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ARTICLE

Vulnerability to psychotogenic effects of ketamine is associated with elevated $D_{2/3}$-receptor availability

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Abstract

Previous positron emission tomography (PET) studies employing competition paradigms have shown either no change or substantial declines in striatal [^{11}C]-raclopride binding after challenge with psychotogenic doses of the *N*-methyl-D-aspartate antagonist ketamine. We sought to probe the relationship between the severity of ketamine-induced psychotic symptoms and altered dopamine $D_{2/3}$ receptor availability throughout brain using the high affinity ligand [^{18}F]-fallypride (FP). PET recordings were obtained in a group of 10 healthy, young male volunteers, in a placebo condition, and in the course of an infusion with ketamine at a psychotomimetic dose. Administration of the Positive and Negative Syndrome Scale and the Thought and Language Index in both conditions revealed a substantial emergence of mainly negative symptoms of schizophrenia, persisting until the end of the 3 h PET recordings. The baseline FP binding in cortex, caudate nucleus and other brain regions was highly predictive of the individual severity of psychotic symptoms in the ketamine condition. However, there was no evidence of ketamine-evoked reductions in FP binding. In the context of earlier findings, we speculate that high baseline $D_{2/3}$-receptor availability may impart benefits with regard to cognitive flexibility, but increases the risk of maladaptive information processing in the face of environmental stresses and challenges.

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Introduction

Non-competitive antagonists of the brain *N*-methyl-D-aspartate (NMDA) receptor such as phencyclidine (PCP), MK-801 or ketamine (Ket) at sub-anaesthetic doses have frequently been used to establish behavioural models of schizophrenia; these drugs cause paranoid symptoms as well as schizophrenia-like negative symptoms, thought disorders and disorganization in healthy volunteers (Javitt & Zukin, 1991). In patients suffering from schizophrenia, acute challenge with NMDA antagonists can exacerbate a large proportion of the patient's individual symptom cluster (Malhotra *et al.* 1997). These observations led to the proposal that NMDA-antagonism transiently induces a model of psychosis possessing both face and also construct validity. Furthermore, NMDA-antagonists have also been used to provoke schizophrenia-like behavioural disturbances in animals. For example, PCP administration perturbs the pre-pulse inhibition of the startle reflex in the macaque monkey (Javitt & Lindsley, 2001) in a manner similar to the trait perturbation seen in patients suffering from schizophrenia (Perry & Braff, 1994). In general, behavioural effects of NMDA-antagonists have lent considerable support to a theory of the pathophysiology of schizophrenia roughly described as the 'glutamate hypothesis', which has come to augment or supplant the earlier dopamine hypothesis.

Nevertheless, behavioural disturbances caused by NMDA-antagonists differ from schizophrenia with regard to their lesser therapeutic response to dopamine D_2 receptor antagonists (Krystal *et al.* 1999). The induction of schizophrenia-like symptoms through NMDA-antagonism might be explicable by several mechanisms, including the disruption of inhibitory signalling, the

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dysregulation of cortico-striatal loops and the pharmacologically induced release of dopamine and other neurotransmitters (for review, see Large, 2007). Treatment with NMDA antagonists increases interstitial dopamine levels in the rat nucleus accumbens (Kato *et al.* 2000), although the associated behavioural changes were not resolved by treatment with either D_2- or 5-HT_2-antagonists. Thus, the relationship between dopaminergic and glutamatergic mechanisms and their contribution to psychotic symptoms remains uncertain.

NMDA receptor mediated alterations in brain dopamine transmission have been investigated in a number of molecular imaging studies with positron emission tomography (PET). The reported effects of NMDA-antagonists on presynaptic dopamine function and dopamine release in a living human brain are highly discrepant (Aalto *et al.* 2002; Deep *et al.* 1999; Kegeles *et al.* 2000, 2002; Smith *et al.* 1998; Tsukada *et al.* 2005; Vollenweider *et al.* 2000). These discrepant results may reflect individual differences in the modulation of dopamine transmission by NMDA, as suggested by Vollenweider *et al*. (2000), who reported a positive correlation between the magnitude of Ket-induced [^{11}C]raclopride binding changes (depicting increased dopamine release) and the individual increase in psychopathology scores.

In the present study, we explore in more detail the relationship between baseline and Ket-evoked changes in dopamine $D_{2/3}$ receptor availability and the associated psychopathological disruption evoked by sub-anaesthetic psychotomimetic Ket doses in healthy young volunteers, employing a single-blind design with either placebo (Pbo) or Ket treatment. Whereas several PET studies have investigated effects of Ket on striatal binding of the dopamine $D_{2/3}$ receptor ligand [^{11}C]raclopride, we used the high-affinity dopamine $D_{2/3}$ ligand [^{18}F]fallypride (FP), so as to quantify receptor availability not just in striatum, but also in extrastriatal regions (Vernaleken *et al.* 2011). Extrastriatal regions might be of particular interest given that Moghaddam and colleagues found that significantly higher changes in interstitial dopamine concentrations were evoked by PCP in the prefrontal cortex than in the nucleus accumbens (Adams & Moghaddam, 1998). Furthermore, we assessed the behavioural and cognitive disruption evoked in our subjects by Ket, not only with standard instruments covering psychotic symptom clusters but also by specifically targeting the characteristic thought and speech disturbances.

Method

This investigation was approved by the local ethics committee of the Medical Faculty of the RWTH Aachen University (Aachen, Germany) as well as by the Federal Institute for Pharmaceuticals and Medical Products (BfArM, Bonn), and the German national radiation safety authorities. All subjects gave written informed consent. All PET investigations were performed at the PET Centre of the RWTH Aachen University, Germany.

Subjects

We included a group of 10 healthy male subjects (20–30 yr; mean $\pm$ S.D. 24.4 $\pm$ 3.9). All subjects were native German-speaking persons devoid of any evident speech pathology, as required for performance of the language tests. They underwent physical and mental-state examinations, including a structured diagnostic interview (Structured Clinical Interview for DSM-IV). Furthermore, all subjects underwent electroencephalography and electrocardiography examinations, in order to screen for cerebral and cardiac pathologies. Other exclusion criteria included any evidence of mental disorders or relevant somatic diseases, self-report of illicit drug consumption in the 6 months preceding the PET study or positive urine testing for drugs. In addition, all subjects were free of any centrally acting medication for at least 6 wk and had never been treated with any psychopharmacological agents.

Study schedule

After the screening visit, two FP-scans per subject were scheduled, to be conducted within an interval of 7–21 d (mean $\pm$ S.D. 10.5 $\pm$ 4.8). All pairs of scans were performed at the same time of day. One scan was performed under bolus-constant-infusion conditions (*vide infra*) of Ket and the otherwise identical baseline scan was acquired during Pbo administration. The assignment to the Pbo/Ket scan order was randomized and under single-blind conditions. The Thought and Language Index (TLI; Liddle *et al.* 2002) test was administered after the Ket bolus-infusion and before the FP scans (*vide infra*) and a Positive and Negative Syndrome Scale (PANSS) rating was performed directly after completion of each FP scan.

Radiochemistry and data acquisition

The radiosynthesis of FP was a high-yield modification of the method for the synthesis of [^{18}F]desmethoxyfallypride, as described in detail previously (Gründer *et al.* 2003). Emission images were acquired with a Siemens ECAT EXACT 922/47 whole-body PET scanner (Siemens AG, Germany) in 3D-mode (field-of-view: 16.2 cm; 47 planes; FWHM axial: 4.6 mm, in-plane: 6.0 mm). Dynamic data acquisition consisted of a series of 39 time frames (3×20 s, 3×1 min, 3×2 min, 3×3 min, 21×5 min, 2×8 min, 4×10 min), resulting in a total emission recording period of 180 min. A 15-min transmission scan using a ^{68}Ge source was carried out prior to the Ket/Pbo-infusion for subsequent attenuation correction. During the scanning procedure, each subject's head was comfortably immobilized using a vacuum mask and the occurrence of important head displacement between the transmission scan and the FP-injection, which might

have been provoked by the TLI-procedure or Ket infusions, was excluded by monitoring of fiducial marks. A mean of 211 ± 34 MBq FP was injected (Pbo: 221 ± 29 MBq; Ket: 202 ± 37 MBq). The specific activity was 1474 ± 1370 (range 165–4739) GBq/μmol (Pbo: 1464 ± 1273 GBq/μmol; Ket: 1484 ± 1530 GBq/μmol; $p = 0.96$; paired t test), corresponding in each case to <1 pmol mass injected.

Image and data analysis

Images were reconstructed with filtered back-projection using a Hanning filter (filter width: 4 mm). After frame-by-frame motion-correction, the whole dynamic baseline Pbo-scan was nonlinearly normalized to MNI coordinates using the MEDx software (v3.43; Medical Numerics, USA). The individual transformation parameters were saved for later analysis of the corresponding Ket scans. Templates of polygonal volumes of interest (VOIs) were applied to the spatially normalized dynamic recordings, for extraction of time-activity curves (TACs) for cerebellum, caudate nucleus (CN), putamen, thalamus, dorsolateral prefrontal cortex (dlPFC) and inferior temporal cortex [inferior temporal gyrus (ITG)]. This template was used in several previous FP-investigations (e.g. Gründer *et al.* 2008; Vernaleken *et al.* 2010) and covers the regions with reliably detectable FP-binding. The results are therefore fully contrastable. The summed motion-corrected emission images in the Ket condition were automatically co-registered to the Pbo-scan using a linear 6-parameter algorithm (MEDx software; v3.43; Medical Numerics). Thereby, the normalization parameters for the Pbo scan could also be applied for the resampled Ket scan. TACs were acquired identically as in the Pbo analyses. The binding potentials (BP_{ND}) of these VOIs were calculated using the Simplified Reference Tissue Model (SRTM), with the cerebellum serving as the non-binding reference region (Lammertsma & Hume, 1996).

Ket and Pbo infusions

The Ket challenge was administered as a combined bolus/constant-rate i.v. infusion. The customer *S*-ketamine (Ketanest S®; Pfizer, USA) was formulated by dilution with 0.9% NaCl solution to a final concentration of 0.5 mg/ml for the slow bolus infusion of 8 mg *S*-ketamine for 5 min, corresponding to a mean *S*-ketamine dose relative to the individual body weight of 0.097 mg/kg (S.D. ±0.018 mg/kg). Starting immediately after completion of the bolus, a solution containing 0.25 mg/ml *S*-ketamine was administered as a constant infusion at the (weight-adapted) rate of 0.01 mg/kg.min for a period of approximately 1 h. The initial bolus was not weight-adjusted for practicability reasons; close to 13% of the entire dose was delivered during the bolus infusion. This procedure was designed in order to maintain psychopathological symptoms throughout the initial phase of the subsequent dynamic PET recording. The bolus plus infusion of Ket or Pbo began 35 min before FP administration and was maintained for the first 30 min of the dynamic PET recording. After the PET recording, all participants remained in the research facility under medical supervision for at least 2 h. For the Pbo condition, sterile 0.9% NaCl was administered as a bolus plus infusion emulating the Ket condition. During the investigation, no severe adverse events occurred; there were no drop-outs. Adverse experiences of the participants were according to the expected pharmacological profile of Ket, which was in part subject of the present investigation. In one case the time of somnolence was prolonged; normal vigilance, however, returned within the supervision period.

Psychopathological rating

The presence and extent of psychopathological changes due to Ket administration were assessed with the PANSS. PANSS ratings were performed directly after each FP scan by a trained psychiatrist blind to the drug treatment condition. Furthermore, the subjects were characterized at the screening visit by the use of the Temperament and Character Inventory (TCI; Cloninger, 1994).

Assessment of thought and speech-associated dysfunctions

The assessment of thought and speech-associated dysfunctions caused by Ket was performed using a modified version of the TLI initially described by Liddle *et al.* (2002). In brief, this assessment tool formally measures the extent of thought disruptions with regard to eight items, i.e. poverty of speech (PoS), weakening of goals, perseveration of ideas, looseness, peculiar use of words, peculiar sentences, non-logical reasoning (NlR) and distractibility. The subjects were asked to watch pictures from the Thematic Apperception Test (Murray, 1943). Different selections of pictures were used for the Pbo and the Ket conditions. Subjects were initially instructed to describe the standardized pictures, as rapidly as possible, while speaking clearly. The subject was verbally, but in a non-directive manner, prompted to keep talking for 2 min, in contrast to Liddle *et al.* (2002), who recorded speech for only 1 min. A total of eight pictures were presented, each separated by 30 s of stimulus-free condition. The participants' speech production was digitally recorded and subsequently filtered (Adobe Audition 3; Adobe Systems Software Ireland Limited, http://www.adobe.com/) and transcribed. According to instructions in the TLI manual, these documents were used for qualitative and quantitative analyses of the participants' overt responses in each condition, yielding information about content and number of items evoked by each image. The TLI was performed in the 30-min interval before the bolus-injection of FP. The rater for the TLI

Table 1. Clinical disturbances by ketamine treatment

Condition	Specific activity (GBq/μmol)	Activity (MBq)	PANSS				TLI							
			Total	Positive	Negative	General	PoS	WoG	PoI	Los	PUW	PeS	NIR	Dis
Placebo	1464 ± 1273	221 ± 29	30 ± 0	7 ± 0	7 ± 0	16 ± 0	0.45 ± 0.67	0.28 ± 1.48	0.05 ± 0.16	0.05 ± 0.11	0.20 ± 0.26	0.10 ± 0.17	0.25 ± 0.24	0 ± 0
Ketamine	1484 ± 1530	202 ± 37	46.8 ± 16.6	10.3 ± 2.9	15.0 ± 8.51	21.5 ± 5.74	2.18 ± 2.33	1.48 ± 1.60	0.38 ± 0.70	0.23 ± 0.32	0.35 ± 0.45	0.50 ± 0.57	0.60 ± 0.47	0.30 ± 0.67
Change	+20	−19	+16.8	+3.3	+8.0	+5.5	+1.73	+1.20	+0.33	+0.18	+0.15	+0.40	+0.35	+0.30
	($p = 0.49$)	($p = 0.34$)	($p = 0.0078$)	($p = 0.0140$)	($p = 0.0156$)	($p = 0.0156$)	($p = 0.075$)	($p = 0.058$)	($p = 0.074$)	($p = 0.066$)	($p = 0.336$)	($p = 0.041$)	($p = 0.053$)	($p = 0.180$)

Ketamine induced changes in psychopathology [Positive and Negative Syndrome Scale (PANSS)] and the Thought and Language Index (TLI). Depicted are the TLI subscores poverty of speech (PoS), weakening of goals (WoG), perseveration of ideas (PoI), looseness (Los), peculiar use of words (PUW), peculiar sentences (PeS), non-logical reasoning (NIR) and distractibility (Dis). S-ketamine was administered i.v. as a bolus of 8 mg followed by a constant infusion for 1 h at a rate of 0.01 mg/kg.min. Statistical tests were performed using the Wilcoxon rank-order test.

(as well as for the PANSS) was blind against the Pbo/Ket conditions.

Statistics

Differences between Pbo and Ket conditions were tested using the paired two-sided t test, with Bonferroni's correction for multiple comparisons. Due to the relatively low sample size, correlations were calculated by Spearman's method, except for the case when age was examined as a confounding factor. For the latter, a general linear model was applied as additional method.

Results

Ket and psychopathology

The Ket infusion resulted in pronounced and statistically significant changes in PANSS total scores, which increased from 30 ± 0 (mean ± S.D.) in the Pbo condition to 46.8 ± 16.6 (mean ± S.D.; range: 30–74; paired two-tailed t test: $p = 0.01$) in the Ket condition. With regard to the PANSS-subscores, the strongest differences were observed for the negative symptoms (PANSSneg: +114.2%). Details of changes in PANSS-subscales are presented in Table 1. One of eight TLI-items and two of the subscale scores also showed a significant worsening (i.e. peculiar sentences, disorganized thought/language, non-specific dysregulation). Another set of five items and one subscore showed changes at a trend level (for details see Table 1). None of the significant TLI-differences survived a Bonferroni's correction.

Ket and $D_{2/3}$ receptor availability

The mean baseline BP_{ND}-values were 22.9 ± 3.2 in CN, 24.7 ± 3.5 in putamen, 2.75 ± 0.37 in thalamus, 1.01 ± 0.16 in ITG and 0.29 ± 0.15 in the dlPFC. There were no significant correlations between the BP_{ND} estimates at baseline and the individual FP specific activity. Upon factoring for age of the participants, we found that FP BP_{ND} declined at a rate ranging from 3.1% per decade in dlPFC to 5.4% per decade in thalamus, which was statistically significant only in dlPFC [$p = 0.008$, $F = 12.3$, d.f. numerator (d.f.n) = 1, d.f. denominator (d.f.d) = 8 and ITG ($p = 0.010$, $F = 11.2$, d.f.n = 1, d.f.d = 8]; the decline in the thalamus and the putamen was on trend level. Correlations between the baseline FP BP_{ND} and clinical or neuropsychological parameters from the screening visit were absent for most of the assessments. However, there was a trend-like or nearly significant correlation between the TCI novelty seeking measures from the screening visit and baseline BP_{ND} in dlPFC ($r = 0.633$, $p = 0.0495$ two-tailed Spearman correlation) and CN ($r = 0.620$, $p = 0.060$). In contrasting the Ket and saline conditions, we found significant changes of the FP BP_{ND} only in the CN [+1.35 ± 1.23 (+5.9%); paired two-sided t test: $p = 0.006$], a finding that survived Bonferroni's correction.

Table 2. [^{18}F]fallypride binding potentials

	[^{18}F]fallypride binding (BP_{ND})				
Condition	dlPFC	ITG	Thalamus	CN	Putamen
Placebo	0.29 ± 0.15	1.01 ± 0.16	2.75 ± 0.37	22.9 ± 3.2	24.7 ± 3.5
Change	−0.01 (−1.9%) ($p = 0.77$)	±0 (±0%) ($p = 0.92$)	+0.07 (+2.5%) ($p = 0.63$)	+1.4 (+5.9%) ($p = 0.006$)	+0.9 (+3.5%) ($p = 0.054$)

dlPFC, Dorsolateral prefrontal cortex; ITG, inferior temporal gyrus.
Influence of *S*-ketamine administration on the $D_{2/3}$ receptor availability measured by [^{18}F]fallypride positron emission tomography. All binding potentials (BP_{ND}) were estimated by means of the simplified reference tissue model using the cerebellum as reference region.
Statistical tests were performed using the Wilcoxon rank-order test.
Significant ketamine-induced BP_{ND} elevations observed in the caudate nucleus (CN) survived Bonferroni's correction for multiple testing.

Table 3. Correlations between binding potentials and PANSS changes

	dlPFC		ITG		Thalamus		CN		Putamen	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
PANSS total	+0.750	0.012*	+0.669	0.035*	+0.705	0.023*	+0.778	*0.008**	+0.657	0.039*
–positive	+0.635	0.049*	+0.716	0.020*	+0.599	0.067*	+0.624	*0.054**	+0.508	0.134*
–negative	+0.683	0.029*	+0.522	0.122*	+0.681	0.030*	+0.755	0.012*	+0.607	0.063*
–general	+0.695	0.026*	+0.656	0.039*	+0.755	0.012*	+0.742	0.014*	+0.607	0.063*

dlPFC, Dorsolateral prefrontal cortex; ITG, inferior temporal gyrus; CN, caudate nucleus.
* Statistically significant correlations: $p < 0.05$.
**Statistically significant correlations surviving Bonferroni's correction: $p < 0.01$.
Rank order correlations (two-sided Spearman correlation) between the regional $D_{2/3}$ receptor availability measured by [^{18}F]fallypride (binding potentials) under placebo conditions and the individual increase in Positive and Negative Syndrome Scale (PANSS) ratings. PANSS scores were assessed closely to the placebo and the ketamine scan conditions.

A Ket-evoked increase in FP BP_{ND} in putamen was statistically significant only at trend-level [+0.86 ± 1.22 (+3.5%); paired two-sided *t* test: $p = 0.054$; see Table 2]. Neither the subject's age nor the scanning order for saline and Ket conditions had any influence on the changes in BP_{ND}.

Confounding factors for psychopathological changes

There were significant associations between the Ket-induced changes in psychopathology (measured by PANSS ratings) and the baseline $D_{2/3}$ receptor availability in all VOI regions, with the least association in the putamen. Only the ΔPANSS *vs.* CN-BP_{ND} correlation survived Bonferroni's correction ($r = 0.778$; $p = 0.008$; two-sided Spearman correlation). The association with the dlPFC-BP_{ND} was of comparable magnitude, but just failed significance level after Bonferroni's correction [$r = 0.750$; $p = 0.012$ (uncorrected); two-sided Spearman correlation]. For details and correlations between FP BP_{ND} and PANSS subscore changes, see Table 3 and Fig. 1. In the corresponding correlation of regional baseline BP_{ND}-values with specific dysfunctions of thought and language during Ket administration, significant correlations were found between thalamic FP binding and three items (PoS: $r = 0.693$; $p = 0.026$; NlR: $r = 0.646$, $p = 0.044$; impoverished thoughts: $r = 0.673$, $p = 0.033$; two-sided Spearman correlation), but correlations in other brain regions were not significant. When a general linear model with ΔPANSS as dependent parameter and CN-BP_{ND}/age as independent variable was applied, the baseline CN-BP_{ND} as well as age*CN-BP_{ND} showed significant effects (CN-BP_{ND}: Wald $\chi^2 = 6.1$, $p = 0.013$; age*CN-BP_{ND}: Wald $\chi^2 = 4.6$, $p = 0.032$), whereas age effects alone were on trend level: (age: Wald $\chi^2 = 3.5$, $p = 0.063$).

Discussion

This investigation focuses on the assessment by PET of dopaminergic trait markers for the individual vulnerability for psychosis. Ketamine at the sub-anaesthetic dose is known to evoke transient positive and negative symptoms (Krystal *et al.* 1994; Oye *et al.* 1992; Umbricht *et al.* 2000) even in healthy subjects. In comparison to these investigations, we administered higher doses of Ket

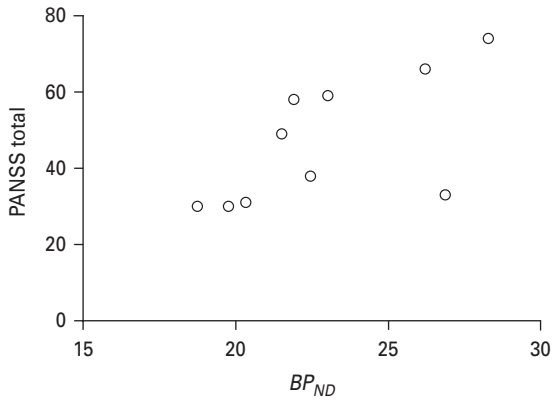

Fig. 1. The relationship between the $D_{2/3}$ receptor availability ([^{18}F]fallypride-positron emission tomography) in placebo conditions (caudate nucleus) and the extent of psychotic symptoms, which acutely emerged during ketamine treatment (8 mg *S*-ketamine as slow bolus followed by a constant infusion rate of 0.01 mg/kg.min). Psychotic symptoms were quantified by the Positive and Negative Symptom Scale (PANSS). BP_{ND}, binding potentials. All subjects ($n = 10$) scored with PANSS = 30 in the placebo condition.

(8 mg bolus plus an average of 51 mg over 60 min) so as to provoke a persistent and substantial challenge during the initial phase of the FP-PET recording and extending throughout the 3 h session. The extent of psychotic symptoms, however, was no more pronounced than in previous studies, but was highly variable, ranging from no observable disturbances to transient schizophrenia-like intensity in one individual. Using the same *S*-ketamine injection protocol within an functional magnetic resonance imaging investigation, Nagels *et al.* (2011) reported similar heterogeneous clinical effects on PANSS ratings.

The psychotogenic actions of Ket are usually attributed to its NMDA-antagonistic properties and consequent potentiating of dopaminergic transmission, although direct partial agonist as well as antagonistic effects at D_2 receptors have also been reported (Anand *et al.* 2000; Kapur & Seeman, 2001; Krystal *et al.* 2005). Some (Smith *et al.* 1998; Vollenweider *et al.* 2000) – but not all (Aalto *et al.* 2002; Kegeles *et al.* 2002) – PET investigations using [^{11}C]raclopride, a dopamine $D_{2/3}$ ligand of moderate affinity, have reported that Ket challenge decreased striatal receptor availability in association with clinical changes. Insofar as declining receptor availability is usually interpreted to indicate increased competition from endogenous dopamine, these earlier PET findings suggest a tonic NMDA receptor modulated change of dopamine emission rates.

The main finding of this study was the observation of a positive correlation between the baseline $D_{2/3}$ receptor availability in several brain regions with the individual extent of psychopathological changes during Ket administration. This finding suggests that one's receptor availability could be predictive for vulnerability to emergence of psychotic symptoms upon pharmacological or other challenges. In previous PET studies of normal subjects, $D_{2/3}$ receptor availabilities have been shown to correlate with the sensation-seeking trait (Gjedde *et al.* 2010), cognitive performance and reaction times (Gjedde *et al.* 2010; Volkow *et al.* 1998) and (in animals) with the vulnerability to seek a psychostimulant drug (Dalley *et al.* 2007; Nader *et al.* 2006). Also in PET studies of healthy volunteers with the presynaptic markers FDOPA-PET and 6-[^{18}F]-fluoro-*meta*-tyrosine-PET investigations, presynaptic dopaminergic parameters were positively associated with frontal lobe function of healthy subjects as well as with individual vulnerability to pharmacologically induced disturbances (Braskie *et al.* 2011; Vernaleken *et al.* 2007, 2008).

The present finding that the individual vulnerability to experience psychotic symptoms from Ket challenge is predictable from the baseline $D_{2/3}$ receptor availability was evident not only in the basal ganglia (especially in the CN), but also in cerebral cortex. In spite of the small group size, these correlations reached statistical significance, especially in the CN ($r = 0.778$) and notably also in subcortical regions (ITG, thalamus) and even in the dlPFC, a region characterized by very low ligand binding and low signal:noise ratio. These correlations were, however, region specific, as baseline FP BP_{ND} in putamen showed a less pronounced and consistent association, in spite of its high magnitude of ligand binding. This pattern of VOI-based findings suggests that Ket's effects on psychopathology may be subserved by dopamine receptors in a network comprising cerebral cortex, the thalamus and the CN. The functional interplay between fronto-caudate functions and the dopaminergic system has previously been linked with optimal information processing (Klostermann *et al.* 2012). In fact, the CN and the dlPFC-VOI showed the highest correlations with Ket-induced impairments within the present investigation. The finding of strong correlations between an isolated biological *in vivo* parameter (i.e. FP BP_{ND}) and complex behavioural measures may have been favoured by the comparably homogenous group demographics; all were male students of very circumscribed age range and only moderate inter-individual differences in cognitive performance. This minimizes important and well-known factors influencing the end-points of dopamine PET studies (Vernaleken *et al.* 2007; Volkow *et al.* 1998).

An early hypothesis held that positive (as well as negative symptoms) of schizophrenia may be related to impaired ability to segregate relevant from irrelevant information (Arieti, 1955; Storms & Broen, 1969). Contemporary models suggest that predominant signalling by D_2-like (rather than D_1) receptors leads to more liberal information processing, such that 'attention' is less focused, but correspondingly broadened to less salient stimuli (Durstewitz *et al.* 2000). Cortico-striatal information processing is mediated by a highly complex

system of glutamatergic (including NMDA receptors), dopaminergic and GABAergic transmission, which entails counter-regulatory modulation of neuronal excitability and complex feedback mechanisms (Seamans & Yang, 2004). It is to be supposed that a dopamine system characterized by relatively high $D_{2/3}$ receptor availability and high dopamine synthesis capacity is fitter for adjusting to environmental needs through self-regulation of dopamine transmission but may be disadvantageous under circumstances of pathological dysregulation (e.g. schizophrenia; see Kumakura *et al.* 2007; Meyer-Lindenberg *et al.* 2002) or pharmacological provocations.

Several molecular imaging investigations were performed to find disturbances in the dopamine receptor system focusing on D_2 receptors. These studies yielded heterogeneous results (e.g. Buchsbaum *et al.* 2006; Wong *et al.* 1986). Glenthoj *et al.* (2006) reported positive correlations between individual psychopathological impairments and corresponding $D_{2/3}$ receptor availabilities. While we did not replicate this association with FP-PET, we found that patients at the beginning of their disease showed the highest $D_{2/3}$ receptor binding (Vernaleken *et al.* 2009). Consistent with that clinical finding in patients, the present results show that relatively high baseline availability of $D_{2/3}$ receptors in normal subjects is an important factor in the individual vulnerability to Ket-induced expression of psychotic symptoms. Compiling results of this and other PET studies of the dopamine system in healthy subjects and schizophrenia patients, we suggest that high $D_{2/3}$ receptor availabilities may bring benefits with regard to cognitive flexibility, but increase the risk of maladjustment of information processing in the face of environmental, pathological or pharmacological stresses and challenges. Certainly, the intended small group of participants were forced to highly select the subjects in respect of important criteria that are known to be associated with the dopamine system (i.e. gender, age and cognition). Therefore, it needs to be considered that the data are limited in generalizability and in predictive value for the whole population.

Another goal of this investigation was to assess the Ket-induced stimulation of striatal and possibly extrastriatal dopamine transmission. Contrary to our expectations, we did not find any Ket-evoked reductions in the BP_{ND} values in any brain region, but rather an increase of small magnitude, which was significant in the caudate and nearly significant in the putamen. The extent of the latter increase, however, was only in a range of the expected striatal test–retest reliability ($\approx 4\%$), which was reported for FP (Cropley *et al.* 2008). For this reason, the positive and contra-intuitive result in the CN needs to be interpreted very carefully. On the other hand, the 5.9% difference was above the theoretical 5.6% border for rejecting the null hypothesis according to our power analysis (*vide infra*). The lack of BP_{ND}-reductions stands in contrast to previous striatal results using [^{11}C]raclopride (Breier *et al.* 1998; Smith *et al.* 1998; Vollenweider *et al.* 2000). Some published results that the high affinity ligand FP used in the present study is as sensitive to competition from endogenous dopamine as is the lower affinity compound DMFP (Rominger *et al.* 2010), other investigations reported a lower sensitivity in respect of [^{11}C]raclopride (e.g. Slifstein *et al.* 2010). The need for long PET acquisition times due to the slow tracer kinetics (Vernaleken *et al.* 2011) might be an important factor, especially for the unexpected inverse results in the high-binding regions. A 1 h Ket infusion may not have maintained a stable effect during the 3 h recording although psychotomimetic effects of Ket were maintained throughout the entire PET scan. The relatively shorter Ket infusion time in comparison to the PET acquisition time was chosen in order to limit the cumulative Ket dose and the respective side-effects. During the initial protocol planning it was considered that, in challenge paradigms using D_2 receptor ligands, an increase of the dopamine concentration is timely dissociated from the changes of the $D_{2/3}$ receptor availability, which is most likely explained by receptor internalization following the initial displacement by endogenous dopamine. Skinbjerg *et al.* (2010) performed [^{11}C]MNPA and FP scans after amphetamine challenge in arrestin3 knockout mice (no receptor internalization possible). The results suggest that the reduction of $D_{2/3}$ receptor availability at later time-points of the PET scan is mainly due to receptor internalization. Nevertheless, in the absence of BP_{ND} reductions the short Ket infusion protocol limits conclusiveness. The fact that FP shows sufficient vulnerability to amphetamine stimuli in rat striatum may therefore reflect the somewhat faster kinetics in that species, in which 2 h PET recordings sufficed for stable BP_{ND} estimates (Rominger *et al.* 2010). The present 3-h recording time is at the lower end of published protocols. However, a recent kinetic analysis of striatal FP binding in a large series of patients revealed that transient equilibrium was obtained within 2 h, thus justifying our decision to refrain from recording longer than 3 h. Further artificial reductions of acquisition times led to initial small changes (-0.58% for a 10 min reduction) of striatal BP_{ND} estimates. Furthermore, the time to equilibrium as estimated by detection of the peak of the VOI minus cerebellum curve was observed at approximately 120 min for the putamen (Vernaleken *et al.* 2011). An additional *caveat* of the present study is raised by the presence of specific cerebellar D_2 binding (Pinborg *et al.* 2007), which may bias SRTM results. This is especially a concern if the pharmacological impact of the challenging agent is different in the reference region compared to the VOI (Pinborg *et al.* 2007; Vandehey *et al.* 2010). However, a direct comparison between FP and [^{11}C]FLB457 found the BP_{ND} results of FP to be less influenced by specific cerebellar binding. Notwithstanding the aforementioned methodological arguments, some earlier findings (Kegeles *et al.* 2000) suggest that the effect of Ket might be much more pronounced in situations of increased dopaminergic turnover/release

(i.e. pharmacological challenges by stimulants or pathological states such as schizophrenia), which was not the subject of the present investigation.

This investigation was designed to study only a small group of subjects, due to the high risks for complaints that we anticipated arising from the comparatively high Ket dose. In order to optimize the statistical power of this investigation, we therefore tried to reduce the heterogeneity of potentially confounding inter-individual factors, as noted above. Therefore, we do not think that the absence of a reduction in striatal FP binding is not inevitably explained by a low group size. A power analysis ($\alpha_{Err} = 0.05$; $1 - ß_{Err} = 0.8$) revealed a required effect size of $dz > 0.996$ for detection of group differences. Using the present distribution parameters, a 5.6% change in a high-binding region (i.e. putamen) would have been detectable, whereas in low-binding regions (ITG) a 15.8% difference would have been required. The latter difference is beyond expectations; however, a striatal BP_{ND} reduction of $>5.6\%$ was in accordance with the initial hypotheses. Furthermore, it could be speculated whether the use of structural magnetic resonance data for co-registration and – even more relevant – partial volume correction might have increased the quality of the data. In fact, Kegeles *et al*. (2010) exemplified the substantial impact of partial volume correction on FP-PET in the course of a schizophrenia/control comparison. It is noteworthy that, contrary to positive findings (Breier *et al.* 1998; Smith *et al.* 1998; Vollenweider *et al*. 2000), previous investigations reported negative results. An anaesthetic dose of Ket was without effect on [^{11}C]raclopride binding in the cat (Hassoun *et al.* 2003) and a sub-anaesthetic dose was likewise without effect in other studies of healthy humans (Aalto *et al.* 2002; Kegeles *et al.* 2000, 2002). Particular attention was placed on consideration of the equilibrium condition in the latter study. Thus, the factors determining the effects of Ket in the PET competition paradigm remain elusive.

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Statement of Interest

None.

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