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Brain serotonin 2A receptor binding predicts subjective temporal and mystical effects of psilocybin in healthy humans

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Abstract

Background: Psilocybin is a serotonergic psychedelic with psychoactive effects mediated by serotonin 2A receptor (5-HT2AR) activation. It produces an acute psychedelic altered state of consciousness with a unique phenomenology that can be temporally characterized by three intensity phases: onset of psychoactive effect, a peak plateau and return to normal consciousness.

Aims: We evaluated whether pre-drug brain 5-HT2AR binding predicted the three phases of psilocybin subjective drug intensity (SDI) and retrospective self-report of mystical type experiences in healthy individuals.

Method: Sixteen participants completed a pre-drug [^{11}C]Cimbi-36 positron emission tomography scan to assess 5-HT2AR binding. On a separate day, participants completed a single psilocybin session (oral dose range 0.2–0.3 mg/kg), during which SDI was assessed every 20 min. The Mystical Experience Questionnaire (MEQ) was completed at the end of the session. The three SDI phases were modelled using segmented linear regressions. We evaluated the associations between neocortex 5-HT2AR binding and SDI/MEQ outcomes using linear regression models.

Results: Neocortex 5-HT2AR was statistically significantly negatively associated with peak plateau duration and positively with time to return to normal waking consciousness. It was also statistically significantly negatively associated with MEQ total score.

Conclusion: This is the first study to investigate how individual brain 5-HT2AR binding predicts subjective effects of a single dose of psilocybin. Our findings reinforce the role of cerebral 5-HT2AR in shaping the temporal and mystical features of the psychedelic experience. Future studies should examine whether individual brain levels of 5-HT2AR have an impact on therapeutic outcomes in clinical studies.

Keywords

Serotonin 2A receptor, psilocybin, subjective drug intensity, psychedelics, mystical type experience, positron emission tomography

Introduction

Psilocybin is a serotonergic psychedelic drug (Nichols, 2016) which has recently been explored as a novel therapeutic for psychiatric disorders alongside other psychedelic substances (Dos Santos and Hallak, 2020; Johnson et al., 2019; Johnson and Griffiths, 2017). Results from a series of recent clinical trials provide evidence that psilocybin can be used therapeutically for, for example, major depression (Carhart-Harris et al., 2016, 2018), obsessive compulsive disorder (Moreno et al., 2006), anxiety and depression associated with life-threatening cancer (Griffiths et al., 2016; Ross et al., 2016) and addiction (Bogenschutz et al., 2015; Johnson et al., 2014). At medium to high doses (i.e. $>$ 0.2 mg/kg), psilocybin induces an acute psychedelic experience, characterized by three experiential phases: (a) the onset of psychoactive effects followed by (b) a peak plateau and (c) a gradual return to normal waking consciousness (i.e. the subjective state that research participants identify as their typical state of awake consciousness, free from any psychoactive drugs) (Madsen et al., 2019; Preller and Vollenweider, 2018). In contrast to normal waking consciousness, perceptual alterations and changes in sense of self, affect, meaning and cognition are typically experienced during the psilocybin psychoactive phases within a spectrum of what users of psychedelic drugs and researchers have termed 'peak' (Maslow, 1959), 'mysticomimetic' (Nielson et al., 2018) or 'mystical type' experiences (Griffiths et al., 2011). We collectively refer to these terms as 'mystical type experiences' in the present paper.

Mystical type experiences have been reported for centuries with or without the aid of psychoactive drugs (Barrett and Griffiths, 2018; Pahnke, 1963), and have a 'common core' of phenomenological features, including unity of experience, deep-felt blissful mood, transcendence of time and space and ineffability (Pahnke, 1963). Several studies attest to the significance of mystical type experiences for long-term beneficial effects of

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psilocybin in healthy research participants (Griffiths et al., 2006, 2011; MacLean et al., 2011), suggesting that the core features of such experiences are important to understand beyond the acute phenomenology of psychedelic experiences. Previous studies indicate that dose, personality and set and setting predict psilocybin responses (Studerus et al., 2012), however, it is not known whether differences in key serotonergic targets in the brain predict subjective psychedelic intensity and/or mystical type experiences. Such knowledge would advance our understanding of the neurobiological underpinnings of individual differences in the psychedelic experience and implicate potential pre-drug neurobiological markers of lasting effects. From a clinical perspective, identifying neurobiological markers that can predict treatment response to psilocybin would help guide and optimize treatment choice (i.e. who is likely to benefit from this type of treatment), should the drug be approved for clinical practice.

The psychedelic effects of psilocybin are mediated primarily by its actions as an agonist at the serotonin 2A receptor (5-HT2AR) (Gonzalez-Maeso et al., 2007; Kometer et al., 2012; Vollenweider et al., 1998). Our lab recently demonstrated that moderate to high doses of oral psilocybin leads to brain 5-HT2AR occupancy of 60–70%, which correlates with a real-time estimate of subjective drug intensity (SDI) during the experience (Madsen et al., 2019). This is consistent with 5-HT2AR agonism as a critical determinant of the acute psychedelic experience. 5-HT2AR is an excitatory 5-HT receptor, widely expressed throughout the human cerebral cortex (Beliveau et al., 2017; Varnas et al., 2004). It has been implicated in cognition (Zhang and Stackman, 2015), personality (Frokjaer et al., 2008), processing emotionally salient stimuli (Fisher et al., 2009) and physiological processes such as sleep–wake behaviour (Monti, 2011). As such, this receptor affects important features of the human psyche that are altered by stimulation with psilocybin. Based on this, we hypothesize that 5-HT2AR binding in the human brain determines the course and intensity of the psychedelic drug effects.

Brain 5-HT2AR binding can be accurately measured with [^{11}C]Cimbi-36 positron emission tomography (PET), an agonist radiotracer validated for 5-HT2AR human *in-vivo* imaging (Beliveau et al., 2017; Ettrup et al., 2014). In the present paper, we examine the association between pre-drug (baseline) 5-HT2AR binding and acute temporal features of subjective psychedelic intensity and self-reported mystical type experiences following a single psilocybin administration in 16 healthy research participants. We estimate the temporal features of SDI using a three-phase model: (a) onset of psychoactive effects, (b) duration of peak plateau and (c) return to normal waking consciousness.

Methods

Participants and study design

Sixteen healthy participants (mean age ± SD (range): 30.3 ± 6 (24.2–49.8), six females) were recruited from a list of individuals who expressed interest in participating in a psilocybin brain scanning study. Exclusion criteria were: (a) present or previous psychiatric disorder or immediate family history of psychiatric disorder including substance abuse disorder; (b) present or previous neurological illness or significant somatic illness; (c) *use of hallucinogenic drugs within the preceding six months and use of any other intoxicant, with the exception of alcohol, within the preceding month*; (d) non-fluent Danish language skills or learning disability; (e) vision or hearing impairment; (f) pregnancy or breastfeeding for women; (g) magnetic resonance imaging (MRI) contraindications; (h) allergy to the test drugs; (i) significant exposure to radiation within the past year, for example medical imaging investigations; (j) *intake of medication that prolongs the* interval between the Q and T parts of an electrocardiogram (ECG) or ECG results indicative of heart disease; (k) blood donation less than 3 months before project participation; (l) bodyweight less than 50 kg; (m) low plasma ferritin levels (< 12 μg/L); and (n) ⩾ 18 years of age.

The data included in the present study were pooled across two studies from our research group investigating psilocybin 5-HT2AR occupancy (Madsen et al., 2019) and changes in 5-HT2AR one week after psilocybin intervention (Madsen et al., 2020), respectively. These studies were open label and included pre-drug (baseline) 5-HT2AR imaging and psilocybin interventions which followed the same regimen, as described in the experimental procedures section. For detailed descriptions of the studies, please see the above cited references. Written informed consent was obtained from all individuals after a complete description of the study. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee for the capital region of Copenhagen (journal ID: H-16028698, amendments: 56023, 56967, 57974, 59673, 60437, 62255) and Danish Medicines Agency (EudraCT: 2016-004000-61, amendments: 2017014166, 2017082837, 2018023295).

Measures

Psychometric rating of SDI. On the intervention day, SDI was measured approximately every 20 min using a 0–10 Likert scale (question: 'How intense is your experience right now?'; 0 = 'Not at all', 10 = 'Very much') to quantify and monitor the subjective experience of intensity in real time. Measurements were obtained from the time of drug administration to the end of the session. Participants responded orally and the supporting psychologists noted their responses.

Mystical Type Experience Questionnaire. At the end of the intervention day and approximately 6 h after ingestion of psilocybin, participants completed the revised Mystical Type Experience Questionnaire (MEQ) (Barrett et al., 2015). The MEQ is a self-reported inventory comprising 30 items that measure the core features of a mystical type experience relating to a single, discrete event (e.g. psychedelic session). It contains four subscales: Mystical quality, Positive mood, Transcendence of time and space and Ineffability as well as a total score, which is calculated by averaging all items. The participants rated each item on a 6-point Likert scale from 0 (none, not at all) to 5 (extreme, more than any other time in my life and more than a rating of 4).

PET and MR imaging. [^{11}C]Cimbi-36 was produced as described previously (Ettrup et al., 2014). Participants were scanned with an HRRT PET scanner (CTI/Siemens, Knoxville, TN, USA) with an approximate in-plane resolution of 2 mm. Following a 6-min transmission scan, [^{11}C]Cimbi-36 was administered as a bolus and a dynamic 120-min emission scan was acquired and reconstructed into 45 frames (6 × 10 s, 6 × 20 s, 6 × 60 s, 8 × 120 s, 19 × 300 s). Dynamic PET images were reconstructed using an iterative

OP-OSEM 3D method with resolution modelling (10 iterations and 16 subsets). Independent of the PET scan, participants completed an MRI scan session on a Siemens 3T Prisma scanner (Erlangen, DE). A high-resolution T1-weighted structural brain scan (TE/TR/TI, 2.58/1900/900 ms, flip angle, 9°; in-plane matrix, 256 × 256; slices, 224; voxel-size, 0.9 × 0.9 × 0.9 mm) was acquired for each participant. MR images were used for segmenting the brain into gray matter, white matter, and cerebrospinal fluid and region of interest (ROI) delineation.

Brain image analysis and outcomes. Single-subject within PET scan motion and realignment was determined with the automatic image registration (AIR) algorithm (Woods et al., 1992). PET scans were smoothed using a 10 mm within-frame Gaussian filter before realignment. Non-filtered PET images were realigned using these parameters. Statistical parametric mapping (SPM) was used to co-register high-resolution PET and MR images and segment high-resolution MR images. Accurate co-registration and segmentation was confirmed visually for all datasets. We used PVElab to automatically delineate ROIs on the participant's high-resolution T1-weighted MR scan (Svarer et al., 2005). Mean time–activity curves were extracted from the grey-matter voxels within ROIs.

The primary outcome measure for [^{11}C]Cimbi-36 imaging was non-displaceable binding potential (BP_{ND}) with a cerebellum reference region, computed using the simplified reference tissue model (SRTM), which is validated for this tracer (Ettrup et al., 2014, 2016). BP_{ND} is defined as: $BP_{ND} = f_{ND}(B_{avail}/K_D)$, where f_{ND} is the fraction of non-displaceable binding, B_{avail} is the number/L of brain tissue of 5-HT2ARs available for binding and K_D is the dissociation constant. For this study, we chose a neocortex ROI as our predictor of SDI and MEQ. 5-HT2AR is highly expressed within neocortex and displays a high degree of inter-regional correlation across neocortical subregions, indicating that a single neocortex region is highly informative (Erritzoe et al., 2010). The neocortex ROI comprised occipital, orbitofrontal and parietal cortex, pre/post central, middle/inferior frontal, middle/inferior temporal, superior frontal and superior temporal gyrus as defined in PVElab. Subcortical [^{11}C]Cimbi-36 binding was not considered because the signal is low and noisy.

Experimental procedures

Screening and baseline. Prior to inclusion, participants were thoroughly informed about the study, including potential side-effects and risks associated with intake of psilocybin. Participants also underwent a physical and neurological examination, including ECG, blood screening for pathology, and a screening for present or previous psychiatric disorders using the Danish Mini-International Neuropsychiatric Interview (Sheehan et al., 1998).

After inclusion, participants underwent baseline [^{11}C]Cimbi-36 PET and MR imaging. On the scan day and on the intervention day, participants were instructed to abstain from alcohol (including the day before), to consume a light breakfast, and abstain from caffeine. All participants were also screened for drug use (i.e. amphetamines, opioids, benzodiazepines, barbiturates, tetrahydrocannabinol, cocaine, ketamine, phencyclidine and gamma hydroxybutyrate), using a urine test (Rapid Response, BTNX Inc., Markham, Canada). Prior to the intervention day, all participants attended a preparatory consultation with the two psychologists who would assist them on the intervention day. The purpose of the preparatory consultation was to familiarize the participants with the assisting psychologists and included a talk about the motivation for participating and the personal background of the participants. It further included a more detailed talk about drug effects and how to approach these in order to optimize safety and facilitate a good experience.

Intervention day. On the intervention day and in accordance with proposed guidelines (Johnson et al., 2008), participants were again informed about potential side-effects and risks of psilocybin and safety precautions were repeated before they received the drug. Psilocybin was administered orally in 3 mg capsules with a glass of water. Dose was adjusted for bodyweight and ranged from 0.2 ($n = 7$) to 0.3 ($n = 9$) mg/kg (absolute dose range: 12–30 mg). Interpersonal support was provided throughout the intervention by two trained psychologists, and a standardized selection of supportive music was played to assist participants. The music playlist was adapted from one kindly provided by Professor Roland Griffiths, Johns Hopkins University (playlist available upon request) and contained mostly classical music pieces. A light lunch and beverages were offered to the participants. Among the 16 participants, six completed at least one PET scan on the intervention day, during the psychedelic session (Madsen et al., 2019). For this sub-set of the sample, we also obtained blood samples to determine plasma psilocin levels. Notably, all 5-HT2AR PET data presented in the current paper was collected on a day prior to the psilocybin session. All participants returned one day after the intervention day for a post-session consultation with the assisting psychologists.

Data analyses

Consistent with the conceptualization of a three-phase psychedelic experience, SDI time courses were modelled separately for each participant using a segmented linear regression model with two breakpoints modelling the three phases of the psychedelic experience. The intercept, slope parameters, and the position of the breakpoints were estimated by maximum likelihood according to a previously described algorithm (Muggeo, 2003). Based on this model, we derived three outcomes for each participant: (a) an onset slope coefficient, (b) duration of peak plateau, and (c) a return slope coefficient. The onset and return slopes are expressed in SDI change per 10 min, and duration of peak plateau is expressed in minutes. One participant had missing data such that none of these outcomes could be estimated.

Visits to the toilet substantively, but temporarily, disrupted the psychedelic experience. Therefore, SDI ratings acquired immediately following visits to the toilet were excluded from the model, as were SDI scores repeated more than three times at the end of the session (see excluded points denoted in Figure 2).

The associations between neocortex [^{11}C]Cimbi-36 BP_{ND} and the three SDI outcomes were evaluated using three separate linear regression models. *p*-values were family-wise error rate (FWER)-corrected using the Bonferroni method for three tests. The association between neocortex [^{11}C]Cimbi-36 BP_{ND} and MEQ total score was investigated using a linear regression

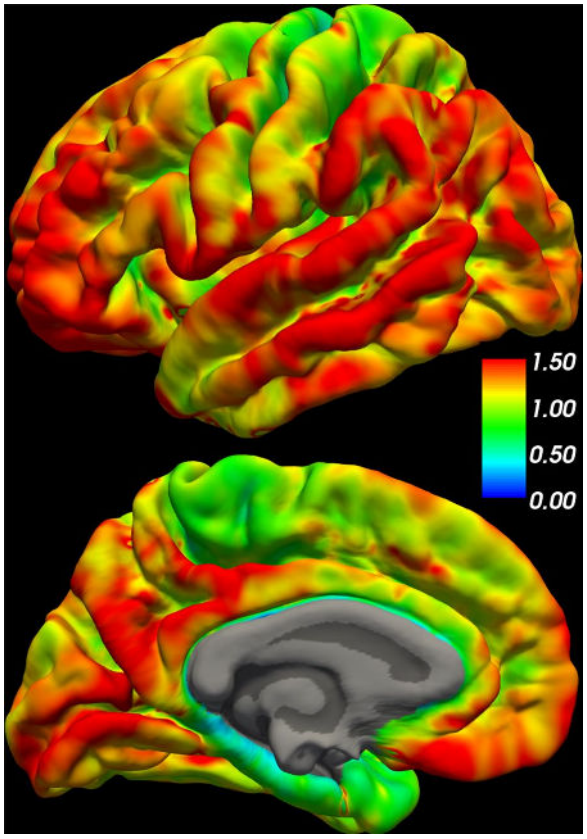

Figure 1. Map of average [^{11}C]Cimbi-36 binding potential (BP_{ND}). Surface illustration of mean neocortex [^{11}C]Cimbi-36 BP_{ND} in the left hemisphere across all sixteen participants.

model. Contingent on a significant result for the MEQ total score, post-hoc analyses were conducted to investigate associations between neocortex [^{11}C]Cimbi-36 BP_{ND} and MEQ sub-scales. *p*-values for these post-hoc analyses were not calculated.

We considered including the following covariates in the linear regression models: dose, sex, setting (PET or not) and previous experience (psychedelic-naïve or not). These were not statistically significantly associated with the SDI and MEQ outcomes and not included in the final models. To evaluate whether 5-HT2AR binding had an effect on the pharmacokinetics of psilocybin, we further conducted an exploratory analysis on the association between neocortex [^{11}C]Cimbi-36 BP_{ND} and time-to-peak (T_{MAX}) in plasma psilocin for a sub-set of the sample ($n=6$).

Associations between the three SDI parameters and MEQ total score were assessed using Pearson's correlation coefficient, *p*-values were FWER-corrected for six tests.

Model assumptions were assessed visually and showed no evidence of model misspecification. We reported uncorrected *p*-values (p_{UNC}) and FWER-corrected *p*-values (p_{FWER}) where necessary. All statistical analyses were conducted in the statistical software R (version 3.3.1; Team RC, 2019).

Surface [^{11}C]Cimbi-36 BP_{ND} maps were estimated using the PETSurfer tool within Freesurfer as described previously and used for visualization purposes only (Greve et al., 2014) (Figure 1).

Results

Descriptive statistics

Table 1 shows descriptive data for the study sample. The sample included 16 healthy research participants (mean age ± SD (range), 30.3 ± 6 (24.2–49.8), six females). Thirteen participants were psychedelic-naïve prior to the study and three participants had previous experience with psychedelic drugs (self-reported drug use (number of years since last use): two participants, 1 ayahuasca (3.5 years); one participant, 25 psilocybin (0.5 year) and 30 lysergic acid diethylamide (1 year)). Inclusion/exclusion of this 'experienced user' did not substantively affect associations and all reported effects include this dataset. [^{11}C]Cimbi-36 PET scans were acquired near in time to the psilocybin intervention (median (range), 25 days (1–68)). No adverse reactions to the drug were observed and no participants required interpersonal crisis support.

Estimation of SDI parameters

Figure 2 shows the temporal modeling of SDI for each participant. Our segmented two-breakpoint model showed excellent fit, capturing nearly all variance in SDI (average variance explained (range): 94% (87–98%)). On average, participants reached SDI peak at 71 min post ingestion (range, 43–167 min), plateaued at peak for 82 min (range, 32–143 min) and took 143 min (range, 77–333 min) to return from peak to normal waking consciousness (Table 1).

5-HT2AR binding and subjective drug effects

Effects of pre-drug 5-HT2AR binding and SDI. Table 2 shows results from the regression models. Figure 3 shows scatterplots of neocortex [^{11}C]Cimbi-36 BP_{ND} against the three SDI outcomes. Pre-drug neocortex [^{11}C]Cimbi-36 BP_{ND} was statistically significantly negatively associated with peak plateau duration (estimate (95% confidence intervals (CI)), *p*-value: −143.6 min/unit 5-HT2AR binding (−238; −49.6), $p_{UNC}=0.006$, $p_{FWER}=0.018$) and positively associated with the return slope coefficient (1.2 SDI units/10 min/unit 5-HT2AR binding (0.6; 1.9), $p_{UNC}=0.001$, $p_{FWER}=0.003$). Thus, lower brain 5-HT2AR was associated with a longer peak plateau period and more rapid decrease in SDI. 5-HT2AR binding was not statistically significantly associated with the onset slope coefficient ($p_{UNC}=0.9$, $p_{FWER}=1$).

Effects of pre-drug 5-HT2AR binding and MEQ. Figure 3 shows a scatterplot of neocortex [^{11}C]Cimbi-36 BP_{ND} against MEQ total score. Neocortex [^{11}C]Cimbi-36 BP_{ND} was statistically significantly negatively associated with the MEQ total score (estimate (95%CI), *p*-value: −3.4 MEQ units/unit 5-HT2AR binding (−6.2; −0.7), $p_{UNC}=0.02$).

We explored post-hoc associations between neocortex [^{11}C]Cimbi-36 BP_{ND} and the four MEQ sub-scales. Similar effects were observed for the sub-scales: positive mood changes (−3.3 MEQ units/unit 5-HT2AR binding (−6.2; −0.5)), mystical (−3.8 MEQ units/unit 5-HT2AR binding (−7.6; −0.02)) and transcendence of time and space (−3.5 MEQ units/unit 5-HT2AR binding (−7.4; −0.4)), whereas a smaller effect was observed for the

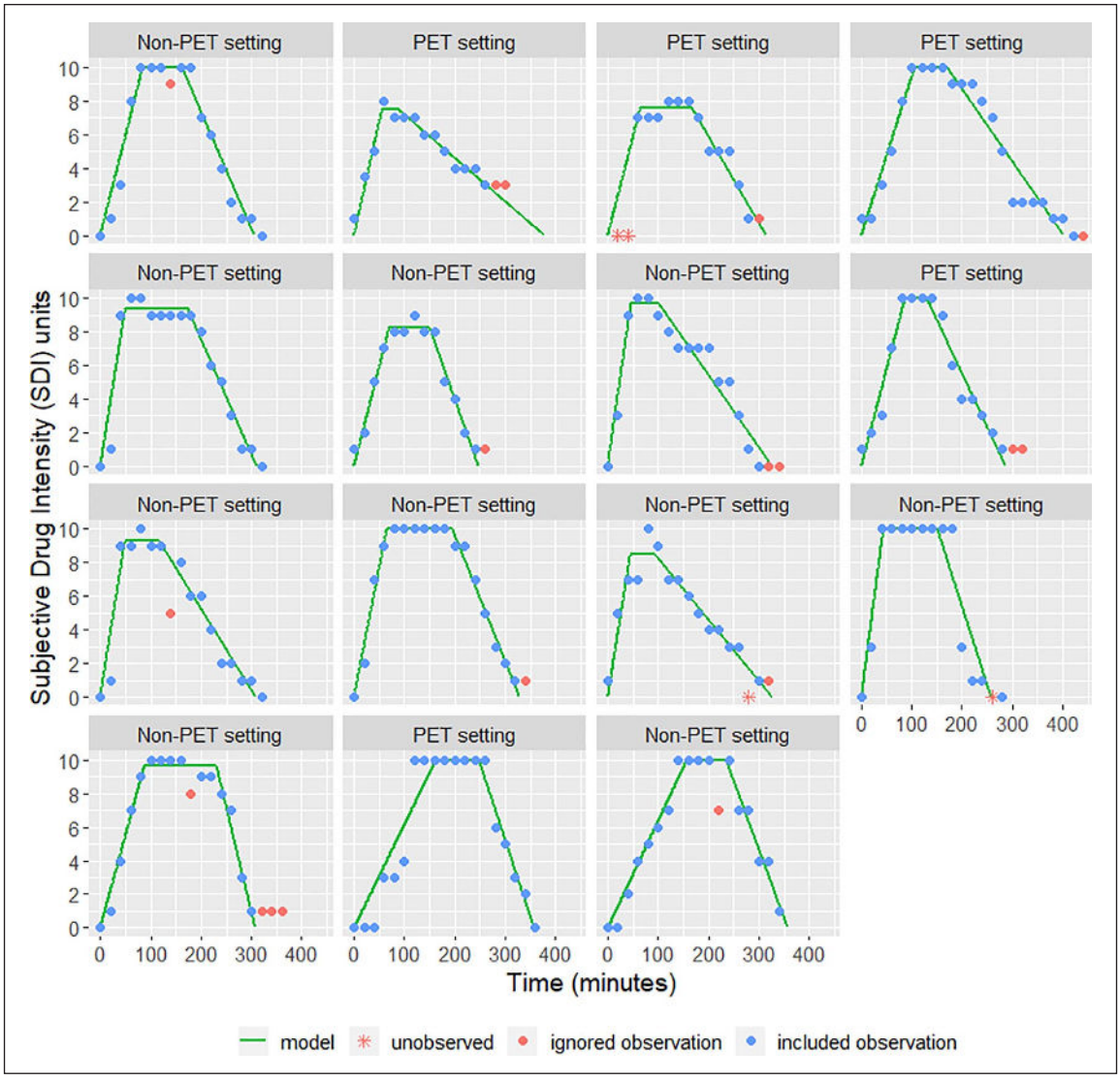

Figure 2. Subjective drug intensity (SDI) time courses. Illustration of the segmented two-breakpoint modelling of the SDI time courses for each participant. The green line represents our three primary model outcomes: (1) onset slope of psychoactive effects, (b) duration of peak plateau and (c) slope of decrease in psychoactive effects. PET, positron emission tomography.

sub-scale Ineffability (−1.9 MEQ units/unit 5-HT2AR binding (−5.2; 1.4)).

Exploratory analysis of pre-drug 5-HT2AR binding and T_{MAX}. In a subset of the sample ($n=6$), we had available data to explore the association between neocortex [^{11}C]Cimbi-36 BP_{ND} and the time to reach peak in plasma psilocin concentration (T_{MAX}). Although a small sample, we found little evidence of an association (estimate (95% CI), standardized regression coefficient, R^2: 1.7 T_{MAX} units/unit 5-HT2AR binding (426.8; 430.1), 0.005, $R^2 < 0.0001$).

Correlations between SDI and MEQ

Figure 4 shows the correlation matrix of the SDI and MEQ outcomes. Duration of peak plateau was negatively correlated with the return slope coefficient ($r=-0.76, p_{UNC}=0.002, p_{FWER}=0.012$) and positively correlated with MEQ total score ($r=0.75$, $p_{UNC}=0.003$, $p_{FWER}=0.018$). The return slope coefficient was negatively correlated with MEQ total score ($r=-0.84$, $p_{UNC}<0.0001$, $p_{FWER}<0.001$). The onset slope coefficient was not correlated with any of the other measures (all $p_{UNC}>0.3$, $p_{FWER}=1$).

Discussion

This is the first study to investigate the association between *in-vivo* brain 5-HT2AR PET binding and psilocybin-induced psychedelic effects in healthy individuals. Consistent with our hypotheses, pre-drug neocortex 5-HT2AR binding was statistically significantly associated with the temporal dynamics of SDI and retrospective MEQ responses. Lower pre-drug 5-HT2AR binding was associated with longer peak effects, a more rapid decrease in SDI effects and higher MEQ scores. Together these

Table 1. Descriptive information about the study participants.

Measures	Study sample, $n=16$		
	Mean ± *SD* or %	Median	Range, min, max
Age (years)	30.3 ± 6	28.6	24.2, 49.8
BMI (kg/m^2)	25.1 ± 3.4	24.4	21.2, 33.2
Sex (female)	38%		
Education	16.7 ± 0.8	17	14, 17
Weight-adjusted psilocybin dose (mg/kg)	0.24 ± 0.05	0.26	0.15, 0.31
Neocortex 5-HT2AR BP_{ND}	1.1 ± 0.2	1.2	0.8, 1.4
Onset slope coefficient/10 min ($n=15$)[a]	1.4 ± 0.5	1.2	0.6, 2.3
Duration of peak plateau (min) ($n=15$)[a]	82.4 ± 33.1	77.5	32, 142.8
Return slope coefficient per 10 min ($n=15$)[a]	−0.7 ± 0.3	−0.7	−1.3, −0.3
Mystical type experience total score	3.6 ± 1	3.8	1.9, 4.9
MEQ30 mystical sub-scale	3.5 ± 1.2	3.7	0.9, 4.9
MEQ30 positive moods sub-scale	3.6 ± 0.9	3.6	2.2, 4.8
MEQ30 transcendence sub-scale	3.7 ± 1.2	4.4	1.8, 5
MEQ30 ineffability sub-scale	4.2 ± 0.9	4.3	1.7, 5
Days from PET to intervention	26.7 ± 16	25	1, 68

5-HT2AR, serotonin 2A receptor; BMI, body mass index; BP_{ND}, non-displaceable binding potential; MEQ, Mystical Type Experience Questionnaire; PET, positron emission tomography.
[a]Due to missing data, parameter was not estimated for one participant.

Table 2. Associations between baseline neocortex serotonin 2A receptor (5-HT2AR) binding and perceptual effects.

Outcomes	Study sample, $n=16$				Stand. Beta
	Point estimate	*SE*	95% CI	*p*-value (UNC, FWER)	
Onset slope coefficient/10 min	0.2	0.9	−1.8, 2.1	0.9, 1	0.05
Duration of peak plateau	−143.7	43.5	−238, −49.6	0.006, 0.02	−0.7
Return slope coefficient/10 min	1.3	0.3	0.6, 1.9	0.001, 0.003	0.8
MEQ30 total score	−3.4	1.3	−6.2, −0.7	0.02	−0.6

FWE, family-wise error corrected; Stand. Beta: standardized regression coefficient; UNC, uncorrected; CI, confidence interval.

results provide a novel perspective on the neuro-molecular mechanisms mediating perceptual effects of psilocybin, highlighting a prominent role for 5-HT2AR in shaping the temporal dynamics of subjective psychedelic effects and self-reported mystical-type experiences.

We evaluated the temporal dynamics of a simple, real-time self-report of psilocybin-induced SDI in healthy individuals by assessing SDI every 20 min. Although subjective, this provides a quantitative, within-subject SDI time course that minimally disrupts the psychedelic session. We modelled this time course to estimate the three phases proposed in the phenomenological description of the psychedelic experience (Preller and Vollenweider, 2018). Our results indicate that this approach effectively parameterized SDI time courses (average of 94% variance explained) and that these parameters appear to map onto MEQ scores, a clinically relevant self-report outcome associated with long-term outcome of psilocybin intervention (see below). In particular, our results suggest that a longer peak plateau and a more rapid return to normal waking consciousness, as measured with SDI, are temporal subjective building blocks upon which a more profound mystical experience can unfold.

Although the importance of the mystical-type experience for long-term transformative benefits of psilocybin in healthy research participants is well established (Griffiths et al., 2006, 2011; MacLean et al., 2011), further studies are warranted to elucidate whether the temporal structure of SDI is similarly predictive of beneficial long-term changes. As we observed a strong negative correlation between peak plateau length and return slope coefficient, these measures may not be independent of each other but rather contingent within the relatively fixed time period of the psychedelic experience, and hence the potential importance of either for long-term effects should be established. In addition to the baseline PET scans used in this paper, six of our participants completed a PET scan during the psychedelic session (Madsen et al., 2019). Interestingly, we did not find evidence that the PET environment disrupted or otherwise significantly affected SDI and MEQ scores, despite previous reports that a PET setting increases risk of having a more anxious experience

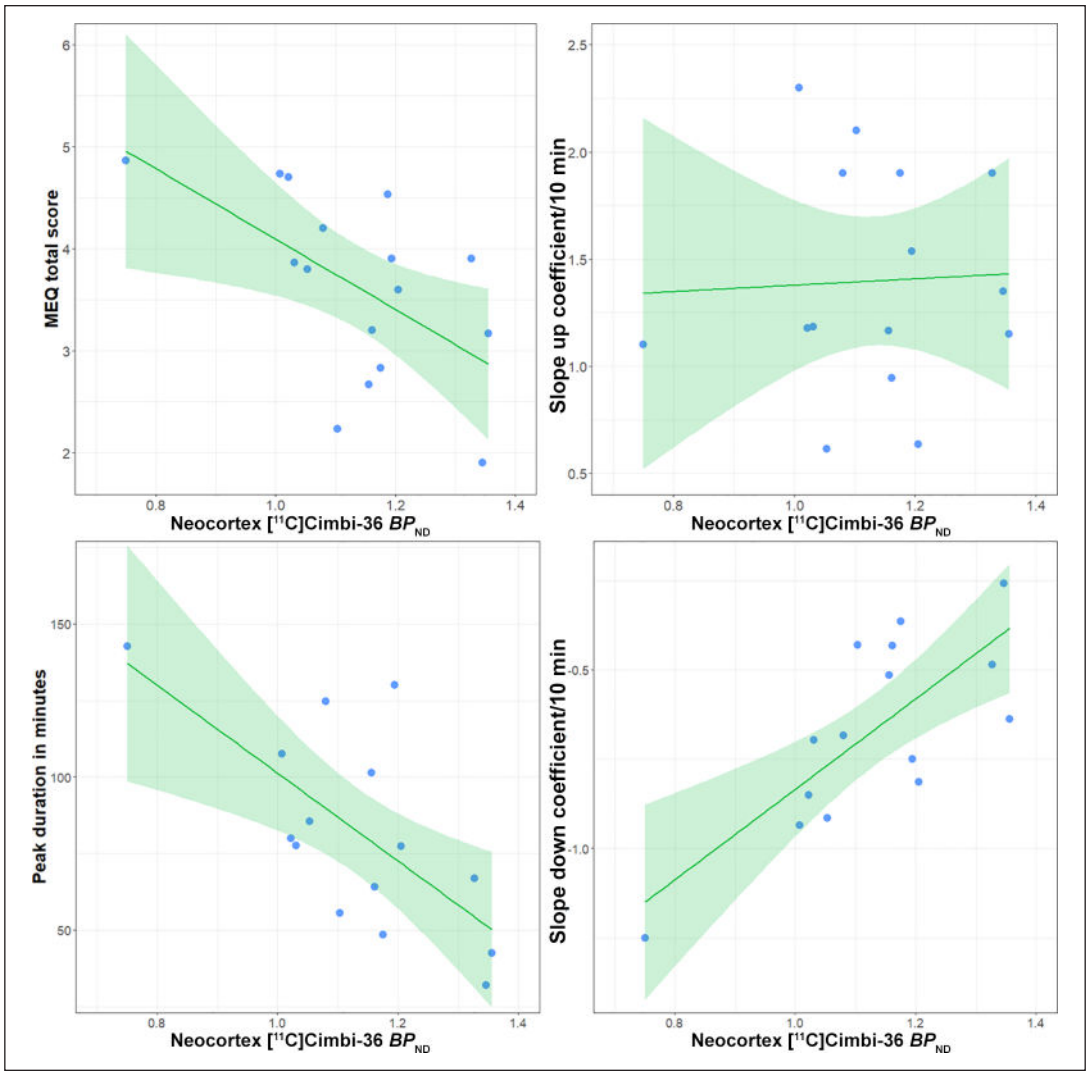

Figure 3. Serotonin 2A receptor (5-HT2AR) and the study outcomes subjective drug intensity (SDI) and Mystical Type Experience (MEQ). Four scatterplots of neocortex 5-HT2AR binding against our three primary SDI model outcomes and MEQ total score. Blue dots represent individual measurement points and green lines and shading for each line represent slope estimates and 95% confidence intervals.

(Studerus et al., 2012). As an important note in this regard, we attempted to create a private and comfortable space within the PET environment, including cushions, LED candles and blankets, etc., similar to the non-PET setting. Furthermore, we employed the same music playlist within the PET setting (including during scans) as in the non-PET setting and the same supporting psychologists were present throughout the PET setting (including during scans) as in the non-PET setting. Importantly, it thus seems that in a highly controlled and supported environment, PET imaging can be used without significantly disrupting the psychedelic experience.

We found that lower pre-drug 5-HT2AR binding was associated with a protracted peak plateau and an accelerated return to normal waking consciousness together with a greater mystical experience. In the above mentioned PET study, we showed that intake of psilocybin leads to a significant 5-HT2AR occupancy in the human brain and that higher 5-HT2AR occupancy is closely associated with higher SDI ratings (Madsen et al., 2019). However, these types of occupancy studies do not allow for a resolution of the temporal dynamics of the receptor stimulation. Assuming that the free fraction of radioligand in the tissue and the affinity of the radioligand to the 5-HT2AR are similar across individuals, then a lower BP_{ND} corresponds to a lower 5-HT2AR density. One explanation for our observation of an inverse association between 5-HT2AR binding and a more profound psychedelic experience could be that the temporal psilocin-5-HT2AR interaction dynamic is different; the occupancy peaks more quickly and because of lower tissue 5-HT2AR density, psilocin is more rapidly cleared from the interstitial fluid. Another explanation may be that 5-HT2AR binding is associated with plasma psilocin pharmacokinetics, that is, the observed effects are a function of plasma psilocin. We have previously shown an association between psilocin concentration and SDI (Madsen et al., 2019), as have two previous studies using lysergic acid diethylamide (LSD) (Dolder et al., 2017; Holze et al., 2019), supporting the utility of subject-specific pharmacokinetics when investigating subjective effects

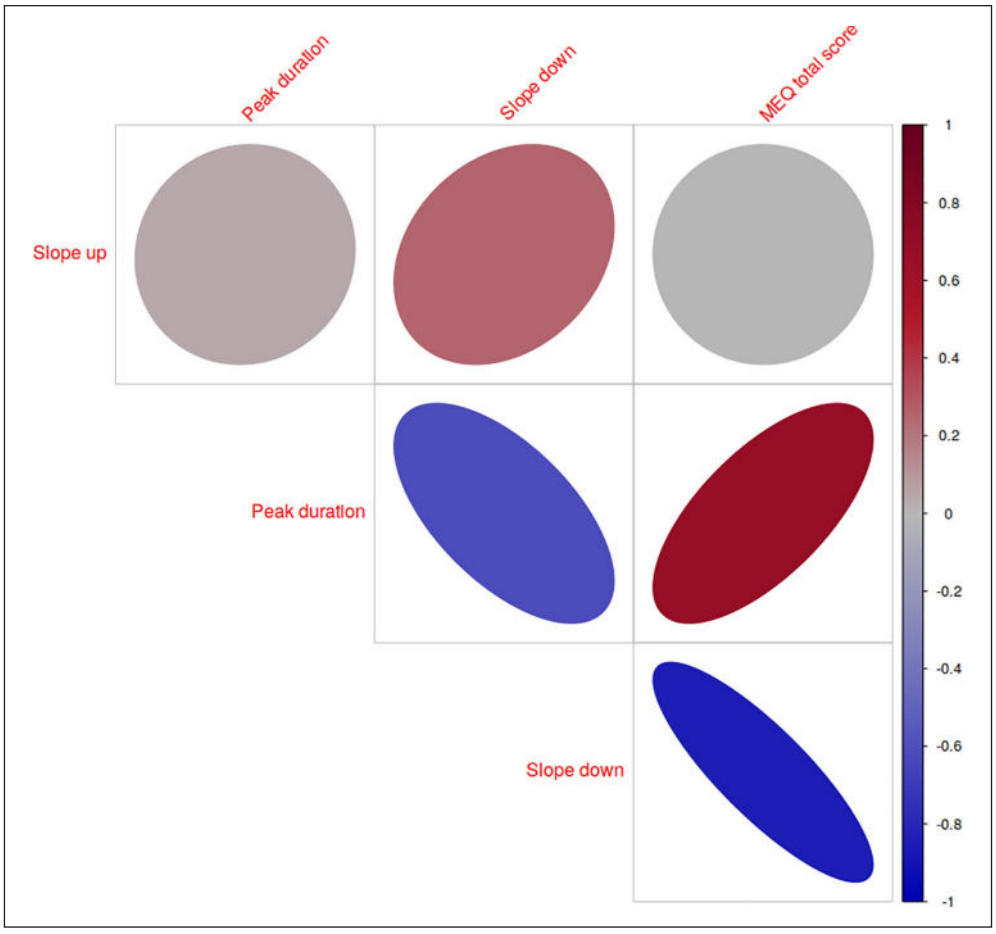

Figure 4. Correlations between subjective drug intensity (SDI) and Mystical Type Experience (MEQ) outcomes. Correlogram of the three SDI outcomes: (a) onset slope of psychoactive effects, (b) duration of peak plateau and (c) slope of decrease in psychoactive effects and MEQ total score. On a spectrum from grey to red, higher degree of red indicates a more positive correlation (rho) and on a spectrum from grey to blue, higher degree of blue indicates a negative correlation. Strength of the correlation is also indicated by ellipse shape.

of psychedelics. In the subset of participants for which plasma psilocin levels were available ($n=6$), we found no evidence of an association between pre-drug 5-HT2AR binding and T_{MAX} in plasma psilocin. However, this subsample is small, and it is difficult to draw a clear conclusion from it. Insofar as possible, future studies should measure plasma psilocin concentrations to account for pharmacokinetic differences between participants.

Lower 5-HT2AR binding may be a marker of higher volume-distributed serotonin in the brain. This is partially supported by preclinical evidence that antidepressants downregulate 5-HT2R including 5-HT2AR levels (Gray and Roth, 2001), as shown for tricyclic antidepressants (Kellar et al., 1981; Peroutka and Snyder, 1980; Sanders-Bush et al., 1989), monoamine oxidase inhibitors (Kellar et al., 1981; Peroutka and Snyder, 1980) and serotonin reuptake inhibitors (Sanders-Bush et al., 1989; Stolz et al., 1983), the latter although with mixed results (Hrdina and Vu, 1993). Downregulation of 5-HT2AR binding after tricyclic antidepressant treatment in patients diagnosed with depression has also been shown (Yatham et al., 1999). Given that antidepressants are thought to increase serotonin availability in the brain, this would seem to suggest an inverse coupling between available serotonin in the brain and 5-HT2AR levels. As such, it could be that lower 5-HT2AR binding indicates higher pre-drug serotonin availability and that this combined with intake of psilocybin leads to more effective receptor stimulation and hence a more profound psychedelic experience. On the other hand, it is hypothesized and has been reported via retrospective survey responses that serotonin reuptake inhibitors blunt the response to serotonin psychedelics (Bonson et al., 1996), which would suggest that lower 5-HT2AR levels are associated with a reduced response to psychedelics. Notably, pharmacologically-induced modulation of 5-HT2AR levels may not accurately represent homeostatic individual differences in 5-HT2AR levels, which may stem from, for example, genetic regulation. Overall, our results reinforce 5-HT2AR as a central neuro-molecular mechanism in mediating the psychedelic experience and we suggest future studies examine whether pre-drug 5-HT2AR binding predicts therapeutic effects of psilocybin in clinical populations.

Methodological considerations

The results presented in this paper should be interpreted in light of some limitations. As with many PET studies, our sample size is relatively small, and the results should therefore be replicated in a larger study. Given that we have previously shown a high correlation between plasma psilocin and 5-HT2AR occupancy,

we suggest that larger studies make use of plasma psilocin levels as a surrogate for 5-HT2AR stimulation to evaluate how this receptor plays a role in the psychedelic experience. We used a weight-adjusted dose, which a recent study suggested may not substantively affect the pharmacokinetics of plasma psilocin, vis-a-vis a fixed dose (Brown et al., 2017). Future studies should seek to elucidate sources of individual variability in psilocin pharmacokinetics, as it is related to the subjective experience (Madsen et al., 2019). In terms of SDI estimation, we do not know upon which experiential content participants based their ratings (e.g. imagery, changes in perception, mood or somatic sensations, etc.). However, we argue that SDI is a meaningful summary measure, supported by the previously mentioned correlations between SDI and 5-HT2AR occupancy and with retrospective questionnaire evaluation (Madsen et al., 2019), but also by the strong correlations observed in this study between SDI and MEQ. A major advantage of the SDI approach is the minimal cognitive load on participants and minimal disturbance of the psychedelic experience while still obtaining a real-time measure of perceptual effects. In particular, we argue that this approach is useful when combined with neuroimaging since it can be more closely related in time to the imaging data of interest.

In summary, we found that in humans lower pre-drug 5-HT2AR binding was associated with longer peak psychedelic effects, a more rapid return to normal waking consciousness together with a greater mystical type experience. These findings reinforce the role of 5-HT2AR binding in shaping critical temporal and mystical features of the psychedelic experience.

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