# Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels

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Short title: Psilocybin experience & 5-HT2AR occupancy

**Abstract**

The main psychedelic component of *magic mushrooms* is psilocybin, which shows promise as a treatment for depression and other mental disorders. Psychedelic effects are believed to emerge through stimulation of serotonin 2A receptors (5-HT2ARs) by psilocybin's active metabolite, psilocin. We here report for the first time the relationship between intensity of psychedelic effects, cerebral 5-HT2AR occupancy and plasma levels of psilocin in humans.

Eight healthy volunteers underwent positron emission tomography (PET) scans with the 5-HT2AR agonist radioligand [$^{11}$C]Cimbi-36: one at baseline and one or two additional scans on the same day after a single oral intake of psilocybin (3-30 mg). 5-HT2AR occupancy was calculated as the percent change in cerebral 5-HT2AR binding relative to baseline. Subjective psychedelic intensity and plasma psilocin levels were measured during the scans. Relations between subjective intensity, 5-HT2AR occupancy, and plasma psilocin levels were modelled using non-linear regression.

Psilocybin intake resulted in dose-related 5-HT2AR occupancies up to 72%; plasma psilocin levels and 5-HT2AR occupancy conformed to a single-site binding model. Subjective intensity was correlated with both 5-HT2AR occupancy and psilocin levels as well as questionnaire scores.

We report for the first time that intake of psilocybin leads to significant 5-HT2AR occupancy in the human brain, and that both psilocin plasma levels and 5-HT2AR occupancy are closely associated with subjective intensity ratings, strongly supporting that stimulation of 5-HT2AR is a key determinant for the psychedelic experience. Important for clinical studies, psilocin time-concentration curves varied but psilocin levels were closely associated with psychedelic experience.

Keywords: Psilocybin, psilocin, 5-HT2AR, PET, psychedelic

## Introduction

Psilocybin is a classic serotonergic psychedelic drug and is the primary psychoactive compound in *magic mushrooms* (Hofmann *et al*, 1958). Its effects are in many ways similar to those of LSD and mescaline (Wolbach *et al*, 1962). Recent clinical trials have shown that psilocybin may be an effective treatment for neuropsychiatric disorders, including treatment-resistant major depressive disorder (MDD)(Carhart-Harris *et al*, 2016), cancer-related anxiety and depression (Griffiths *et al*, 2016; Ross *et al*, 2016), and for addiction to nicotine (Johnson *et al*, 2014) and alcohol (Bogenschutz *et al*, 2015). Thus, psilocybin is an emerging and promising drug for a range of mental disorders where existing drugs have shown shortcomings.

Preclinical findings (González-Maeso *et al*, 2007), human blocking studies (Kometer *et al*, 2012; Vollenweider *et al*, 1998) and preliminary data from a PET study (Quednow *et al*, 2010) strongly suggest that serotonergic psychedelics exert their psychoactive effects through the serotonin 2A receptor (5-HT2AR). However, 5-HT2AR target engagement of psilocybin's active metabolite, psilocin, as well as the pharmacodynamics, i.e., the relation between plasma psilocin levels and 5-HT2AR occupancy, still remain to be established. Importantly, the relationship between the subjective psychedelic experience, plasma psilocin levels and 5-HT2AR occupancy in the human brain is currently unknown.

Positron emission tomography (PET) is an imaging technique capable of quantifying receptor binding *in vivo* (Innis *et al*, 2007; Lammertsma and Hume, 1996). Coupled with drug administration and appropriate radiotracer selection, PET-studies can provide valuable knowledge about relationships between drug levels, drug target occupancy, and associations with clinical response or side-effects (Mamo *et al*, 2007). In the present study we took advantage of the recent development of a 5-HT2R agonist radioligand, [$^{11}$C]Cimbi-36 (Ettrup *et al*, 2014, 2016), to elucidate the direct

role of 5-HT2ARs in psilocybin's psychedelic effects in humans. Here, we for the first time describe the relationships between subjective psychedelic effects, 5-HT2AR occupancy and psilocin plasma concentrations.

## Methods and Materials

**Participants.** Eight healthy participants (three females, mean age ± SD 33.0 ± 7.1 years) were recruited from a database of individuals interested in participating in a human neuroimaging study investigating psilocybin. After providing written informed consent, participants underwent a screening procedure including screening for present or previous psychiatric disorders using Mini-International Neuropsychiatric Interview, Danish translation version 6.0.0 (Sheehan *et al*, 1998), neurological illness or significant somatic illness. Participants were healthy, see **Supplementary data** for complete exclusion criteria and individual participant descriptive data. History of serotonergic psychedelic drug use was noted for the five subjects with such experience (number of times used: 1 [0-55] (median [range]), time since last intake: 42 [6-156] months; **Supplementary data, Table 1**). Participants were thoroughly informed about the study prior to inclusion, including effects of psilocybin, potential side-effects and risks. On the day of information and screening (prior to intervention day), all participants attended a preparatory meeting with at least one of the psychologists present on intervention days to familiarize with the study setting and establish a rapport. The study was approved by the ethics committee for the capital region of Copenhagen (journal identifier: H-16028698, amendments: 56023, 56967, 57974, 59673, 60437, 62255) and Danish Medicines Agency (EudraCT identifier: 2016-004000-61, amendments: 2017014166, 2017082837, 2018023295).

**Procedures.**

Participants underwent a physical exam, including ECG, blood screening for pathology, and a screening for psychopathology. Participants completed baseline [$^{11}$C]Cimbi-36 PET (PET 0) and

MR imaging prior to the psilocybin intervention day (mean ± SD: 49 ± 12 days). A screening procedure for amphetamines, opioids, benzodiazepines, barbiturates, tetrahydrocannabinol, cocaine, ketamine, phencyclidine, and gamma hydroxybutyrate was done using a urine test (Rapid Response, BTNX Inc., Markham, Canada). Participants were asked to be well-rested, refrain from alcohol the day before neuroimaging, have only a light breakfast and abstain from caffeine on study days. On the intervention day and before psilocybin administration, participants were informed again about potential psilocybin effects and safety precautions, as suggested previously (Johnson *et al*, 2008). Two psychologists providing interpersonal support were present on intervention days. During all PET scans (including baseline), a standardized list of music was played on a stereo system in the PET room. The playlist was adapted from one kindly provided by Prof. Roland Griffiths, Johns Hopkins Medicine.

**Psilocybin interventions.** On the intervention day, participants ingested between 3 and 30 mg psilocybin (3 mg capsules) approximately one hour prior (mean ± SD: 58 min ± 13) to the first [$^{11}$C]Cimbi-36 post-drug PET scan (PET 1). Subjects 1-5 underwent a second post-drug PET scan (PET 2) later the same day (344 min ± 41 after psilocybin ingestion), while subjects 6, 7 and 8 underwent only PET 1 on the intervention day. Participants were blind to the dose of psilocybin they were given. Each scan lasted 120 min, descriptive data pertaining to PET scans are available in supplementary data (**Supplementary Table 2**). For assessment of plasma psilocin levels, venous blood samples were taken simultaneously with the [$^{11}$C]Cimbi-36 injection and at 20-minute intervals throughout each scan session. Subjective psychedelic intensity ratings (0-10 Likert scale, 0 = not intense at all, 10 = very intense) were assessed at 20-minute intervals throughout the day until effects had waned. Between the two intervention scans, participants listened to music in the scanner room with staff support as appropriate. This three-scan protocol enabled the determination of 5-HT2AR occupancy during high and low plasma psilocin levels in five individuals. At the end of the

intervention day (mean ± SD: 468 ± 80 min after psilocybin), participants filled out questionnaires capturing aspects of psychedelic experiences: 11-dimension altered states of consciousness questionnaire (11D-ASC) (Dittrich *et al*, 2006; Studerus *et al*, 2010), the 30-item mystical experiences questionnaire (MEQ30) (Barrett *et al*, 2015) and the ego-dissolution inventory (EDI) (Nour *et al*, 2016). All questionnaires were administered in Danish, having been translated and back-translated to English by native Danish, English and bilingual speakers.

**Psilocin plasma concentrations.** Plasma psilocin concentrations were determined using ultra performance liquid chromatography and tandem mass spectrometry. Analysis was performed in units of μg/kg, although data are here presented in units of μg/L. For detailed description of analysis, see supplementary data**.**

**Magnetic resonance imaging.** High resolution 3D T1- and T2-weighted images were acquired on a 3T Prisma scanner (Siemens, Erlangen, Germany) using a 64-channel head coil for the purpose of PET-image coregistration and segmentation (T1-weighted images: inversion time = 900ms, echo time = 2.58ms, repetition time = 1900ms, flip angle = 9°, in-plane matrix = 256x256, in-plane resolution = 0.9x0.9mm, 224 slices and a slice thickness of 0.9mm, no gap; T2-weighted images: echo time = 408 ms, repetition time = 3200ms, in-plane matrix = 256x256, in-plane resolution = 0.9x0.9mm, 208 slices and a slice thickness of 0.9mm, no gap).

**[$^{11}$C]Cimbi-36 PET data acquisition, processing and kinetic modelling.** Acquisition and processing of [$^{11}$C]Cimbi-36 PET data has been described previously (Ettrup *et al*, 2014, 2016), a similar pipeline was used here. PET images were acquired for 120-minutes on a high-resolution research tomography PET-scanner (CTI/Siemens, Knoxville, USA) after a bolus injection of [$^{11}$C]Cimbi-36 (**Supplementary data, Table 2**). Regions of interest were defined using Pvelab, a fully automated regional delineation procedure, and regional time-activity curves were extracted for kinetic modelling (Ettrup *et al*, 2014; Svarer *et al*, 2005).

Kinetic modelling was performed using the simplified reference tissue model (SRTM) (Ettrup *et al*, 2014; Lammertsma and Hume, 1996) with neocortex (a volume-weighted average of all cortical regions) chosen *a priori* as the region of interest due to the high expression of 5-HT2ARs and the consequent beneficial signal-to-noise ratio within this region (Beliveau *et al*, 2017). Cerebellum was chosen as the reference region (Ettrup *et al*, 2014). Non-displaceable binding potential ($BP_{ND}$) was the primary outcome measure (Innis *et al*, 2007).

**[$^{11}$C]Cimbi-36 metabolism.** Analysis of [$^{11}$C]Cimbi-36 radiometabolites was described in recent publications by our lab (Ettrup *et al*, 2014; Johansen *et al*, 2017). We did not observe effects of the psilocybin intervention on [$^{11}$C]Cimbi-36 radiometabolism or protein binding (see **Supplementary data** for details).

**Data analysis.** Within-scan plasma psilocin area under curve ($psilocin_{AUC}$) was calculated from psilocin plasma concentration time curves (**Fig. 1**), using the trapezoid method in GraphPad Prism (version 7.01, GraphPad Software, Inc., CA, USA) and normalized by 120 minutes (duration of blood sampling and PET scan) to yield a mean psilocin concentration, which was used for statistical analyses and figures **(Table 1)**.

Neocortical [$^{11}$C]Cimbi-36 $BP_{ND}$ was plotted against mean psilocin concentration and the relationship modelled using the following equation:

$$Occupancy = \frac{Occ_{max} * C_P}{EC_{50} + C_P}$$

where $Occ_{max}$ denotes the predicted highest attainable occupancy, $C_P$ is plasma psilocin concentration and $EC_{50}$ is the plasma psilocin concentration at 50% $Occ_{max}$ (Gunn and Rabiner, 2017) Modelling and curve fitting was performed in GraphPad Prism.

Subject 1 psilocin concentrations were below limit of quantification (LOQ, 0.5 μg/kg) but above limit of detection (LOD, 0.1 μg/kg) during all second scan time points. We evaluated psilocin-occupancy relations considering LOQ and LOD. Model parameters were similar ($Occ_{max}$ =

75.5% vs. 77.9%, $EC_{50}$ = 1.81 μg/L vs. 2.12 μg/L, respectively). Due to the minor difference in outcomes, we set plasma psilocin concentrations for all time points to the mean value (0.3 μg/kg).

We calculated the $EC_{50}$ (Kenakin, 2016) corresponding to PET 1 and PET 2 for each participant (mean $EC_{50}$ ± SD: PET 1 = 4.5 ± 1.9 μg/L, PET 2 = 6.2 ± 6.0 μg/L). The determined $EC_{50}$ did not differ between the two intervention scans (paired t-test, mean difference = -1.7, 95%CI [-10.2, 6.7], p = 0.6).

All statistical tests apart from non-linear modelling were performed in the statistical software package R (version 3.3.1).

We chose to assess associations between occupancy, plasma psilocin levels and subjective intensity ratings because the latter single, compound measure of drug-intensity was acquired simultaneously with PET 1 and PET 2, which was not the case with the MEQ-30, 11D-ASC and EDI questionnaires. Intensity ratings have previously been used in psychedelics research (Carhart-Harris *et al*, 2012). The questionnaires were not obtained until the end of the last scan session as we did not want to induce suggestive experiences by such a detailed questionnaire. Further, we believed that the intensity ratings would 1) be less sensitive to non-pharmacological modulators of psilocybin-induced altered states of consciousness (i.e., the context in which the drug is administrated (Carhart-Harris *et al*, 2018)), 2) be feasible to administer during scans, and 3) yield a better temporal resolution. Intensity rating was stopped before the end of PET 2 for all participants (n = 5). Thus, for the purpose of calculating mean within-PET 2 intensity, participants were asked if intensity had changed during PET 2 compared to the last recorded rating. All participants responded that intensity had not changed during PET 2, and thus the last recorded score was extrapolated and used to calculate mean PET 2 intensity. For the purpose of modelling the association between occupancy and intensity, a quadratic function was used $Intensity = \beta 1 * occupancy + \beta 2 * occupancy^2$ ), and for the purpose of modelling the association between

psilocin levels and intensity, a non-linear stimulus-response function similar to the occupancy model was used: $Intensity = \frac{Intensity_{max} * C_P}{EC_{50} + C_P}$. 95% Wald-type confidence intervals were computed for $\beta 1$ and $\beta 2$ using quantiles of the Student's t-distribution.

*Post-hoc* linear regression analyses of the association between mean PET 1 intensity ratings and three questionnaire responses (MEQ30, 11-D ASC, EDI) were performed. *Our main hypothesis was that the outcome of the questionnaires would correlate with intensity ratings during PET 1.* For these analyses, we report the unadjusted ($p_{unc.}$) and Bonferroni-adjusted ($p_{FWE}$) p-values. Further exploratory *post hoc* linear regression analyses are available in Supplementary data. The coefficient of determination ($R^2$) is reported as a measure of data variance explained by the respective model.

Voxel-level [$^{11}$C]Cimbi-36 $BP_{ND}$ maps were estimated using the PETSurfer tool within Freesurfer (Greve *et al*, 2014) as described previously (Beliveau *et al*, 2017) and used for visualization purposes only.

**Results**

**Psilocin occupancy at neocortical 5-HT2ARs.** Psilocybin intake was in all PET scans associated with considerable dose-related 5-HT2AR occupancies (PET 1 range: 43%-72%). Occupancies at PET 2 were also substantial (range: 27%-47%) with the exception of Subject 1 for which occupancy was 2% **(Table 1, Fig. 3)**.

**Psilocin levels and receptor occupancy relations.** We found a high inter-individual variability in the dose response curves (e.g. maximum concentration ($C_{max}$) median [range]: 11.9 [2.3-19.3] µg/L) **Fig. 1)**. The relation between plasma psilocin levels and neocortex 5-HT2AR occupancy conformed well to the non-linear regression model. $Occ_{max}$ [95% CI] determined from this model was 76.6 [67.3;88.0] %, $EC_{50}$ [95% CI] was 1.95 [1.17;3.15] µg/L, and $R^2$ was 0.92 (**Fig. 2**).

**Subjective intensity ratings correlate with occupancy and psilocin levels.** Subjective intensity ratings had a qualitatively similar time course compared to plasma psilocin levels (**Fig. 1**). We

found a positive nonlinear association between mean within-scan intensity ratings and psilocin levels. $Intensity_{max}$ [95% CI] was 10.8 [8.6;14.7] and $EC_{50}$ [95% CI] was *4.5* [2.1;9.8] µg/L, and $R^2$ was 0.35 (**Fig. 4**). We also observed a positive association between intensity ratings and occupancy that was well described by a quadratic relationship (β1 [95% CI]: -0.02 [-0.13;0.1], β2 [95% CI]: 0.002 [0.0006;0.003], $R^2$: 0.81, **Fig. 4**).

**Psychedelic questionnaire responses.** As expected, psilocybin had profound effects on the mental state of the participants (MEQ30 total score median [range]: 2.9 [1.6-4.5], 11D-ASC global score (sum of all dimensions) median [range]: 428.1 [35.1-772.1], EDI median [range]: 52 [4.0-97.9]) (see **Fig. S1-2 & Table S3** for detailed responses). *Post hoc* linear regressions showed positive associations between mean PET 1 intensity ratings and total MEQ30 score (β-estimate [95% CI]: 0.34 [0.044; 0.64], $p_{unc.}$ = 0.03, $p_{FWE}$ = 0.09, $R^2$: 0.57), global 11-D ASC score (β-estimate [95% CI]: 76.4 [27.8; 125], $p_{unc.}$ = 0.008, $p_{FWE}$ = 0.024, $R^2$: 0.71) and EDI score (β-estimate [95% CI]: 11.1 [2.23; 20], $p_{unc.}$ = 0.02, $p_{FWE}$ = 0.06, $R^2$: 0.61). For further information, see **Figure S3**.

**Discussion**

We here show that psilocybin ingestion of between 3 mg and 30 mg is associated with dose-dependent occupancy of cerebral 5-HT2ARs. Further, plasma psilocin concentration and 5-HT2AR occupancy are positively associated and the relationship conforms with a single-site binding model. Lastly, subjective intensity ratings are positively correlated with both neocortical 5-HT2AR occupancy and plasma psilocin levels, strongly supporting that stimulation of cerebral 5-HT2ARs is paramount for the psychedelic effects of psilocybin.

Similar to previous 5-HT2AR PET-imaging occupancy studies with other 5-HT2AR drugs (Gründer, M.D. *et al*, 1997; Nordstrom *et al*, 2008), we found that the single-site binding model provided a good fit of the relation between drug blood levels and 5-HT2AR occupancy, and predicted maximum occupancies were similar. Here, it is important to emphasize that the

occupancies detected with an agonist radioligand (such as [$^{11}$C]Cimbi-36) may differ from that of antagonist radioligands because an agonist radioligand may bind preferentially to receptors in the high-affinity state (Fitzgerald *et al*, 1999; López-Giménez *et al*, 2001). Thus, given that high-affinity receptors are believed to be most important for neurotransmission, an agonist radioligand may yield a more relevant estimate of receptor levels.

We found the $EC_{50}$ of psilocin to be 1.95 μg/L. This corresponds to 10 nM, which is in the same range of $K_i$ values from *in vitro* studies (rat cortex) performed with another 5-HT2AR agonist, [$^{125}$I]DOI: 6 nM (McKenna *et al*, 1990) or 25 nM (Blair *et al*, 2000).

The *post hoc* linear regressions showed positive associations between mean PET 1 intensity ratings and MEQ30, global 11-D ASC score, and EDI score, and intensity ratings correlated also with both occupancy and with psilocin levels **(Fig. 4)**. Thus, although the participants scored their overall intensity of the psychedelic experience based on a number of different components (e.g., imagery, changes in perception, stimulation of mood, feeling of enhanced meaning, somatic sensations, etc.), and probably also as a function of previous drug experience and psychological make-up ("set"), including personal coping style, our results show that intensity ratings constitute a meaningful global measure of psychedelic experience that is feasible to obtain with high temporal resolution.

Previous studies in humans reported that antagonists at 5-HT2A and 2C receptors can prevent perceptual effects after subsequent ingestion of psilocybin (Kometer *et al*, 2012; Vollenweider *et al*, 1998). Our data show that psilocin plasma levels correlate with occupancy **(Fig. 2)**, that psilocin levels and occupancy correlate with intensity **(Fig. 4)**, and that intensity correlates with scores of MEQ30, 11D-ASC and EDI. Thus, our findings strongly support that 5-HT2AR stimulation is central for psychedelic experiences in humans, and adding our findings to the existing literature, the

evidence is by now strong that the 5-HT2AR is indeed the critical molecular mediator of psychedelic effects of psilocybin.

Our model can in future studies assist to estimate psilocin brain 5-HT2AR receptor occupancy without the use of PET-imaging, by determining plasma psilocin levels. For example, Brown and colleagues recently reported that ingestion of 25 mg psilocybin results in a mean $C_{max}$ of about 15 ng/mL (Brown *et al*, 2017). Assuming analysis methods of similar quality, similar stability of psilocin samples and a plasma density of 1.02 g/ml (Trudnowski and Rico, 1974), this plasma psilocin level corresponds to 69% occupancy. There is considerable inter-individual variability in psilocybin pharmacokinetics (Brown *et al*, 2017; Hasler *et al*, 1997; Lindenblatt *et al*, 1998). Consistent with this, $C_{max}$ for Subject 3 (12 mg, 0.14 mg/kg) was higher than $C_{max}$ values for Subjects 4, 5 and 6 (15, 18 and 24 mg, respectively; 0.2, 0.2 and 0.3 mg/kg). Importantly, our data convincingly demonstrate that plasma psilocin levels correlate closely with the overall psychedelic experience, and it is possible that future clinical trials may benefit from relating psilocin levels and/or estimated occupancies to clinical effects, rather than absolute doses.

Recently, it has been argued that psychedelic "microdosing" , entailing a dose small enough to avoid noticeable perceptual effects (Fadiman and Korb, 2017), comes with benefits such as enhanced creativity, social interaction and mood. Although a dose range of 0.5-2 mg psilocybin has been suggested as a psilocybin microdose (Dr. James Fadiman, Institute of Transpersonal Psychology, personal communication), there are currently no data available to identify such a cut-off. Subject 1 received 3 mg (0.05 mg/kg), had noticeable perceptual effects and an occupancy of 43%. This indicates that a smaller dose/lower occupancy would be needed for microdosing studies. Based on our data, a dose range of 0.5-2 mg is a reasonable suggestion for potential psilocybin microdose studies.

A few limitations of the study should be noted. When fitted to a single-site binding model without constraining $Occ_{max}$ = 100%, we found $Occ_{max}$ = 77%. Possible explanations for this include violations of kinetic modelling assumptions (Lammertsma and Hume, 1996; Salinas *et al*, 2015), rapid internalization of 5-HT2AR or psilocybin-associated lowering of brain 5-HT levels. Although weaker than for 5-HT2AR, psilocin has also affinity to 5-HT 2B, 5-HT 2C, and 5-HT 1A receptors (Blair *et al*, 2000; Rickli *et al*, 2016); the affinity for the serotonin transporter (SERT) is about 100 times lower (Rickli *et al*, 2016). A net decrease in cerebral 5-HT levels due to psilocin agonist activity at 5-HT1A autoreceptors could lead to an underestimation of occupancy due to decreased competition at 5-HT2ARs during intervention scans (Jørgensen *et al*, 2016). *In vitro* studies reported that 5-HT2AR stimulation led to 5-HT2AR internalization (Buckholtz *et al*, 1985, 1988, 1990; Karaki *et al*, 2014). We cannot exclude that [$^{11}$C]- Cimbi-36, being an agonist radioligand, has different affinity to internalized 5-HT2AR, leading to an underestimation of occupancy. We did not observe a difference between $EC_{50}$ values of PET 1 and 2, suggesting that if internalization occurred, it occurred either very rapidly (within a few minutes) or very slowly (days after). For Subject 1 who received only 3 mg, occupancy was 43% at PET 1 and 2% at PET 2, speaking against 5-HT2AR internalization. Nevertheless, it would be interesting to investigate long-term effects of a single psilocybin dose on cerebral 5-HT2AR levels, as a potential molecular mediator of the long-term effects on personality and mood (Carhart-Harris *et al*, 2016; Griffiths *et al*, 2016; Maclean *et al*, 2011; Ross *et al*, 2016). Such a study is currently ongoing in our lab.

We did not observe statistically significant median head motion during PET 1 or PET 2 compared to baseline scans (**Supplementary Methods and Materials**). Participants 7 and 8 exhibited maximum motion of up to 35 and 20 mm during PET 1, respectively. Although this could affect the kinetic modelling, model fits were acceptable and comparable to baseline scans. Our conclusions are based on only eight participants, but five were investigated three times which generated two

occupancy measures for each of these participants. The majority of male participants, that participants were recruited as specifically interested in a neuroimaging study investigating psilocybin, and the narrow age range decreases generalizability of our findings to the extent there are sex- or age-dependent differences in psilocybin (Tylš *et al*, 2016) or radioligand kinetics and differences in psilocin levels, occupancy or intensity ratings as a function of propensity to seek study participation in a psychedelics research study. PET-environment was positively correlated with anxiety during a psilocybin intervention (Studerus *et al*, 2012) and we cannot exclude that the PET-environment influenced the psychedelic experience (Carhart-Harris *et al*, 2018), making experiences less comparable to therapeutic or naturalistic settings. Yet, our participants experienced anxiety only to a very limited extent (11-D ASC anxiety subscale (median [range]: 4.25 [0; 17.3]). The study was not placebo-controlled and it is possible that this may have ultimately affected intensity ratings. Also, we cannot rule out that metabolites of psilocin or expectation-induced changes in 5-HT levels could affect the occupancy estimates, although we are unaware of evidence suggesting this.

In summary, we find that in humans, psychedelic effects of psilocybin are closely correlated with psilocin stimulation of the 5-HT2AR, and our data allows for an objective assessment of psilocybin effects on 5-HT2AR in future studies, by measuring plasma psilocin levels.

**Funding and Disclosure.** The study was supported by Innovation Fund Denmark (grant ID 4108-00004B), Independent Research Fund Denmark (grant ID 6110-00518B), and Ester M. og Konrad Kristian Sigurdssons Dyreværnsfond (grant ID 850-22-55166-17-LNG). MKM was supported through a scholarship stipend from Rigshospitalet's Research Council (grant ID R130-A5324). DB was supported by a scholarship stipend from the Lundbeck Foundation. BO was

supported by the Lundbeck foundation (grant ID R231-2016-3236) and Marie-Curie-NEUROMODEL (Grant ID 746850). The authors declare no conflict of interest.

**Acknowledgments.** We gratefully acknowledge the assistance of Lone Freyr, Gerda Thomsen, Svitlana Olsen, Josephine Torp, Annette Johansen, Camilla Larsen, Hanne Hansen, Vibeke Dam, Simone Pleinert, Sophia Armand, Dorthe Givard, and Peter Jensen. The John and Birthe Meyer Foundation is gratefully acknowledged for the donation of the Cyclotron and PET-scanner. We also gratefully acknowledge the University of Chemistry and Technology and the National Institute of Mental Health (Prague, Czech Republic) for production of psilocybin and Glostrup Apotek (Glostrup, Denmark) for encapsulation.

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**Figure legends.**

**Fig. 1. Psilocin and intensity rating time course.** (*A*) Plasma psilocin levels. Individual data points are measured plasma psilocin concentrations, fitted with spline fits. (*B*) Time course of subjective intensity ratings. Time = 0 indicates time of psilocybin ingestion.

**Fig. 2. Relationship between mean within-scan plasma psilocin levels and neocortical 5-HT2AR occupancy**. Estimated $EC_{50}$ [95% CI]: 1.95 [1.16;3.15] µg/L and $Occ_{max}$ [95% CI]: 76.6 [67.3;88.0] %.

**Fig. 3. Psilocybin occupancy of 5-HT2AR.** [$^{11}$C]Cimbi-36 $BP_{ND}$ map of the cortical surface of the left hemisphere of Subject 5 at baseline and at the first post-psilocybin intervention scan. Color bar in units $BP_{ND}$.

**Fig. 4. Subjective intensity of the psychedelic experience at the time of the PET scan, neocortical 5-HT2AR occupancy and plasma psilocin concentration.** *A)* Relationship between intensity ratings and neocortical 5-HT2AR occupancy. The fitted line was obtained using a quadratic function. *B)* Relationship between intensity and psilocin concentration, fitted to a single site receptor binding model.

| ID | Dose (mg) | Weight-adjusted dose (mg/kg) | $C_{max}$ (µg/L) | Mean psilocin PET 1 (µg/L) | Mean psilocin PET 2 (µg/L) | Occupancy PET 1 (%) | Occupancy PET 2 (%) |
|---|---|---|---|---|---|---|---|
| Subject 1 | 3 | 0.05 | 2.3 | 1.9 | <LOQ* | 42.9 | 1.8 |
| Subject 2 | 6 | 0.07 | 4.4 | 3.5 | 0.7 | 56.2 | 26.7 |
| Subject 3 | 12 | 0.14 | 16.7 | 12.6 | 3.4 | 66.4 | 42.9 |
| Subject 4 | 15 | 0.2 | 11.7 | 10.5 | 2.3 | 63.2 | 30.9 |
| Subject 5 | 18 | 0.2 | 11.8 | 10.6 | 2.6 | 72.4 | 47.0 |
| Subject 6 | 24 | 0.27 | 12.0 | 9.0 | NA | 60 | NA |
| Subject 7 | 24 | 0.3 | 18.9 | 11.5 | NA | 66 | NA |
| Subject 8 | 30 | 0.3 | 19.3 | 15.6 | NA | 65.2 | NA |

A

Plasma psilocin concentration (µg/L)

20
18
16
14
12
10
8
6
4
2
0

0 60 120 180 240 300 360 420 480

Time (min)

B

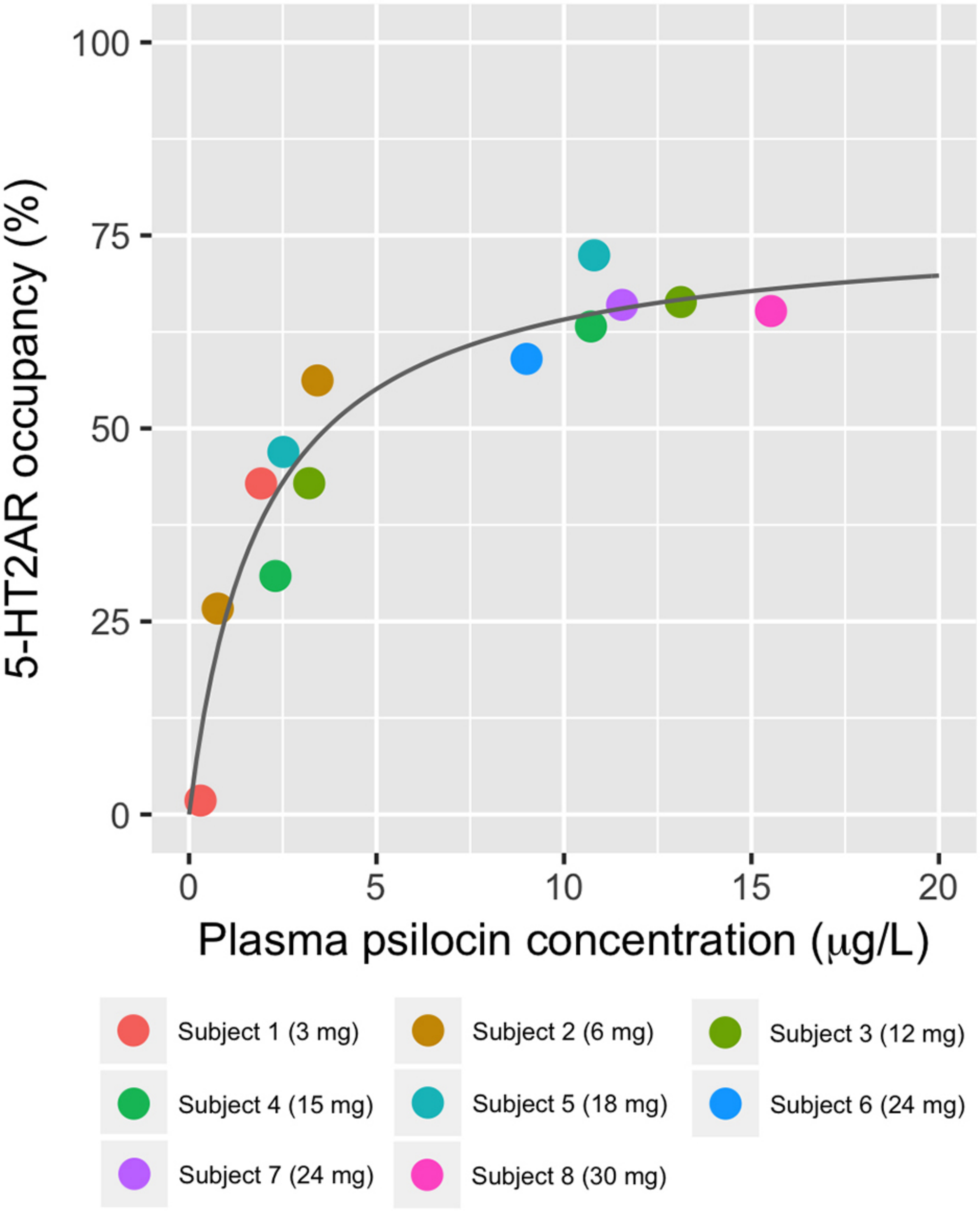
Intensity rating

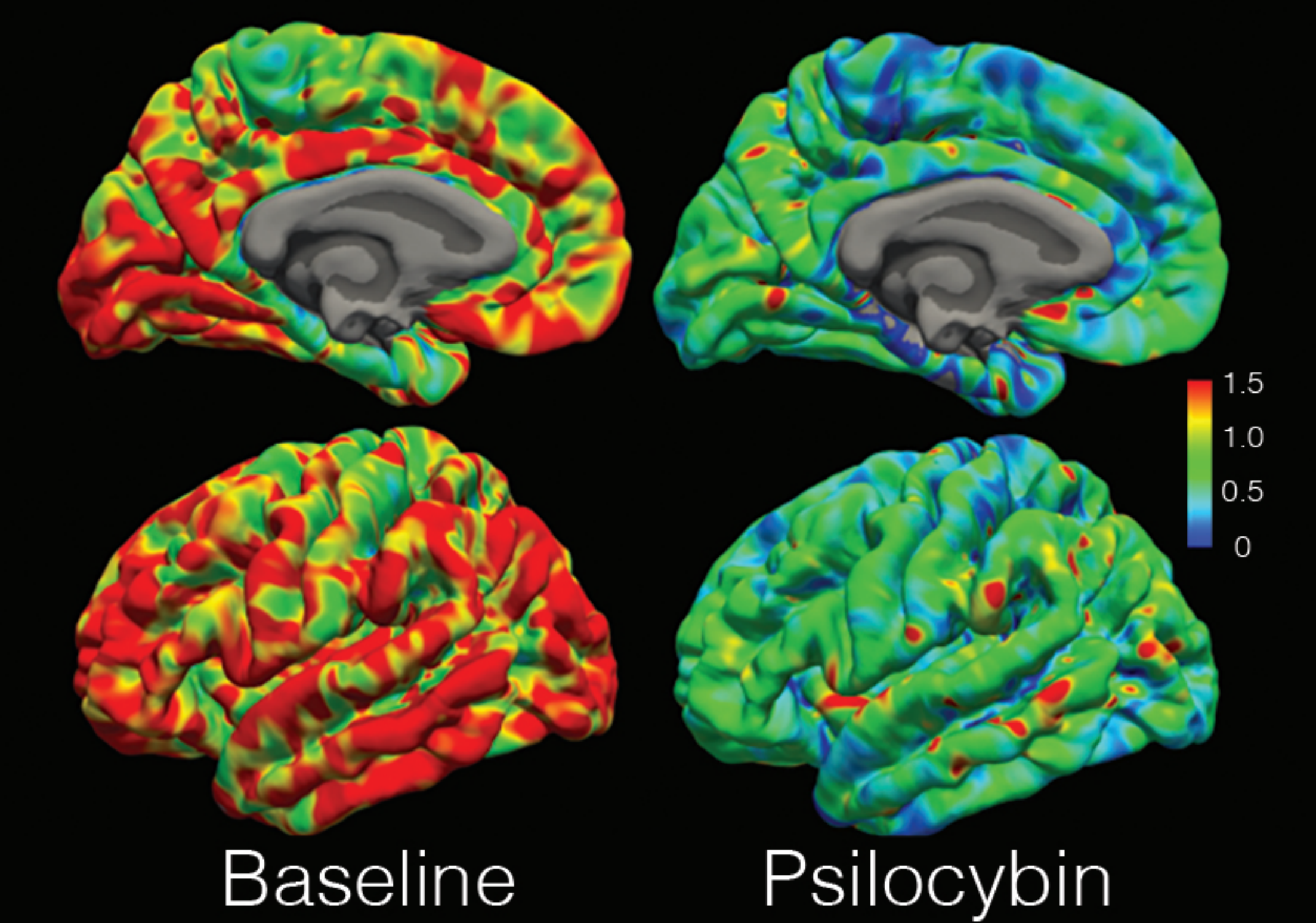
10
9
8
7
6
5
4
3
2
1
0

0 60 120 180 240 300 360 420

Time (min)

Subject 1 (3 mg)
Subject 2 (6 mg)
Subject 3 (12 mg)
Subject 4 (15 mg)
Subject 5 (18 mg)
Subject 6 (24 mg)
Subject 7 (24 mg)
Subject 8 (30 mg)


100
75
50
25
0
5-HT2AR occupancy (%)
0
5
10
15
20
Plasma psilocin concentration (µg/L)
Subject 1 (3 mg)
Subject 2 (6 mg)
Subject 3 (12 mg)
Subject 4 (15 mg)
Subject 5 (18 mg)
Subject 6 (24 mg)
Subject 7 (24 mg)
Subject 8 (30 mg)


1.5
1.0
0.5
0
Baseline
Psilocybin

A

B

Intensity rating

10.0
7.5
5.0
2.5
0.0

0
20
40
60
80

Occupancy (%)

0
5
10
15
20

Plasma psilocin concentration (μg/L)

Subject 1 (3 mg)
Subject 2 (6 mg)
Subject 3 (12 mg)
Subject 4 (15 mg)
Subject 5 (18 mg)
Subject 6 (24 mg)
Subject 7 (24 mg)
Subject 8 (30 mg)

# Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels

## Supplementary information.

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### Exclusion criteria.

Exclusion criteria included: 1) personal or immediate family history of psychiatric disorder including substance misuse disorder, 2) present or previous neurological condition/disease, significant somatic condition/disease or intake of drugs suspected to influence test results; 3) non-fluent Danish language skills; 4) vision or hearing impairment; 5) learning disability; 6) pregnancy; 7) breastfeeding; 8) MRI contraindications; 9) alcohol or drug abuse 10) allergy to test drugs; 11) significant exposure to radiation within the past year (e.g., medical imaging investigations); 12) intake of QT-prolonging medication or electrocardiogram (ECG) results indicative of heart disease, 13) history of significant adverse response to a hallucinogenic drug. 14) use of hallucinogenic drugs less than 6 months prior to inclusion; 15) blood donation less than 3 months before project participation; 16) bodyweight less than 50 kg; 17) low plasma ferritin levels (< 12 µg/L).

### Individual participant characteristics.

| | Sex | Age (years) | Bodyweight (kg) | Past psychedelic drug use* |
|---|---|---|---|---|
| Subject 1 | Female | 33 | 57 | Psilocybin: 2 (10)<br>LSD: 1 (13) |
| Subject 2 | Male | 32 | 86 | Psilocybin: 2 (2) |
| Subject 3 | Male | 31 | 83 | Ayahuasca: 1 (3.5) |

| | | | | |
|---|---|---|---|---|
| Subject 4 | Male | 29 | 75 | Ayahuasca: 1 (3.5) |
| Subject 5 | Male | 30 | 88 | None |
| Subject 6 | Female | 28 | 89 | None |
| Subject 7 | Female | 50 | 79 | None |
| Subject 8 | Male | 32 | 97 | Psilocybin: 25 (0.5)<br>LSD: 30 (1) |

**Table S1:** Subject characteristics. *Drug: number of times used (number of years between last intake and study participation).

## [$^{11}$C]Cimbi-36 PET-imaging data.

| | Baseline $BP_{ND}$ | PET 1 $BP_{ND}$ | PET 2 $BP_{ND}$ | Baseline scan | PET 1 | | PET 2 | |
|---|---|---|---|---|---|---|---|---|
| | | | | Dose (MBq) | Mass (μg) | Dose (MBq) | Mass (μg) | Dose (MBq) | Mass (μg) |
| Subject 1 | 1.02 | 0.59 | 1.00 | 454.9 | 0.9 | 546.7 | 0.48 | 586.5 | 0.70 |
| Subject 2 | 1.58 | 0.69 | 1.16 | 580. 9 | 1.18 | 526.0 | 0.62 | 530.0 | 0.49 |
| Subject 3 | 1.16 | 0.39 | 0.66 | 585.9 | 1.02 | 452.2 | 1.54 | 570.95 | 0.83 |
| Subject 4 | 1.16 | 0.43 | 0.80 | 391.4 | 1.23 | 177.9 | 1.17 | 189.2 | 0.74 |
| Subject 5 | 1.35 | 0.37 | 0.72 | 596.4 | 0.6 | 590.99 | 1.0 | 593.5 | 1.19 |
| Subject 6 | 1.19 | 0.49 | NA | 279.4 | 1.45 | 439.8 | 1.46 | NA | NA |
| Subject 7 | 1.052 | 0.36 | NA | 513.0 | 0.53 | 524.8 | 1.24 | NA | NA |
| Subject 8 | 1.36 | 0.47 | NA | 550.5 | 0.38 | 508.5 | 0.61 | NA | NA |

**Table S2**. Non-displaceable binding potentials ($BP_{ND}$) in neocortex, injected radioactivity dose and mass dose of Cimbi-36.

### Psychometric evaluation of psilocybin effects

In order to assess the participants' more detailed subjective experience of psilocybin intake, we administered questionnaires immediately after the last [$^{11}$C]Cimbi-36 PET-scan or when participants felt normal (app. 5-8 hours after psilocybin administration). In order to avoid to induce suggestive responses, we deliberately chose not to administer these questionnaires until the end of the study day. Thus, we cannot exclude that the questionnaire scores could be influenced by recall bias. The questionnaires were the 11-dimension altered states of consciousness **(Fig. S1)** (1, 2), the revised mystical experiences questionnaires **(Fig. S2)** (MEQ-30) (3), and the ego dissolution inventory **(Table S3)** (4).

The outcomes of all three questionnaires were congruent with staff members subjective evaluation of the participants' mental states, specifically that apart from subject 1, all participants experienced profound effects of psilocybin.

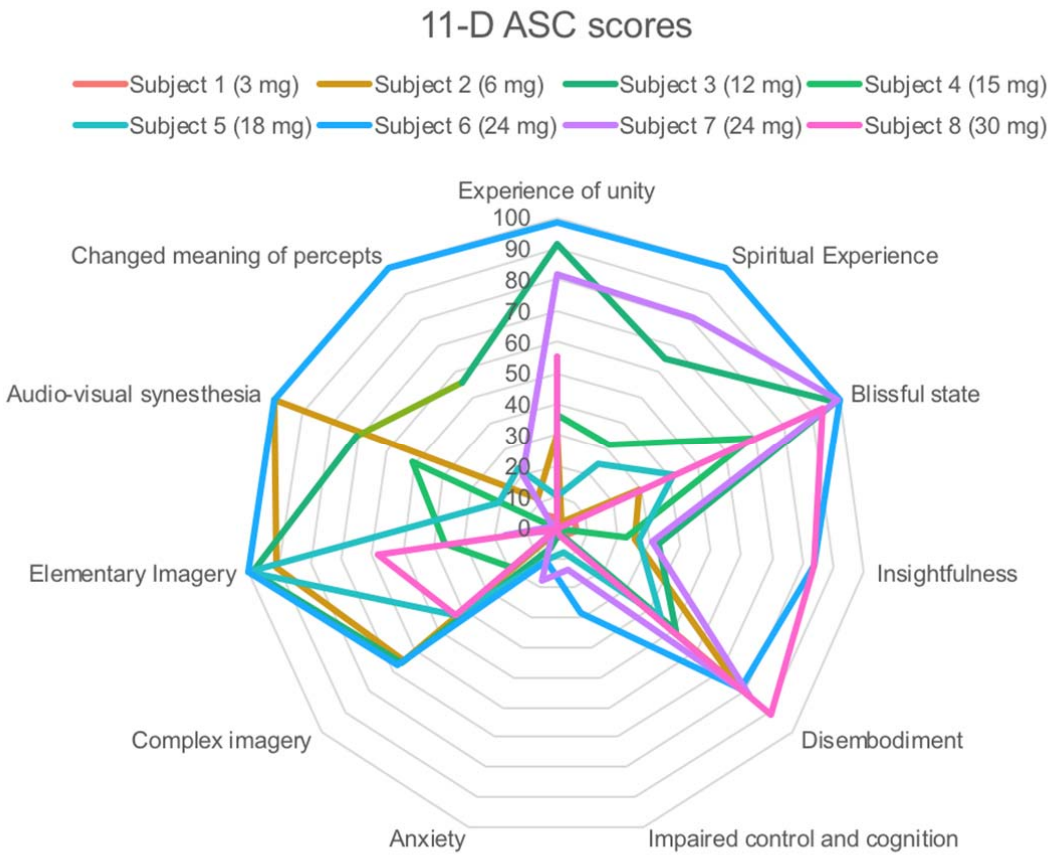


**Fig. S1.** 11-dimensional altered states of consciousness scale questionnaire results, maximum score is 100.

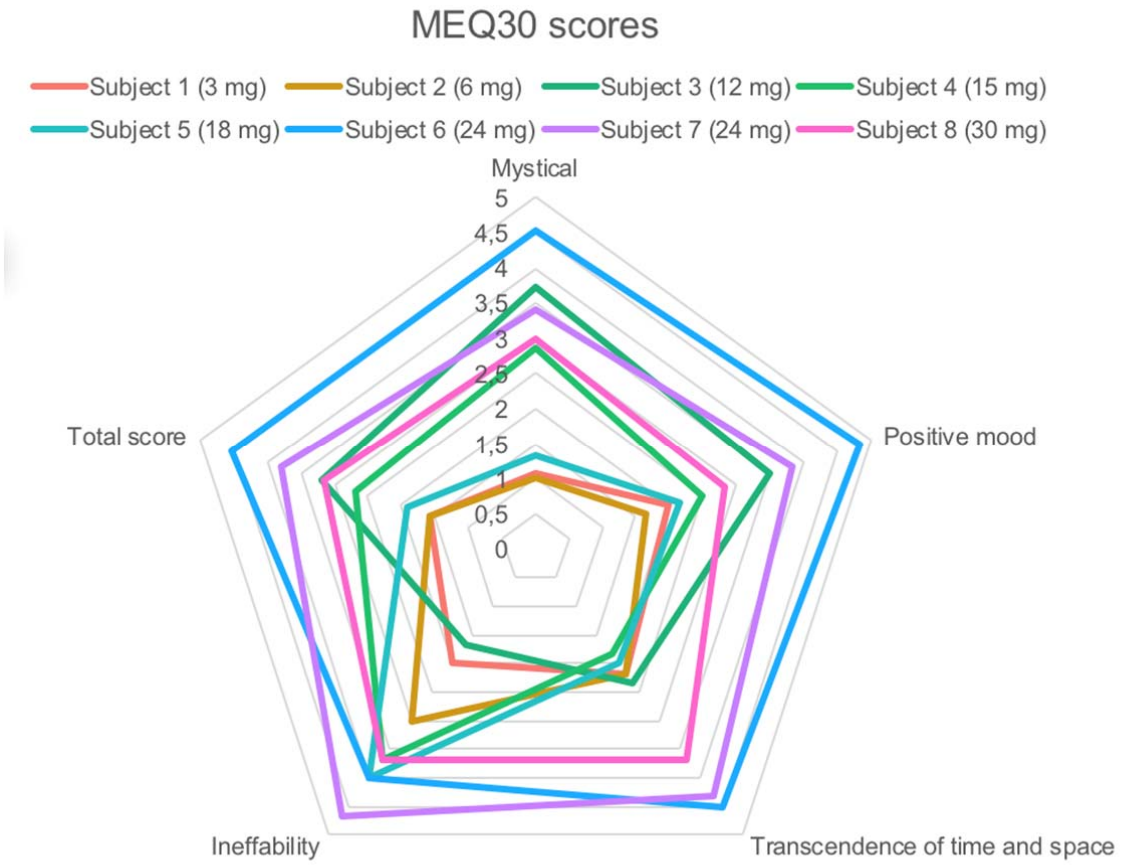


**Fig. S2.** Results of revised mystical experiences questionnaires (MEQ-30), maximum score is 5. Mean total score ± SD: 2.8 ± 1.1

| ID | EDI score |
|---|---|
| Subject 1 (3 mg) | 4 |
| Subject 2 (6 mg) | 38.5 |
| Subject 3 (12 mg) | 65.5 |
| Subject 4 (15 mg) | 37.375 |
| Subject 5 (18 mg) | 18.375 |
| Subject 6 (24 mg) | 80.625 |
| Subject 7 (24 mg) | 86.75 |
| Subject 8 (30 mg) | 97.875 |

**Table S3.** Results of ego dissolution inventory (EDI), maximum score is 100. Mean score ± SD: 53.6 ± 34.1

**Correlations between results of MEQ30, 11-D ASC, EDI, $C_{max}$, mean psilocin levels, and occupancy.**

Exploratory linear regressions were performed to evaluate associations of questionnaire responses of MEQ30, 11-D ASC, EDI with intensity ratings from PET 1, dose, adjusted dose, $C_{max}$, mean psilocin levels (n=8). Total MEQ-score, global ASC-score (i.e., the sum of all ASC dimensions) and EDI scores were selected *a priori* as variables of interest. After controlling Type I error rate, using Bonferroni-correction, no p-values were < 0.05.

| **Dependent variable** | **β** | **95% CI lower bound** | **95% CI upper bound** | **SE** | **$R^2$** | **$p_{unc.}$** |
|---|---|---|---|---|---|---|
| MEQ30 total | 0.34 | 0.0444 | 0.636 | 0.121 | 0.569 | 0.0306 |
| ASC Global | 76.4 | 27.8 | 125 | 19.9 | 0.711 | 0.00853 |
| EDI | 11.1 | 2.23 | 20 | 3.63 | 0.61 | 0.0221 |
| MEQ30 Mystical | 0.453 | 0.131 | 0.775 | 0.132 | 0.663 | 0.0138 |
| MEQ30 Positive mood | 0.298 | -0.0335 | 0.63 | 0.136 | 0.446 | 0.0701 |
| MEQ30 Transcendence of time & space | 0.208 | -0.203 | 0.619 | 0.168 | 0.204 | 0.262 |
| MEQ30 Ineffability | 0.126 | -0.289 | 0.54 | 0.169 | 0.084 | 0.486 |
| ASC Unity | 12.2 | 2.83 | 21.5 | 3.81 | 0.629 | 0.0188 |
| ASC Spiritual experience | 9.25 | -4.19 | 22.7 | 5.49 | 0.321 | 0.143 |
| ASC Blissful state | 14 | 7.41 | 20.5 | 2.68 | 0.819 | 0.00199 |
| ASC Insightfulness | 8.56 | 0.209 | 16.9 | 3.41 | 0.512 | 0.046 |
| ASC Disembodiment | 7.82 | -4.8 | 20.4 | 5.16 | 0.277 | 0.18 |
| ASC Impairment of | 1.31 | -2.44 | 5.07 | 1.53 | 0.109 | 0.425 |
| ASC Anxiety | 0.592 | -1.99 | 3.17 | 1.05 | 0.0499 | 0.595 |
| ASC Complex imagery | 6.46 | -3.02 | 15.9 | 3.88 | 0.317 | 0.146 |
| ASC Elementary Imagey | 9.66 | -4.12 | 23.4 | 5.63 | 0.329 | 0.137 |
| ASC Synaesthesia | 6.28 | -10.7 | 23.2 | 6.93 | 0.12 | 0.4 |
| ASC Changed meaning of percepts | 7.6 | -4.73 | 19.9 | 5.04 | 0.275 | 0.182 |

**Table S4.** Statistical outcome of linear regression. Independent variable: mean PET-1 subjective intensity.

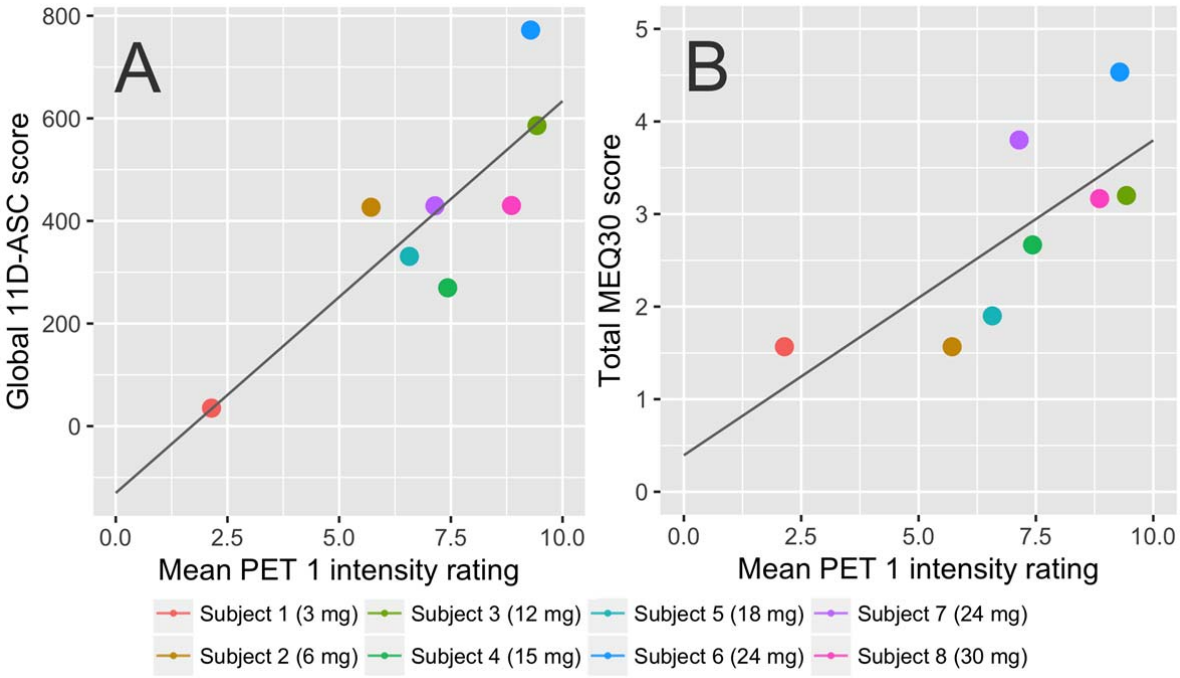


**Fig. S3. Intensity and psychedelic questionnaire scores.** *A)* Relationship between mean PET 1 intensity ratings and global 11-D ASC score, $R^2$ = 0.71, β [95% CI] = 76.4 [27.8;125.0]. *B)* Relationship between mean PET 1 intensity ratings and total score of MEQ30, $R^2$ = 0.57, β [95% CI] = 0.34 [0.04;0.64]. Global 11-D ASC and MEQ30 scores were both obtained after the completion of PET 2.

| Dependent variable | β | 95% CI lower bound | 95% CI upper bound | SE | $R^2$ | $p_{unc.}$ |
|---|---|---|---|---|---|---|
| MEQ30 total | 0.0105 | -0.112 | 0.133 | 0.0502 | 0.00725 | 0.841 |
| ASC Global | 1.56 | -23.2 | 26.3 | 10.1 | 0.00393 | 0.883 |
| EDI | 1.44 | -2.17 | 5.06 | 1.48 | 0.137 | 0.366 |
| MEQ30 Mystical | 0.0189 | -0.132 | 0.17 | 0.0617 | 0.0154 | 0.77 |
| MEQ30 Positive mood | -0.0106 | -0.132 | 0.111 | 0.0497 | 0.0075 | 0.838 |
| MEQ30 Transcendence of time & space | 0.00202 | -0.124 | 0.128 | 0.0516 | 0.000257 | 0.97 |
| MEQ30 Ineffability | 0.028 | -0.0873 | 0.143 | 0.0471 | 0.0556 | 0.574 |
| ASC Unity | -0.0902 | -4.28 | 4.1 | 1.71 | 0.000462 | 0.96 |
| ASC Spiritual experience | -1.42 | -5.66 | 2.81 | 1.73 | 0.101 | 0.443 |
| ASC Blissful state | 1.46 | -2.51 | 5.42 | 1.62 | 0.119 | 0.403 |
| ASC Insightfulness | 1.63 | -1.21 | 4.47 | 1.16 | 0.247 | 0.21 |
| ASC Disembodiment | 0.911 | -3.05 | 4.87 | 1.62 | 0.0501 | 0.594 |
| ASC Impairment of | -0.312 | -1.36 | 0.731 | 0.426 | 0.0818 | 0.492 |
| ASC Anxiety | -0.22 | -0.911 | 0.471 | 0.282 | 0.092 | 0.465 |
| ASC Complex imagery | 0.44 | -2.67 | 3.55 | 1.27 | 0.0196 | 0.741 |
| ASC Elementary Imagey | 0.889 | -3.63 | 5.41 | 1.85 | 0.0372 | 0.647 |
| ASC Synaesthesia | -1.74 | -6.38 | 2.9 | 1.9 | 0.123 | 0.394 |
| ASC Changed meaning of percepts | -1.16 | -4.96 | 2.63 | 1.55 | 0.086 | 0.481 |

**Table S5.** Statistical outcome of linear regression. Independent variable: dose (mg psilocybin).

| Dependent variable | β | 95% CI lower bound | 95% CI upper bound | SE | $R^2$ | $p_{unc.}$ |
|---|---|---|---|---|---|---|
| MEQ30 total | 8.61 | 1.74 | 15.5 | 2.81 | 0.611 | 0.022 |
| ASC Global | 1080 | -847 | 3010 | 788 | 0.239 | 0.219 |
| EDI | 272 | 56.8 | 488 | 88.1 | 0.614 | 0.0213 |
| MEQ30 Mystical | 9.31 | -0.563 | 19.2 | 4.04 | 0.47 | 0.0605 |
| MEQ30 Positive mood | 7.17 | -1.03 | 15.4 | 3.35 | 0.432 | 0.0763 |
| MEQ30 Transcendence of time & space | 8.46 | 1.04 | 15.9 | 3.04 | 0.564 | 0.0316 |
| MEQ30 Ineffability | 8.27 | 1.66 | 14.9 | 2.7 | 0.61 | 0.0222 |
| ASC Unity | 211 | -97.2 | 520 | 126 | 0.319 | 0.145 |
| ASC Spiritual experience | 199 | -147 | 544 | 141 | 0.249 | 0.209 |
| ASC Blissful state | 302 | 75.2 | 528 | 92.5 | 0.639 | 0.0173 |
| ASC Insightfulness | 205 | -3.83 | 413 | 85.2 | 0.49 | 0.0531 |
| ASC Disembodiment | 191 | -117 | 499 | 126 | 0.278 | 0.18 |
| ASC Impairment of | 50.2 | -33 | 133 | 34 | 0.266 | 0.19 |
| ASC Anxiety | 30.4 | -26.7 | 87.5 | 23.3 | 0.22 | 0.24 |
| ASC Complex imagery | -17.2 | -297 | 263 | 114 | 0.00375 | 0.885 |
| ASC Elementary Imagey | 12.1 | -399 | 423 | 168 | 0.000858 | 0.945 |
| ASC Synaesthesia | -110 | -538 | 318 | 175 | 0.0614 | 0.554 |
| ASC Changed meaning of percepts | 80.1 | -265 | 425 | 141 | 0.0511 | 0.59 |

**Table S6.** Statistical outcome of linear regression. Independent variable: adjusted dose (mg psilocybin per kg bodyweight).

| Dependent variable | β | 95% CI lower bound | 95% CI upper bound | SE | $R^2$ | $p_{unc.}$ |
|---|---|---|---|---|---|---|
| MEQ30 total | 0.123 | -0.00334 | 0.249 | 0.0516 | 0.486 | 0.0546 |
| ASC Global | 17.4 | -13.4 | 48.2 | 12.6 | 0.242 | 0.215 |
| EDI | 4.52 | 1.28 | 7.75 | 1.32 | 0.66 | 0.0142 |
| MEQ30 Mystical | 0.153 | -0.0019 | 0.307 | 0.0631 | 0.493 | 0.0521 |
| MEQ30 Positive mood | 0.101 | -0.041 | 0.243 | 0.0581 | 0.335 | 0.132 |
| MEQ30 Transcendence of time & space | 0.0966 | -0.0554 | 0.249 | 0.0621 | 0.287 | 0.171 |
| MEQ30 Ineffability | 0.0708 | -0.0831 | 0.225 | 0.0629 | 0.174 | 0.303 |
| ASC Unity | 3.97 | -0.501 | 8.45 | 1.83 | 0.44 | 0.0728 |
| ASC Spiritual experience | 2.9 | -2.77 | 8.58 | 2.32 | 0.207 | 0.257 |
| ASC Blissful state | 5.37 | 2.63 | 8.12 | 1.12 | 0.793 | 0.00303 |
| ASC Insightfulness | 2.49 | -1.46 | 6.45 | 1.62 | 0.284 | 0.174 |
| ASC Disembodiment | 2.86 | -2.18 | 7.91 | 2.06 | 0.243 | 0.215 |
| ASC Impairment of | 0.261 | -1.27 | 1.79 | 0.626 | 0.0282 | 0.691 |
| ASC Anxiety | 0.411 | -0.539 | 1.36 | 0.388 | 0.158 | 0.33 |
| ASC Complex imagery | 0.171 | -4.31 | 4.65 | 1.83 | 0.00145 | 0.929 |
| ASC Elementary Imagey | 0.722 | -5.81 | 7.26 | 2.67 | 0.012 | 0.796 |
| ASC Synaesthesia | -1.86 | -8.68 | 4.95 | 2.79 | 0.0694 | 0.528 |
| ASC Changed meaning of percepts | 0.896 | -4.7 | 6.49 | 2.29 | 0.025 | 0.709 |

**Table S7.** Statistical outcome of linear regression. Independent variable: Cmax.

| Dependent variable | β | 95% CI lower bound | 95% CI upper bound | SE | $R^2$ | $p_{unc.}$ |
|---|---|---|---|---|---|---|
| MEQ30 total | 0.137 | -0.0559 | 0.33 | 0.0789 | 0.335 | 0.133 |
| ASC Global | 20.8 | -22 | 63.6 | 17.5 | 0.191 | 0.28 |
| EDI | 5.16 | -0.232 | 10.6 | 2.21 | 0.478 | 0.0577 |
| MEQ30 Mystical | 0.183 | -0.0438 | 0.41 | 0.0928 | 0.394 | 0.0957 |
| MEQ30 Positive mood | 0.105 | -0.104 | 0.315 | 0.0856 | 0.201 | 0.266 |
| MEQ30 Transcendence of time & space | 0.0826 | -0.145 | 0.31 | 0.093 | 0.116 | 0.409 |
| MEQ30 Ineffability | 0.0795 | -0.134 | 0.293 | 0.0872 | 0.122 | 0.397 |
| ASC Unity | 4.24 | -2.6 | 11.1 | 2.79 | 0.277 | 0.18 |
| ASC Spiritual experience | 2.68 | -5.47 | 10.8 | 3.33 | 0.0973 | 0.452 |
| ASC Blissful state | 6.62 | 1.94 | 11.3 | 1.91 | 0.666 | 0.0135 |
| ASC Insightfulness | 3.39 | -1.91 | 8.68 | 2.16 | 0.29 | 0.169 |
| ASC Disembodiment | 2.78 | -4.52 | 10.1 | 2.98 | 0.126 | 0.388 |
| ASC Impairment of | 0.0992 | -1.99 | 2.19 | 0.853 | 0.00225 | 0.911 |
| ASC Anxiety | 0.287 | -1.07 | 1.65 | 0.556 | 0.0426 | 0.624 |
| ASC Complex imagery | 0.793 | -5.18 | 6.77 | 2.44 | 0.0173 | 0.756 |
| ASC Elementary Imagey | 1.97 | -6.65 | 10.6 | 3.52 | 0.0495 | 0.596 |
| ASC Synaesthesia | -2.2 | -11.4 | 7.05 | 3.78 | 0.0534 | 0.582 |
| ASC Changed meaning of percepts | 0.808 | -6.76 | 8.38 | 3.09 | 0.0112 | 0.803 |

**Table S8.** Statistical outcome of linear regression. Independent variable: normalized $psilocin_{AUC}$ (mean psilocin concentration)

**Plasma psilocin concentrations.**

Blood samples were drawn from an intravenous access in the antecubital vein and collected in EDTA vials, placed on ice, centrifuged, and plasma was aliquoted and stored at -20°C. Psilocin was obtained from Lipomed (Arlesheim, Switzerland) and Cerilliant (Round Rock, TX, USA) for calibrator and control batches, respectively, while the deuterated internal standard (IS) psilocin-d10 was from Cerilliant. Acetonitrile, methanol HPLC grade and water were obtained from Fishers Scientific (Loughborough, UK). Ascorbic acid was obtained from VWR (Hassrode, Belgium).

Stock solution (1000 mg/l) of psilocin and IS were prepared in acetonitrile and stored in amber ampoules at -20°C until use. Working standard solutions from 0.5 µg/l to 1000 µg/l were freshly prepared in 50% methanol in water for each analysis, and the IS-solution was 100 µg/l in 50% methanol. For preparation of calibration standards and quality controls blank plasma was preserved with 1% fluoride and stored at -20°C. Two quality controls (QC) were prepared at low (5.0 µg/kg) and high (50 µg/kg) levels and stored at -80°C. These two along with a freshly spiked blank plasma sample at 2.5 µg/kg were analyzed in each run.

Protein precipitation was performed on a fully automated Tecan Freedom EVO 200 robotic platform (Tecan group Ltd, Männedorf, Switzerland) that included all pipetting, centrifugation, and evaporation steps. Each plasma sample (100 mg) was transferred to a 96-well 2.0 ml deep-well plate and 20 µl IS-solution was added to each well, followed by precipitation with 700 µl acetonitrile and shaking. The samples were centrifuged at 1000 g for 10 min, and the supernatant was evaporated to dryness under a stream of nitrogen at 35°C. Afterwards, the samples were reconstituted in 100 µl mixture of 12.5% methanol:12.5% acetonitrile:75% 0.05% formic acid in

water, shaken and centrifuged again. Finally, the supernatant was transferred to a 96-well plate and 1 µl was injected into the chromatographic system.

Chromatographic separation was performed on a HSS T3 column (100 x 2.1 mm, 1.8um, Waters, Milford, MA, USA) using an ACQUITY Ultra Performance Liquid Chromatography system (UPLC) from Waters. The mobile phase was composed of solvent A: 1 mM ammoniumformate in 0.1% formic acid in water and B: 0.1% formic acid in 1:1 mixture of acetonitrile:methanol. The column was maintained at 45°C with a flow 0.4 ml/min, and a gradient elution was applied from 2% to 100% B within 3.2 min with a total analysis time of 4.5 min. Detection was done by tandem mass spectrometry using an ACQUITY TQS from Waters. Ionization was achieved by electrospray in positive mode, and the source temperature was set at 150°C and desolvation temperature at 600°C. Two transitions were used for psilocin, m/z 205 -> 58 and 205 -> 160, with a cone voltage of 20 V and collision energy at 14 and 18 eV, respectively. For the IS the transition was m/z 215 -> 164 with cone 20 V and collision energy of 18 eV. Argon was used as collision gas at 0.45 Pa, and desolvation and cone gasflow were fixed at 1000 L/hr and 150 L/hr, respectively. Data were acquired and processed with MassLynx 4.2 software (Waters).
Quantification was performed by an eight-point linear calibration curve (0.1, 0.5, 1.0, 5.0, 25, 50, 100, 200 µg/kg) with weighting 1/x. Limits of detection (LOD) and quantification (LOQ) were 0.1 and 0.5 µg/kg, respectively, while the upper limit of quantification was 200 µg/kg. The overall process efficiency was found to be 63% based on an obtained extraction efficiency of 81% and matrix effect of 25% that the stable isotope labelled IS adjusted for. QC plasma samples were measured in each series with a RSD of 5% and an accuracy of 78% for the low level and less than 5% and an accuracy 89% for the high level. Similar performance was obtained with QC´s preserved with 1 mM ascorbic acid demonstrating that stored plasma QC´s at -80°C without ascorbic acid were stable for at least 6 months. The freshly spiked plasma QC at 2.5 µg/kg analyzed in each series had a RSD of 13% and an accuracy of 88% (n = 11). For final presentation of results, units of concentration (µg/kg) were converted into µg/L using a conversion factor or 1.02 kg per liter plasma.

**[$^{11}$C]Cimbi-36 kinetic modelling.**

We selected neocortex as our region of interest due to the high cortical expression of 5-HT2AR, high correlation in 5-HT2AR across regions (5), and large volume and consequent beneficial signal-to-noise ratio (SNR). In agreement with previous studies, cerebellum was chosen as reference region (6, 7). The outcome measure of the SRTM is non-displaceable binding potential ($BP_{ND}$), which is "the ratio at equilibrium of specifically bound radioligand to that of non-displaceable radioligand in tissue" (8) and is proportional to the number of receptors available for binding in the tissue:

$$BP_{ND} = \frac{f_{ND} * B_{avail}}{K_D}$$

Here, $f_{ND}$ is the free radioligand fraction in the non-displaceable compartment and is assumed to be equal in the reference region (cerebellum) and in the tissue of interest (neocortex). $B_{avail}$ is the number of available receptors, and $K_D$ is the dissociation constant ($K_{off}/K_{on}$) (8).

**[$^{11}$C]Cimbi-36 metabolism and psilocybin intervention**

Venous blood samples were collected 5, 10, 20, 40, 60, and 120 minutes after tracer injection and plasma phase (2 mL) was subsequently analyzed using a column-switching high-performance liquid chromatography (HPLC) system to determine the fractions of unchanged tracer and radiometabolites as previously described (6, 9).[$^{11}$C]Cimbi-36 was metabolized rapidly after

administration **(S11)** and two fractions of radiolabelled metabolites were identified in the radiochromatogram (M1: polar and M2: less polar). Small polar metabolites (M1) may penetrate the blood-brain-barrier and decrease the SNR. To evaluate potential effects of psilocybin intervention on metabolism of [$^{11}$C]Cimbi-36, plasma radiometabolism profiles were plotted and compared to baseline. The free fraction of [$^{11}$C]Cimbi-36 in human plasma was estimated during baseline and the first intervention scan.

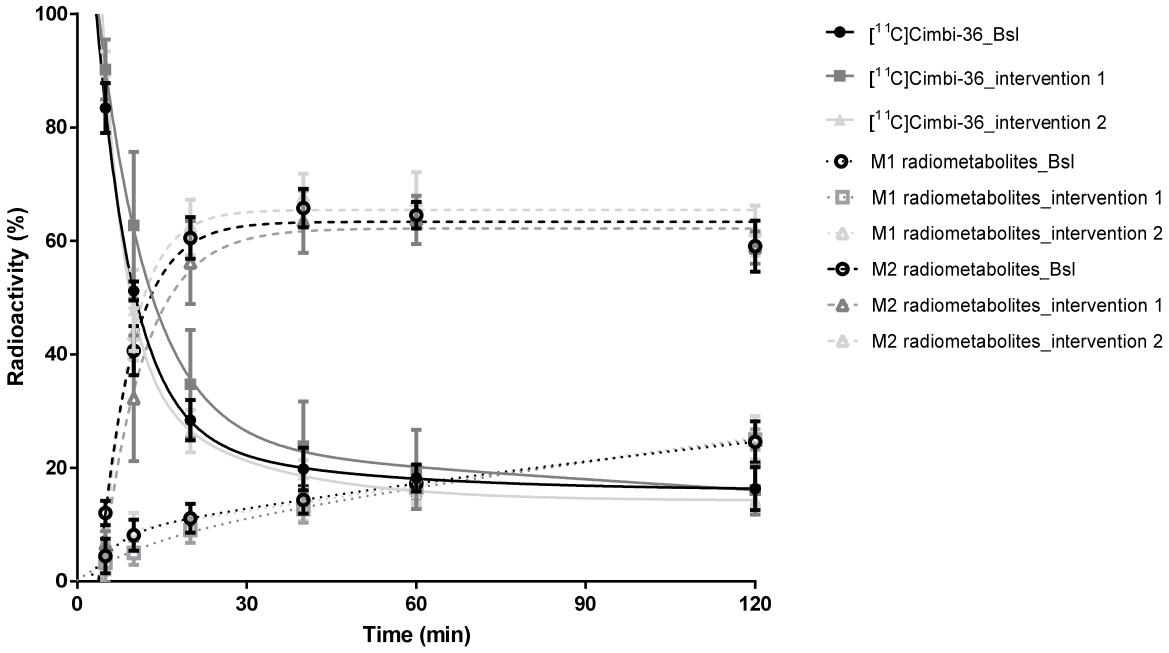


**Fig. S4.** Parent compound ([11C-Cimbi-36]) and M1 and M2 components at baseline (Bsl) and intervention 1 and 2 as a function of time after injection.

The time course of fractions of intact radioligand and radiometabolites were fitted to a non-linear regression curve using a two-phase model. Data are shown as mean ± SD. No significant differences were found when the average fraction of intact [$^{11}$C]Cimbi-36 during baseline (Bsl) was compared to the two interventions scan ($p= 0.76$ and $p=0.98$ for Bsl vs Intervention 1 and Bsl vs Intervention 2, respectively; unpaired t-test of mean values). Also, the levels of M1 radiometabolites were similar across scans. The rate of [$^{11}$C]Cimbi-36 metabolism was much slower for one subject, who was omitted from the present analysis (including the subject did not affect results, $p= 0.77$ and $p=0.77$ for Bsl vs Intervention 1 and Bsl vs Intervention 2, respectively). The free fraction of [$^{11}$C]Cimbi-36 in plasma at equilibrium was 3.6±0.5% at baseline and 2.9±0.4% at first intervention scan, and no significant difference was found between scans ($p=0.30$; unpaired t-test of mean values). All plots and statistical tests were performed using GraphPad Prism (version 7.03, GraphPad Software Inc., CA, USA).
Taken together, these observations indicate that psilocybin administration did not affect the rate of [$^{11}$C]Cimbi-36 metabolism in plasma, making differences in radioligand metabolism unlikely to confound the PET quantification.

**Motion assessment**

We assessed effects of psilocybin on motion (mean median max motion ± SD, baseline: 3.4 ± 0.8 mm; PET1: 3.0 ± 1.5 mm, PET2: 2.6 ± 0.6 mm) using a paired t-test on median maximum motion data from baseline and intervention scans. There was not statistically significant effect of psilocybin on motion: Baseline vs PET 1: p=0.6, Baseline vs PET2: p=0.13. PET1 vs PET2: p = 0.11.

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