
Right caudate nucleus	0.42	(0.2)	0.45	(0.2)	0.22**	(0.2)
Left putamen	0.62	(0.3)	0.45	(0.3)	0.41*	(0.2)
Right putamen	0.56	(0.3)	0.42	(0.1)	0.49	(0.2)

Data are given as means (±SD).
CTRL Control sample, *EPS* ultra-high-risk subjects suspected to be in the early prodromal state, *LPS* ultra-high-risk subjects suspected to be in the late prodromal state, *Plasma activity* concentration attributable to parent compound at equilibrium, *PANSS* positive and negative symptoms scale, *MADRS* Montgomery–Asperg depressive rating scale, *GAF* global assessment of functioning
*Significant $0.01<p<0.05$
**Significant $p<0.01$

Written informed consent was obtained from all participants before study, and study protocols were approved by the ethics committees of the Medical Faculties of the Universities of Bonn and Duesseldorf, the Federal Office for Radiation Protection (BfS), and the Federal Institute for Drugs and Medical Devices (BfArM), Germany.

Image acquisition

T1-weighted cranial magnetic resonance images (MR) images (MP-RAGE) were obtained on a Siemens Sonata 1.5T scanner. Radiosynthesis of [^{18}F]altanserin was performed at the Institute of Nuclear Chemistry, Research Center Juelich, with a radiochemical purity of >99% (Hamacher and Coenen 2006). [^{18}F]Altanserin was infused as a 2-min bolus followed by continuous infusion with a bolus/infusion ratio of K_{bol}=2.1 h. An overview of injected doses, specific radioactivities and plasma radioactivities is presented in Table 2. PET measurements were performed in 3D mode on a Siemens ECAT EXACT HR+ scanner (Siemens-CTI, Knoxville, TN, USA). Scatter from outside the field of view was reduced by a lead ring insert. A 10-min transmission scan (with three ^{68}Ge/^{68}Ga line sources) was obtained for attenuation correction. Dynamic emission data were collected in six frames of 10-min length starting from 120 min after the start of [^{18}F]altanserin application (zero). PET data were corrected for randoms, scatters and attenuation, Fourier rebinned into 2D sinograms, reconstructed by filtered backprojection (Shepp filter, 2.5-mm width) with a voxel size of 2×2×2.43 mm^3 [63 slices; full width of half maximum 5.8, 5.8, 6.6 mm (x, y, z) at 10 cm from the central axis], and decay-corrected. Venous blood samples were taken at 2, 5, 10, 20, 30, 45, 60, 120, 130, 140, 150, 160, 170, and 180 min p.i. The fraction of radioactive parent compound in plasma was determined by selective liquid–liquid extraction with quantitation of the recovery of total radioactivity, followed by thin-layer chromatography (Matusch et al. 2007).

Image processing

Dynamic PET data obtained in ECAT7 format were converted to “analyze” format using PMOD v.2.75 (PMOD Group, Zurich, Switzerland). Frames were realigned using SPM2 (Wellcome Trust Centre for Neuroimaging, University College London, London, UK) to correct for head movements during the scan. MR images were oriented along the anterior commissure–posterior commissure (AC-PC) line. The realigned frames were co-registered to the individual MR images using SPM2. The cerebellar region was manually delineated on the individual MR images using PMOD v.2.75. Based on the cerebellar radioactivity concentration ($C_{Reference}$) and the plasma radioactivity concentration attributable to parent compound (C_{PPC}), PET data sets containing voxel-wise radioactivity concentrations C_{Voxel} obtained from PMOD v.2.75 were parametrized according to Eq. 1 (Pinborg et al. 2003), with all variables C averaged from 120 to 180 min p.i.

$$BP_1' = (C_{Voxel} - C_{Reference})/C_{PPC} \quad (1)$$

Average images were corrected for partial-volume effects according to the algorithm of Muller-Gartner et al. (1992) using its voxel-wise application in PMOD v.2.75 (gray and white matter cutoff 0.5; start regression 0.95). Gray and white matter masks were generated using SPM2 segmentation. Missegmented voxels were manually removed using PMOD v.2.75. Then, the partial-volume corrected images were parametrized according to Eq. 1.

Statistical parametric mapping

SPM2 was employed to compare 5-HT$_{2A}$R binding (BP_1') between groups. Individual partial-volume corrected and uncorrected parametric maps of BP_1' were spatially normalized to the Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) 152 T1 template as supplied with SPM2, with individual 2-mm^3 voxel size MRI as source images. Normalized images were smoothed with a 10×10×10 mm^3 Gaussian kernel. Voxel-wise group comparisons were performed by a two-sample t test (without threshold masking, without sphericity correction, and with the brain extracted from the T1 template, cutoff 0.1, as explicit mask). Given previous observations of reduced 5-HT$_{2A}$R BP_1' in the prefrontal cortex of both at-risk individuals (Hurlemann et al. 2005a) and first-episode patients at onset of schizophrenia (Ngan et al. 2000), SPM analysis was led by an anatomically defined a priori hypothesis, and the level of significance was thresholded at $p<0.001$ uncorrected. Clusters of significant voxels ($k>20$) were rendered to the generic T1 ‘Collin’-brain for visual localization.

Confirmatory VOI method analysis

To confirm SPM results obtained after spatial transformation into the MNI/ICBM 152 space, we performed an additional analysis based on volumes of interest (VOIs) defined a posteriori in individual space. First, accuracy of spatial transformation was verified by exporting binary 3D data sets of significant voxels from the SPM and reconverting into individual space. Then, VOIs were delineated by a blinded observer upon macroanatomical criteria within the co-registered individual MR images using PMOD v.2.75 and covered candidate regions identified by SPM analysis. These individual VOI maps were applied to the BP_1' images

to obtain regional BP_1' values. Statistical analyses were performed using SPSS v.14 (SPSS, Chicago, IL, USA). The influence of age and gender was studied by calculating a univariate analysis of covariance. In analogy to SPM, a series of between-group comparisons were performed using two-sample *t* tests. To account for an inflation of the type I error rate due to multiple testing, the threshold *p* for significance was Bonferroni/Holmes-adjusted. A two-tailed bivariate Spearman (non-parametric) correlation coefficient was calculated for each VOI and the global neocortical BP_1' against diagnostic variables (PANSS, MADRS, GAF) and HTR2A SNP markers.

HTR2A SNP genotyping

Five HTR2A SNP markers were genotyped in all subjects to exclude an imbalance in genotype distributions as a potential cause of between-group differences in BP_1' values. BP_1' values of A- and G-allele (C- and T-allele) carriers were compared with mean BP_1' values of heterozygote subjects. For this purpose, BP_1' values derived from 20 cortical and subcortical VOIs (Table 2) were entered into analysis. A region-wise multivariate 3×3 analysis of variance was performed to test for a differential association of regional BP_1', phenotype (CTRL, EPS, LPS) and genotype.

Analysis of gray matter segments

To estimate the potential influence of regional under- or overcorrection of partial-volume effects, individual gray matter segments underwent the same SPM and VOI method analyses as partial-volume corrected PET data. This additional analysis allowed us to determine the relative contribution of gray matter atrophy to decreases in BP_1' within corresponding regions.

Results

Group characteristics

Groups did not differ with respect to age, education, and gender (*p* values >0.05). As expected, LPS subjects had higher PANSS positive scores compared to EPS subjects (+63%; $p=0.035$). We found no relevant correlations between a subset of diagnostic variables (PANSS, MADRS, GAF) and regional BP_1' values. The five SNP markers tested were concordant with Hardy–Weinberg equilibrium. Genotype distributions and allele frequencies did not differ between groups ($\chi^2<3.1$, $p>0.2$). We did not detect any influence of HTR2A SNP markers on regional BP_1' values across the entire sample ($n=32$) nor within any diagnostic group.

Group comparisons

Averaged parametric BP_1' maps depicted in Fig. 1 demonstrate subtle reductions of 5-HT_{2A}R BP_1' in the EPS group and marked reductions of 5-HT_{2A}R BP_1' in the LPS group. Age and gender as covariates had no influence on regional BP_1'. SPM between-group comparisons revealed BP_1' decreases ($p<0.001$) in right orbital prefrontal cortex and left anterior cingulate cortex of EPS subjects relative to controls (Fig. 2). BP_1' decreases in LPS subjects relative to controls further encompassed left dorsolateral prefrontal cortex, right anterior cingulate cortex, right posterior insula, left temporal pole, left amygdala, and left hippocampus (Fig. 2). With exception of the orbital prefrontal cortex, anterior cingulate cortex, and temporal pole, the above-reported decreases were confirmed by the VOI method analysis (Table 2). Separate SPM and VOI method analyses for subjects who did ($n=5$) and did not ($n=9$) subsequently convert to first-episode psychosis relative to controls both identified a BP_1' decrease in right caudate nucleus. Changes in right caudate BP_1' relative to controls were quantified as follows: EPS subjects, +7.1% (n.s.); LPS subjects, −48.5% ($t=-2.68$, $df=27$ $p=0.012$); converters, −62.2% ($t=-2.79$, $df=24$, $p=0.010$). Furthermore, we observed a 7.5%

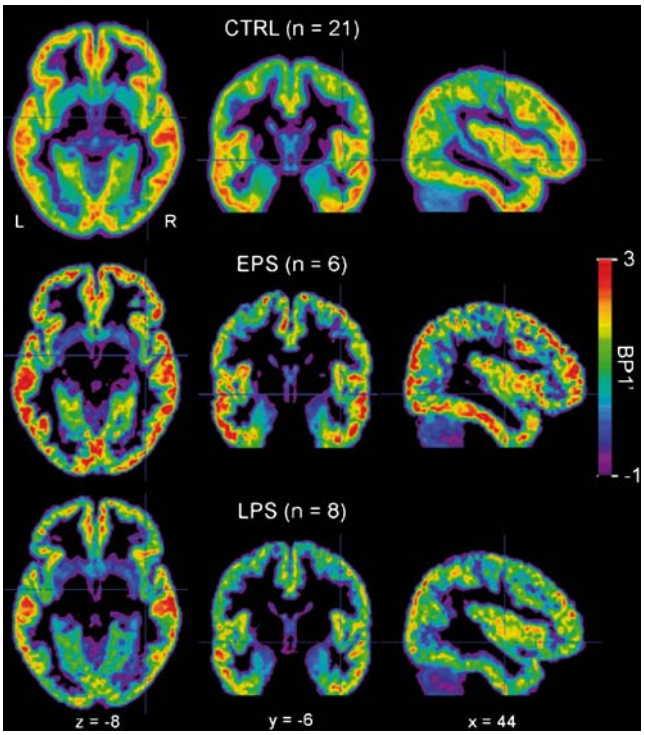

Fig. 1 Averaged parametric BP_1' images from 21 controls (CTRL), six EPS subjects, and eight LPS subjects. Presented are sections crossing the center of the cluster of highest significance within the right posterior insula at the MNI coordinates indicated. *BP_1'* Binding potential, *EPS* ultra-high-risk subjects suspected to be in the early prodromal state, *LPS* ultra-high-risk subjects suspected to be in the late prodromal state, *MNI* Montral Neurological Institute

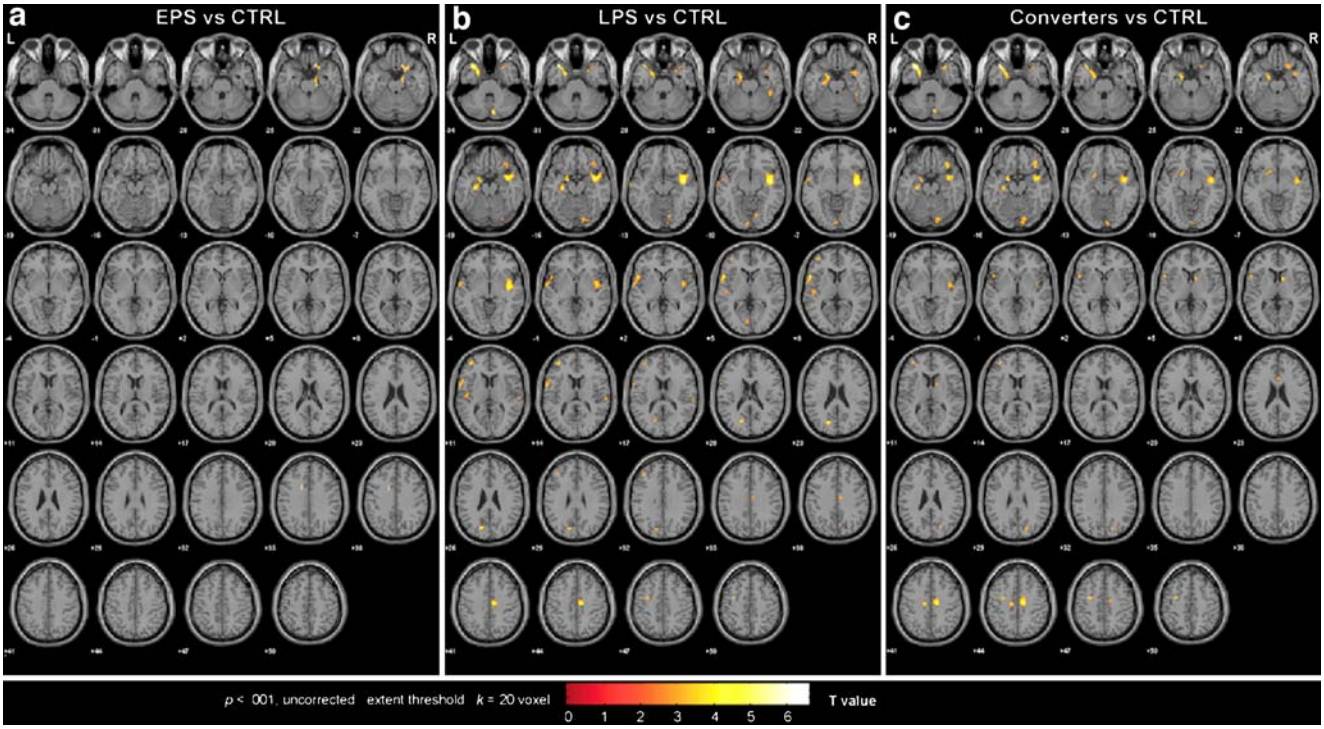

Fig. 2 SPM *T* maps (thresholded at $p=0.001$) displaying the results of a comparison of parametric BP_1' images from six EPS subjects (**a**), eight LPS subjects (**b**), and five converters (**c**) relative to 21 controls (CTRL). Our findings implicate progressive decreases in 5-HT_{2A}R density as a surrogate biological measure of increased risk for schizophrenia, irrespective of conversion. *EPS* Ultra-high-risk subjects suspected to be in the early prodromal state, *LPS* ultra-high-risk subjects suspected to be in the late prodromal state, *SPM* statistical parametric mapping

increase of global neocortical BP_1' in the EPS group and a 3.6% decrease in global neocortical BP_1' in the LPS group; however, none of these changes reached statistical significance. EPS-vs-LPS between-group comparisons yielded no significant differences on the voxel level; however, the VOI-based analysis identified a −51.9% decrease in right caudate BP_1' of LPS subjects compared to EPS subjects ($t=-2.67$, $df=12$, $p=0.021$). Moreover, converters had a −60.7% lower BP_1' in right caudate nucleus than non-converters ($t=-2.74$, $df=12$, $p=0.018$). The SPM of partial-volume corrected (Figs. 2 and 3) and uncorrected data sets yielded similar results, with the former reaching higher levels of significance.

Contribution of gray matter atrophy to group differences

Analysis of PET data with and without partial-volume correction in conjunction with the fraction of gray matter (f_{GM}) within each VOI allowed us to estimate the region-wise contribution of atrophy effects. While f_{GM} reductions were symmetrically distributed within both hemispheres, reductions of PET signal were located almost unilaterally and non-symmetrically. Generally, reductions of f_{GM} in EPS and LPS groups relative to the CTRL group did not exceed 10% (orbital prefrontal cortex). No f_{GM} reductions were observed in amygdala, hippocampus, and caudate nucleus, thus, excluding a bias of gray matter atrophy in these regions. In all other VOIs, relative decreases in uncorrected BP_1' exceeded relative decreases in f_{GM} by a factor of 3–5. The algorithm for partial-volume correction as used in the present study tended to overcorrect lateral frontotemporal regions. Thus, the observed decreases in lateral frontotemporal 5-HT_{2A}R BP_1' are likely to be even underestimated.

Discussion

First, consistent with our a priori hypothesis, [^{18}F]altanserin PET revealed regional decreases in 5-HT_{2A}R BP_1' that predate the transition to schizophrenia and disseminate across frontotemporal gray matter as a function of increasing levels of risk. Second, compared to controls, additional decreases in caudate 5-HT_{2A}R BP_1' were present in converters to first-episode psychosis, but absent in non-converters. Together, these findings identify a serotonergic component of vulnerability to schizophrenia.

Decreases in 5-HT_{2A}R density as suggested by decreased BP_1' could, in principle, reflect reduced synapto- and/or neurogenesis, accelerated cell loss (apoptosis), dendritic/axonal pruning/de-arborization (Keshavan et al.

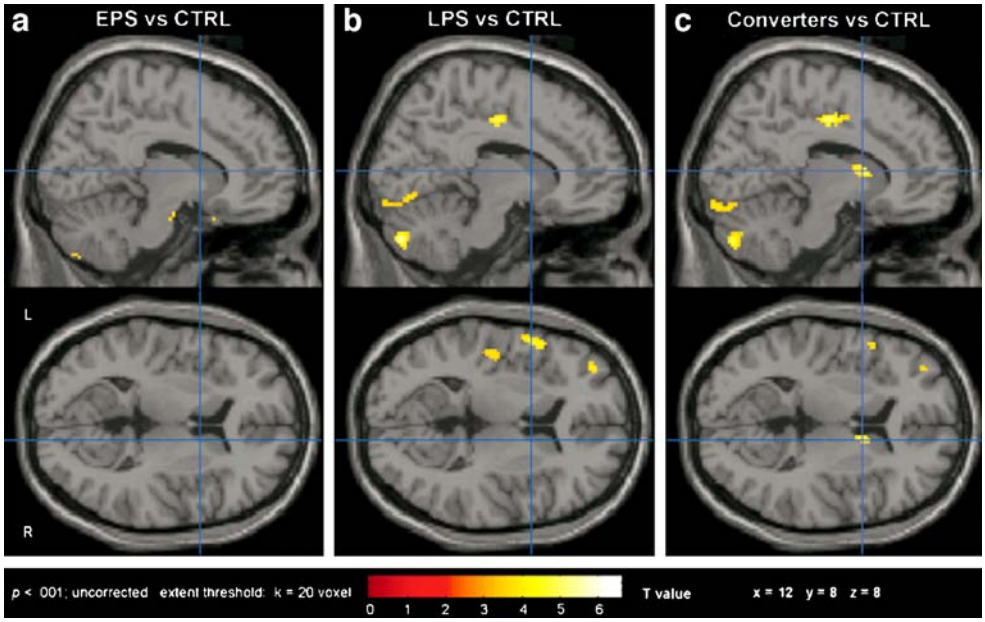

Fig. 3 SPM *T* maps (thresholded at $p=0.001$) displaying the results of a comparison of parametric BP_1' images from six EPS subjects (**a**), eight LPS subjects (**b**), and five converters (**c**) relative to 21 controls (CTRL). Presented are sections crossing the center of the cluster of highest significance within the right caudate nucleus at the MNI coordinates indicated. A reduction of caudate BP_1' in converters might serve as a predictor of imminent transition to first-episode schizophrenia. *BP_1'*, Binding potential, *EPS* ultra-high-risk subjects suspected to be in the early prodromal state, *LPS* ultra-high-risk subjects suspected to be in the late prodromal state, *MNI* Montral Neurological Institute, *SPM* statistical parametric mapping

2005), or 5-HT_{2A}R down-regulation/internalization in response to altered serotonergic signaling during the premorbid and/or peri-onset phase of schizophrenia. Substantial evidence for a degenerative pathology comes from longitudinal morphometric studies which revealed progressive frontotemporal gray matter decreases in ultra-high-risk subjects whose illness proceeded from the ARMS to first-episode psychosis (Pantelis et al. 2003). Moreover, there is accumulating evidence for continued and interrelated changes in brain structural and functional indices after onset of schizophrenia (Salisbury et al. 2007). In light of this evidence, dynamic models of the pathophysiology of schizophrenia have been conceptualized wherein an early (pre- or perinatal) static lesion is posited to synergistically interact with some form of later progressive gray matter lesion to cause the illness (Pantelis et al. 2003; Salisbury et al. 2007). The underlying biological mechanisms are unclear. However, postmortem histological findings suggest that regional volumetric changes in schizophrenia do not necessarily result from decreases in total cell number (Heckers and Konradi 2002; Hurlemann et al. 2005b; Walker et al. 2002) but could reflect a dendritic/synaptic pathology (Feinberg 1983; McGlashan and Hoffman 2000). While our findings of reduced 5-HT_{2A}R BP_1' would be compatible with a dendritic/synaptic degeneration in the schizophrenia prodrome, a progressive decline in 5-HT_{2A}R BP_1' within the same individuals over time remains to be documented by longitudinal scan/re-scan data.

The question emerges whether the present decreases in 5-HT_{2A}R BP_1' could be primarily driven by gray matter atrophy. Indeed, cross-sectional morphometric studies have demonstrated that compared to ultra-high-risk subjects who did not develop psychosis, those who did develop psychosis had less gray matter in insular cortex (Borgwardt et al. 2007) where we observed the most significant decreases in 5-HT_{2A}R BP_1'. In contrast, reductions of 5-HT_{2A}R BP_1' also occurred in the amygdala, which seems to be unaffected by volumetric changes in the ARMS (Velakoulis et al. 2006). Analysis of regional 5-HT_{2A}R BP_1' (with and without correction for partial-volume effects) in conjunction with the corresponding gray matter fractions revealed that the present decreases in 5-HT_{2A}R BP_1'' either exceed gray matter atrophy or manifest in regions devoid of gray matter atrophy. Reductions of 5-HT_{2A}R density in the ARMS thus appear to result from a primary illness process rather than representing an epiphenomenon due to gray matter atrophy in cortical (Honea et al. 2005; Kasai et al. 2003) or subcortical (Kreczmanski et al. 2007) brain regions.

Our results add to pharmacological (Gonzalez-Maeso et al. 2007; Meltzer et al. 1989, 2003) and genetic (Arranz et al. 1995, 2000; Norton and Owen 2005) evidence implicating the 5-HT_{2A}R in the pathophysiology of schizophrenia. The observed decreases in frontotemporal 5-HT_{2A}R BP_1' accord with the majority of postmortem autoradiography studies, which demonstrated reduced 5-

$HT_{2A}R$ binding in the frontotemporal cortex of schizophrenic patients (Dean 2003; Matsumoto et al. 2005), a finding consistent with lower HTR2A expression evidenced by postmortem mRNA hybridization assays (Lopez-Figueroa et al. 2004; Norton and Owen 2005). However, potential confounds of aging, illness chronicity, and antipsychotic drug action limit the extrapolation of these postmortem findings towards the pathophysiology of schizophrenia. By focusing on a sample of never-medicated individuals at ultra-high-risk for schizophrenia, the present study avoids such confounds. Our findings obtained with [^{18}F]altanserin complement evidence of reduced [^{18}F]setoperone binding in the prefrontal cortex of drug-naïve patients at onset of schizophrenia (Ngan et al. 2000), but conflict with reports of unchanged [^{18}F]setoperone and [^{18}F]spiperone binding in mixed samples of drug-free and drug-naïve schizophrenic patients (Lewis et al. 1999; Okubo et al. 2000; Trichard et al. 1998; Verhoeff et al. 2000). However, as compared to the combined 5-$HT_{2A}R$/DA D_2R ligands [^{18}F]setoperone and [^{18}F]spiperone, [^{18}F]altanserin shows a higher selectivity for 5-$HT_{2A}R$ and is sensitive towards even subtle reductions of 5-$HT_{2A}R$ density in subcortical areas rich in DA D_2Rs. We interpret our observation of decreases in caudate 5-$HT_{2A}R$ BP_1′—with maximum reductions in converters—as providing preliminary evidence of a neurochemical indicator of illness activity that might help to predict transition to first-episode psychosis. In this context, we note that up-regulation of caudate DA D_2Rs determined with [^{11}C]raclopride PET has been associated with enhanced genetic risk for schizophrenia (Hirvonen et al. 2005). Together, these data implicate subcortical monoaminergic dysregulation as an important vulnerability marker of psychosis.

Interestingly, inter-individual variation in 5-$HT_{2A}R$ BP_1′ is less in sub- and archicortical regions than in neocortical regions, a finding consistent with previous bolus/infusion [^{18}F]altanserin PET studies involving a total of 84 healthy controls aged between 18 and 74 years (Adams et al. 2004; Haugbol et al. 2007). Based on these studies, a minimum sample size of 27 has been suggested to detect a 20% difference in regional 5-$HT_{2A}R$ density (Haugbol et al. 2007). A relative lack of power in our study is compensated by a focus on young individuals aged between 21 and 36 years, a twice-as-high spatial resolution of the applied PET camera, and a restrictive threshold for regional between-group differences that exceeds 20%.

With respect to the functional significance of regional deficits in 5-$HT_{2A}R$ density, it should be noted that single-unit recordings in nonhuman primates indicate a modulatory role of prefrontal 5-$HT_{2A}R$ in the working memory domain (Williams et al. 2002), dysfunction of which is considered as a cognitive endophenotype for schizophrenia (Glahn et al. 2003). In addition, pharmacological challenges in rodents provide evidence for a direct influence of prefrontal and hippocampal 5-$HT_{2A}R$ density on performance in associative learning tasks, which is impaired in schizophrenic patients (Harvey 2003; Romano et al. 2006). Moreover, local reductions of 5-$HT_{2A}R$ density may contribute to abnormal insular cortex activity in schizophrenic patients, which has been related to the formation of hallucinations (Nagai et al. 2007). This finding is consistent with the putative role of the insular cortex—and the paralimbic belt—as a locus of multimodal convergence, serving to integrate extrapersonal gustatory, olfactory, auditory, visual, and somatosensory stimuli into the intrapersonal milieu (Mesulam and Mufson 1985). However, decreases in insular 5-$HT_{2A}R$ density, as detected in the present study, are not specific to the pathophysiology of schizophrenia, but are also implicated in unipolar depression (Biver et al. 1997). [^{18}F]Fluorodesoxyglucose PET in depressed patients indicates that insular cortex metabolism normalizes after treatment with selective 5-HT reuptake inhibitors (SSRIs; Kennedy et al. 2001; Mayberg et al. 1999). These findings are compatible with a hypothesized role of the insular cortex (Damasio et al. 2000) and the 5-$HT_{2A}R$ (Weisstaub et al. 2006) in generating fear. [^{11}C]Flumazenil PET in panic disorder patients identified the paralimbic belt as the region of largest decrease in $GABA_A$-benzodiazepine receptors, suggesting that disinhibition of this circuitry is involved in mediating pathological fear states (Malizia et al. 1998). Together, these findings imply that consistent with an overlap of psychopathology and functional deficits at initial stages, schizophrenia and affective spectrum disorders might share a common serotonergic deficit in paralimbic regions.

In portraying the ARMS as a period of progressive serotonergic dysregulation, our results contribute to the biological validation of a diagnostic distinction between early and late prodromal states (Hafner et al. 2004) and to the clarification of neurochemical changes associated with the transition to first-episode psychosis. Such clarification is critical for psychopharmacological efforts to forestall illness progression and prevent morbidity from increasing. As delayed treatment entails unnecessary suffering and correlates with an unfavorable prognosis (Norman and Malla 2001; Keshavan et al. 2003; Wyatt 1991), the schizophrenia prodrome has become a prime target for the conceptualization of preventive psychopharmacological strategies (Cornblatt et al. 2002; McGorry et al. 2002; Woods et al. 2003). Our findings substantiate the rationale for establishing a phase-specific psychopharmacological intervention that includes the serotonergic component of vulnerability to schizophrenia.

In summary, our cross-sectional data implicate decreases in cortical 5-$HT_{2A}R$ density as a surrogate biological measure of increased risk for schizophrenia, irrespective of conversion. Progressive reductions of subcortical 5-

$HT_{2A}R$ density could provide an indicator of illness activity and help to predict imminent conversion to schizophrenia.

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