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Published in: NeuroImage

DOI: 10.1016/j.neuroimage.2022.119716

Publication date: 2022

Document version
Publisher's PDF, also known as Version of record

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Citation for published version (APA):
Psilocybin modulation of time-varying functional connectivity is associated with plasma psilocin and subjective effects

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\section{A R T I C L E   I N F O}

Keywords:
Psilocybin
Psilocin
FMRI
Connectivity
Dynamics
TvFC

\section{A B S T R A C T}

\textbf{Background:} Psilocin, the neuroactive metabolite of psilocybin, is a serotonergic psychedelic that induces an acute altered state of consciousness, evokes lasting changes in mood and personality in healthy individuals, and has potential as an antidepressant treatment. Examining the acute effects of psilocin on resting-state time-varying functional connectivity implicates network-level connectivity motifs that may underlie acute and lasting behavioral and clinical effects.

\textbf{Aim:} Evaluate the association between resting-state time-varying functional connectivity (tvFC) characteristics and acute psilocin levels (PPL) and subjective drug intensity (SDI) before and right after intake of a psychedelic dose of psilocybin in healthy humans.

\textbf{Methods:} Fifteen healthy individuals completed the study. Before and at multiple time points after psilocybin intake, we acquired 10-minute resting-state blood-oxygen-level-dependent functional magnetic resonance imaging scans. Leading Eigenvector Dynamics Analysis (LEiDA) and diametrical clustering were applied to estimate discrete, sequentially active brain states. We evaluated associations between the fractional occurrence of brain states during a scan session and PPL and SDI using linear mixed-effects models. We examined associations between brain state dwell time and PPL and SDI using frailty Cox proportional hazards survival analysis.

\textbf{Results:} Fractional occurrences for two brain states characterized by lateral frontoparietal and medial frontoparietal-cingulate coherence were statistically significantly negatively associated with PPL and SDI. Dwell time for these brain states was negatively associated with SDI and, to a lesser extent, PPL. Conversely, fractional occurrence and dwell time of a fully connected brain state partly associated with motion was positively associated with PPL and SDI.

\textbf{Conclusion:} Our findings suggest that the acute perceptual psychedelic effects induced by psilocybin may stem from drug-level associated decreases in the occurrence and duration of lateral and medial frontoparietal connectivity motifs. We apply and argue for a modified approach to modeling eigenvectors produced by LEiDA that more fully acknowledges their underlying structure. Together these findings contribute to a more comprehensive neurobiological framework underlying acute effects of serotonergic psychedelics.

\section{1. Introduction}

Psilocybin is a psychedelic compound that has gained significant interest over the last decade with promising evidence for therapeutic efficacy in treating several neurological and neuropsychiatric disorders, including depression (Carhart-Harris et al., 2021, 2018; Davis et al., 2021), anxiety (Vargas et al., 2020), substance abuse (Bogenschutz et al., 2022; García-Romeu et al., 2019), migraine (Schindler et al., 2021), and cluster headache (Madsen et al., 2022; Sewell et al., 2006).

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Received 8 February 2022; Received in revised form 11 October 2022; Accepted 25 October 2022
Available online 27 October 2022.
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Through stimulation of the serotonin 2A receptor (5-HT2AR), psilocin, the psychoactive metabolite of psilocybin, potently and acutely induces an altered state of consciousness (Griffiths et al., 2006; Hasler et al., 2004; Madsen et al., 2019; Stenbak et al., 2021). Psilocybin also induces rapid and lasting positive effects on mood, well-being, and personality (Carhart-Harris et al., 2018; Erritzoe et al., 2018; MacLean et al., 2011; Madsen et al., 2020). These intriguing effects precipitate the need to resolve associated and perhaps mediating neurobiological mechanisms. Such information can potentially inform future drug development programs and identify patient subgroups that may benefit from psychedelic therapy or predict potential adverse drug effects.

Previous studies have characterized distributed functional brain connectivity and macroscale cerebral networks acutely affected by a single administration of a serotonin psychedelic compound such as psilocybin with resting-state functional magnetic resonance imaging (rs-fMRI) (McGuire et al., 2022; Vollenweider and Preller, 2020). Studies suggest modulation of distributed connectivity patterns includes alterations in thalamic connectivity (Preller et al., 2019), whole-brain connectivity (Madsen et al., 2021; Preller et al., 2020), decreased segregation and integration of canonical resting-state networks (Madsen et al., 2021; Mason et al., 2020), and macroscopic measures such as entropy (Carhart-Harris et al., 2014). However, most studies have focused on “static” functional connectivity, estimated as the correlation between pairs or across sets of areas over the duration of the scan session. This approach assumes signal stationarity for the entirety of the 5–10-minute rs-fMRI scan session, which may neglect relevant and observable neural dynamics arising from, e.g., mind-wandering or ephemeral experiences.

Time-varying functional connectivity (tvFC) has emerged as a method for extracting informative, time-varying brain connectivity patterns (Lauri et al., 2020; Petti et al., 2017). Unsupervised machine learning methods are employed to cluster instantaneous or small time-window connectivity metrics into discrete groups of distinct coactivation. Such metrics are usually model-based and include lagged and zero-lag correlation coefficients and various estimates of interregional functional synchrony (Bastos and Schoffelen, 2016; Glerean et al., 2012). An appealing aspect of tvFC strategies is that they attempt to model dynamics of connectivity processes that occur within a resting-state scan session, which is particularly relevant to the evaluation of psychedelics, which induce a dynamically evolving psychological experience.

Acute psychedelic effects on time-varying functional brain connectivity have been previously examined in only two separate datasets (Lord et al., 2019; Lappi et al., 2021; Tagliazucchi et al., 2014). Lord and colleagues applied Leading Eigenvector Dynamics Analysis (LEIDA) (Cabral et al., 2017) to rs-fMRI data acquired before and after a single intravenous dose of psilocybin in nine subjects. The authors reported that the probability of occurrence (“fractional occurrence”) of a discrete brain state comprising frontoparietal network elements was significantly lower after psilocybin infusion (Lord et al., 2019). Notably, plasma psilocin levels were not measured, which we have shown is tightly coupled to 5-HT2AR drug occupancy (Madsen et al., 2019) at the time of functional brain imaging. Moreover, there was only partial agreement between the discrete brain states identified and canonical resting-state networks, suggesting that acute psilocin effects may be informatively characterized by approaches that group sets of regions in a data-driven manner (e.g., clustering) as opposed to a priori defined network structures. It is critical to evaluate whether similar findings are observed in an independent sample and to evaluate this effect following oral psilocybin administration, as this is how it is administered clinically. During oral administration, the psychedelic effects are protracted over approximately six hours. Examining psilocybin effects on functional connectivity in alignment with an assessment of plasma psilocin levels and subjective effects throughout this period provides a novel perspective on its dynamic effects on the brain.

Furthermore, we see an opportunity to improve LEIDA and associated statistical evaluations. Typically, LEIDA clusters leading eigenvectors of instantaneous phase coherence maps using Euclidean k-means. Prior to clustering, eigenvectors are flipped so that the majority of elements are negative. However, eigenvectors are, in practice, normalized to have unit length and have arbitrary sign, attributes not acknowledged by Euclidean k-means nor preserved by the aforementioned flip procedure (see Supplementary Figure S1). Further, in the case of an eigenvector with similar numbers of positive and negative loadings, slight variations can result in sign flips that place otherwise similar eigenvectors in different areas of this region space, which can affect clustering results when antipodal symmetry is not considered. This leads to sub-optimal clustering. More recent approaches to address the issue of spherical distribution of eigenvectors include “k-medoids”, which labels observed data points as centroids (Farinha et al., 2022). However, this leaves unresolved the limitation of the sign-flip procedure. Directional statistics is a branch of statistics that deals with data where the direction holds more information than the amplitude, typically represented as normalized vectors distributed on some geometric manifold (Mardia and Jupp, 1999). Specifically, the Watson distribution (Watson, 1965) models data distributed on the antipodally symmetric (P – 1)-dimensional unit hypersphere, corresponding to the distribution of independent and identically distributed eigenvectors. Diametrical clustering (Dhillon et al., 2003) is the k-means equivalent of Watson mixture models (Sra and Karp, 2013) and may offer more suitable clustering of eigenvectors in LEIDA by respecting the spherical manifold and sign ambiguity of orthonormal eigenvectors (Jensen et al., 2022).

In addition to fractional occurrence, the average duration of brain state occurrences (“dwell time”) can complementarily inform the nature of connectivity dynamics. Previously, studies have analyzed mean dwell time using simple t-tests, which do not account for the exponential decrease in state activation probability as a function of the number of consecutive active time points. We suggest modeling dwell time using survival analysis, which more appropriately captures the conditional dependence of state probability on the previous length of active time (Cox, 1972).

Here we evaluated acute psilocybin effects on tvFC with blood-oxygen-level-dependent (BOLD) rs-fMRI in 15 healthy participants, each of whom completed one 10-min rs-fMRI scan session before intake of a psychedellic dose of psilocybin and multiple 10-min rs-fMRI scan sessions after psilocybin intake (approximately 40, 80, 140 and 300 min post-administration). We applied LEIDA with diametrical clustering to account for the intrinsic spherical geometry and antipodal symmetry in the distribution of eigenvectors. We investigated a range of number of clusters k ∈ {2, …, 20} to analyze the stability of our findings across partitions of the data space, similar to previous studies (Cabral et al., 2017; Figueroa et al., 2019). To establish the association between tvFC characteristics and the psychopharmacological effects of psilocybin, we determined the association between the fractional occurrence of discrete brain states, defined by clustering, and both plasma psilocin level (PPL) and subjective drug intensity (SDI), which we have shown are coupled to 5-HT2AR occupancy and baseline 5-HT2AR (Madsen et al., 2021, 2019; Stenbak et al., 2021). We determined associations between PPL and SDI and discrete brain state dwell time using Cox regression frailty models.

2. Methods

A brief description of experimental procedures is provided here; a full description can be found elsewhere (Madsen et al., 2021).

2.1. Experimental procedures

Fifteen healthy participants (age 34.3 ± 9.8 years, six females) with no or limited prior experience with psychedelics were recruited for a brain imaging study, including a single psilocybin intervention. All participants provided written informed consent and were healthy, including screening for neurological, psychiatric, or somatic illnesses. Two psychologists prepared participants and supported them at all stages of the intervention.
Psilocybin was taken orally in multiples of 3 mg psilocybin capsules, dosed according to body weight (total dose: 0.24 ± 0.04 mg/kg) in a single-blind cross-over study design. Within each cross-over, participants received either psilocybin or a non-psychedelic drug (ketanserin), i.e., there is not a placebo condition; only the psilocybin data (pre- and post-drug) are reported here. Functional neuroimaging data were acquired once before and at regular intervals (approximately 40, 80, 130, and 300 min) after administration. Immediately after each rs-fMRI acquisition, participants were asked to rate their perceived SDI on a Likert scale from 0 to 10 (0 = “not at all intense”, 10 = “very intense”). Following each subjective rating, a blood sample was drawn from an intravenous catheter to determine the concentration of unconjugated psilocin in plasma (Kolacynska et al., 2021; Madsen et al., 2021). The study was approved by the ethics committee for the capital region of Copenhagen (journal identifier: H-16,028,698, amendments: 56,023; 56,967; 57,974; 59,673; 60,437; 62,255) and the Danish Medicines Agency (EudraCT identifier: 2016-004,000-61, amendments: 2017,014,166; 2017,082,837; 2018,023,295).

2.2. Neuroimaging data acquisition

MRI data were acquired on a 3T Siemens Prisma scanner (Siemens, Erlangen, Germany) with a 64-channel head coil. A structural T1-weighted 3D image was acquired at the pre-drug imaging session (inversion time = 900 ms, TE/TR = 2.58/1900 ms, flip angle = 9°, matrix 256 × 256 × 224, resolution 0.9 mm isotropic, no gap). BOLD fMRI data were acquired using a T2*-weighted gradient echo-planar imaging sequence (TE/TR = 30/2000 ms, flip angle = 90°, in-plane matrix = 64 × 64 mm, in-plane resolution = 3.6 × 3.6 mm, 32 slices, slice thickness = 3.0 mm, gap = 0.75 mm), 300 vol (10 min) were acquired in each imaging session. Participants were wearing noise-cancelling headphones, which were powered off, and were instructed to close their eyes and let the mind wander freely without falling asleep. In total, 74 scan sessions were acquired across the 15 participants.

2.3. fMRI data preprocessing

fMRI data preprocessing was performed separately for each of the 10-minute rs-fMRI scan sessions. The data were preprocessed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Steps included 1) slice-timing correction, 2) spatial realignment and field unwarping, 3) co-registration of the T1-weighted structural image to the first functional volume, 4) segmentation of the T1-weighted image into gray matter, white matter, and cerebrospinal fluid (CSF) maps, 5) normalization of the $P = 90$ dimensional Anatomical Automatic Labeling (AAL) atlas (excluding cerebellum) (Tzourio-Mazoyer et al., 2002) to the co-registered structural images, and 6) smoothing of functional images (4 mm FWHM Gaussian kernel). Motion and signal variance artifacts were identified using Artifact detection Tool (ART, https://www.nitrc.org/projects/artifact_detect). As significant motion is to be expected (Carhart-Harris et al., 2012) and something we have acknowledged previously with these data (Madsen et al., 2021), we excluded individual scan sessions where more than 50% of volumes exceeded the ART threshold (global threshold = 4 standard deviations, motion threshold = 2 standard deviations). Based on this criterion, two scan sessions from a single participant were excluded, resulting in 21,600 rs-fMRI volumes included in subsequent analyses. We later provide an analysis of the dependence of our main findings on motion parameters. fMRI time-series were denoised using CONN (https://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon, 2012) by voxel-wise nuisance regression of 1) three translation and three rotation parameters from realignment and their first-order derivatives, and 2) anatomical component correction using the first five principal components and their first-order derivatives from white-matter and CSF time-series (Behzadi et al., 2007). Time-series data were bandpass filtered between 0.008 and 0.09 Hz. We used the AAL atlas to parcellate the denoised functional images into 90 cortical and subcortical regions.

2.4. Leading eigenvector dynamics analysis

LEIDA attempts to summarize instantaneous (i.e., for every acquired sample) functional brain connectivity through an assessment of inter-regional phase coherence. The processing steps are 1) separation of amplitude and phase from every regional signal, 2) establishing instantaneous phase coherence matrices as the cosine of pairwise phase differences, and 3) reducing dimensionality by extracting the first eigenvector for every phase coherence matrix (see Fig. 1). The result is an eigenvector for every time point with the same dimensionality as the input data, which represents the main direction of variation of the corresponding phase coherence matrix. Thus, nodes with the same sign are said to be coherent, and their size represents the strength of coherence with all other nodes.

The Hilbert transform can be used to decompose a signal $s(t)$ into amplitude $a(t)$ and phase $\theta(t)$ through the relation $s(t) = a(t)e^{i\theta(t)}$. Specifically, for each scan session, the analytic signal $z(t) = s(t) + is\hat{x}(t)$ is constructed, where $\hat{x}$ is the imaginary unit and $s\hat{x}(t) = s(t) + \pi i$ is the Hilbert transform, where $\pi$ represents the convolution operator (see Fig. 1). The analytic signal represents a complex extension to the real signal where oscillations in the real signal become circular evolutions in the complex plane, and signal magnitude becomes a distance from the origin (see Fig. 1B). The complex analytic signal can be described in polar coordinates $z(t) = a(t)e^{i\theta(t)}$ with instantaneous amplitude $a(t) = \sqrt{x(t)^2 + y(t)^2}$ and phase $\theta(t) = \arctan \left( \frac{y(t)}{x(t)} \right)$. The instantaneous phase is the angle from the positive x-axis to $z(t)$ and the series is a sawtooth curve with a discontinuity at the jump from $-\pi$ to $\pi$ (see Fig. 1A, 2nd panel). The phase generally contains the oscillatory information in $s(t)$, while $a(t)$ encompasses (potentially spurious) amplitude information, which is discarded in LEIDA. Instantaneous phase coherence between brain region pairs $(j,k) \in \{1, \ldots, P\}^2$, where $P = 90$ is the number of regions in the AAL atlas, is described using the symmetric phase coherence map $A_t$ for every time point $t$ with elements $A_{t,j,k} = \cos(\theta_{t,j} - \theta_{t,k})$. Since $A_t$ has $P^2$ unique elements, we may be well served by describing its information in lower dimensions using the eigendecomposition. Due to the angle difference identity $\cos(\theta_{t,j} - \theta_{t,k}) = \cos \theta_{t,j} \cos \theta_{t,k} + \sin \theta_{t,j} \sin \theta_{t,k}$, for any two regions $j$ and $k$, $A_t$ can be fully decomposed into two P-dimensional orthogonal eigenvectors, which are each a linear combination of the vectors $e = \cos \theta_{t,j}$ and $s = \sin \theta_{t,j}$. For each time point, we retained only the eigenvector $v_{t,j}$ corresponding to the largest eigenvalue, thereby capturing the dominant instantaneous connectivity pattern.

2.5. Clustering

Eigenvectors are generally normalized to unit length and have arbitrary sign, and are thus distributed on the surface of the antipodally symmetric (two points located exactly opposite represent the same entity) unit hypersphere (see Fig. 1D). The (Dimroth-Scheidegger)-Watson distribution models such data (Sra and Karp, 2013; Watson, 1965) and is thus preferable to, e.g., the Gaussian distribution, which assumes Euclidean distance between samples (see Figure S1). If we assume that eigenvectors can be described by a mixture of independent Watson distributions, we can disentangle clusters by estimating a Watson mixture model. Diometrical clustering is derived from mixture modeling of multivariate Watson distributions where only the mean direction is modeled, disregarding cluster variance and covariance structures, simplifying the analysis of brain states (Dhillon et al., 2003; Sra and Karp, 2013). Diometrical clustering can be regarded as the standard k-means algorithm where centroid locations are updated according to the squared Pearson correlation similarity measure: $s \text{im}_{c,t} = \left( v_{t,j}^T \mu_{c} \right)^2$, where $\mu_{c}$ is the centroid of cluster $c$, and $v_{t,j}$ the leading eigenvector of the phase coherence map for any time point $t$ or scan session. Importantly, this approach
addresses two limitations of previously applied clustering techniques, namely 1) the ability to group correlated and anticorrelated unit norm vectors into the same cluster and 2) constraining the optimization to the surface of the associated hypersphere. The squared Pearson correlation is equivalent to the squared cosine similarity for normalized vectors and thus equivalent to finding the squared cosine of the angle between unit vectors. We initialized our algorithm using k-means++ rewritten for diametrical clustering (Arthur and Vassilvitskii, 2007). For each k, 200 replications of the clustering algorithm were run, where the best of the 200 replications (in terms of the sum of squared Pearson correlation to the nearest centroid) was chosen as the output.

To retrieve recurrent interregional phase coherence patterns, we grouped the 21,600 leading eigenvectors concatenated across all scan sessions into k clusters, which we denote “brain states” (see Fig. 1D). The optimal number of brain states is not known or well-defined; therefore, we produced models for k ranging from 2 to 20, with higher k revealing more fine-grained patterns (Cabral et al., 2017; Figueroa et al., 2019).

2.6. Brain network state occurrence

To identify associations between brain state dynamics and PPL and SDI, we calculated the fractional occurrence (FO) for each brain state, as defined by the fraction of time points in a scan session assigned to that brain state. For each state, this produced 72 FO estimates, one for each scan session. We modeled the association between FO and PPL (or SDI) with a random intercept linear mixed-effects model to account for inter-subject variability and a variable number of post-administration scan sessions. The models were fitted using maximum likelihood, and we used the likelihood ratio to test for significance of the fixed effect and generate confidence intervals unadjusted for multiple comparisons (CI_{unadj}). To account for multiple testing across a set of k states, we performed permutation testing with max-T adjustment and 100,000 permutations by scrambling the normalized residuals of the linear mixed-effects model (Lee and Braun, 2012; Westfall and Young, 1993). The initially observed statistical estimates (likelihood ratios) were then compared to the distribution of maximum statistics across the k models for every permutation, and a corresponding p-value was calculated as the number of permutations where the initial likelihood ratio exceeded the maximum statistic. Permutation testing and Max-T correction were performed within-k and separately for the models with PPL and SDI as fixed effects, respectively.

2.7. Brain network state dwell time

We employed survival analysis to model state dwell time, i.e., the time spent in a brain state before switching. Survival analysis explicitly models the exponential decrease in (state) survival probability as a function of the survival time, making it preferable to, e.g., average dwell time using group-wise statistical tests. We looped through all time points, and, whenever the brain state changed within a scan session, we noted the number of preceding samples t and the corresponding subject, PPL, and SDI. The first and last active state from a scan session was excluded since we cannot estimate the true dwell time in this
case. We modeled the dwell time of a brain state using a Cox proportional hazards model, including a frailty element $\zeta$ to account for inter-subject variability: $\lambda(t|x_d, z_\zeta) = z_\zeta \lambda_0(t) \exp(x_d \beta)$, where $n = 1, \ldots, N$ denotes subjects $l = 1, \ldots, L$ denotes sessions for subject $n$, and $x_d$ the corresponding PPL or SDI (Cox, 1972; Vaupel et al., 1979). We report the estimated hazard ratio $H = R = \hat{\beta} = \frac{\lambda(t=1)}{\lambda(t=0)}$, which is proportional in the covariate level (PPL or SDI). The associated confidence interval is defined as $\pm 1.96 \text{ se(}\hat{\beta})$, where $\text{se}(\hat{\beta})$ is the estimated standard error of the coefficient estimate. $\lambda(t)$ represents the common baseline hazard irrespective of subject or covariate level. We could not find any existing permutation test specifically for frailty Cox proportional hazards models. Therefore, we controlled the FWER using Bonferroni-Holm correction (Holm, 1979) applied within-$k$.

2.8. Identification of recurrent brain states

The diatomical clustering algorithm returns $k$ unordered cluster directions and labels of volumes assigned to each model according to the squared Pearson correlation. To match brain states across $k$, the estimated centroids for $k$ were assigned to the closest centroid for $k = 1$, without replacement. To match specific centroids across different $k$, we selected a template $k$, and, for all other $k$, found the centroid that most closely matched the template centroid in terms of squared Pearson correlation. To analyze clustering stability, we ran diatomical clustering 1000 times with five replications each for all $k \in \{2, \ldots, 20\}$, and, identified, for each $k$, the state most closely matching the relevant template states. If the Pearson correlation coefficient between the identified states and the template was negative, the sign of the identified state was inverted. We also compared diatomical clustering output to that of Euclidean k-means, where the output centroids from the latter were normalized to unit length before matching.

2.9. Visualizations, code, and data availability

We have published the MATLAB (The MathWorks, Inc.) and R code used to generate the results presented in this study at (https://github.com/anders-s-olsen/psilocybin_dynamic_FC) We used BrainNet Viewer (Xia et al., 2013) (https://www.nitrc.org/projects/bnv/) to generate connectivity visualizations. The datasets generated and/or analyzed during the current study can be made available upon completion of a formal data-sharing agreement.

3. Results

21,600 leading eigenvectors defined by LEIDA were clustered into $k$ discrete brain states defined by centroids (average eigenvectors across time within cluster) determined with diatomical clustering (see Fig. 1). We explored a range of $k \in \{2, \ldots, 20\}$ centroids, aligned with previous studies (Cabral et al., 2017; Figueroa et al., 2019; Kringlebach et al., 2020). Generally, specific centroid locations were stable across contiguous values of $k$; 90-dimensional projections of all centroids for all values of $k$ can be found in Supplementary Video S2. See Fig. 2 for a visualization of estimated brain states for $k = 7$.

For all values of $k \geq 4$, we observed a “global” brain state characterized by all centroid elements having the same sign (see state 1 in Fig. 2). All other brain states were characterized by coherence loadings in both directions. For example, for $k = 7$, brain state 2 showed strong coherence between areas related to visual processing (red nodes) and regions related to the salience network (blue nodes). Brain state 4 showed coherence between regions related to the frontoparietal, or central executive network, including the dorsolateral prefrontal cortex and posterior parietal cortex. Anti-coherent elements were observed in cingulate (anterior, medial, and posterior) and parietal regions. Coherence may be understood as a parallel to synchronization. Put another way, two nodes of the same sign are synchronous with each other, i.e., their phase differences are between $[-\pi/2, \pi/2]$, and will have a positive edge.

3.1. Psilocybin effects on brain state fractional occurrence and dwell time

All $p$-values are presented along their corresponding centroid in Supplementary Video S2. All centroids across all values of $k$ showing a statistically significant association between either PPL or SDI and either FO or dwell time are listed in Supplementary Table S3.

Figs. 3A and 3C summarize FWER-controlled $p$-values of linear mixed-effects models estimates of the association between fractional occurrence (FO) of individual brain states and PPL or SDI, respectively. Across multiple values of $k \geq 4$, we observed one brain state (“frontoparietal state 1”, green triangle symbols in Fig. 3, see also brain state visualization in Fig. 4) for which the FO was statistically significantly negatively associated with both PPL ($k = 7$: slope = $-0.0064$ FO per $\mu$g/L PPL, 95% CI$_{\text{unadj}} = [-0.0091; -0.0036]$; $\text{FWER}\text{-maxT} < 0.001$) and SDI ($k = 7$: slope = $-0.013$ FO per SDI rating, 95% CI$_{\text{unadj}} = [-0.017; -0.009]$; $\text{FWER}\text{-maxT} < 0.001$). Specifically, the association between brain state FO and PPL was significant for the interval $k \in \{4, \ldots, 8, 10\}$, whereas for SDI, this association was significant for all $k \geq 4$ except $k = 17$. Put another way, the average total time the brains occupies this frontoparietal state during the course of a 10-min rs-fMRI scan was negatively related to PPL and SDI. For $k \geq 8$, we observed a second brain state (“frontoparietal state 2”, red star symbols in Fig. 3, see also Supplementary Figure S5) for which the FO was also statistically significantly negatively associated with both PPL ($k = 11$: slope = $-0.0045$ FO per $\mu$g/L PPL, 95% CI$_{\text{unadj}} = [-0.0064; -0.0026]$; $\text{FWER}\text{-maxT} < 0.001$) and SDI ($k = 11$: slope = $-0.0089$ FO per SDI rating, 95% CI$_{\text{unadj}} = [-0.011; -0.0065]$; $\text{FWER}\text{-maxT} < 0.001$). For PPL, this association was significant for all $k \in \{8, \ldots, 19\}$ except $k = 9$. For $k \geq 4$, we observed a third brain state (“fully connected state”, blue diamond symbols in Fig. 3, see also Supplementary Figure S5) for which the FO was statistically significantly positively associated with SDI for all $k \geq 5$ ($k = 7$: slope = 0.0078 FO per SDI rating, 95% CI$_{\text{unadj}} = [0.0027; 0.013]$; $\text{FWER}\text{-maxT} = 0.026$). We did not observe any statistically significant associations between the fully connected state and PPL ($k = 7$: $\text{FWER}\text{-maxT} = 0.284$).

Fig. 3B and 3D summarize Bonferroni-Holm corrected $p$-values of Cox proportional hazards models of the effect of PPL and SDI on dwell time, respectively. Frontoparietal state 1 dwell time was negatively associated with PPL across several values of $k$ ($k = 7$; Hazard ratio (HR) = 1.018, 95% CI$_{\text{unadj}} = [1.006; 1.028]$; $\text{FWER}\text{-BH} = 0.012$ and SDI ($k = 7$; HR = 1.037, 95% CI$_{\text{unadj}} = [1.019; 1.055]$; $\text{FWER}\text{-BH} < 0.001$). Specifically, the association between brain state dwell time and PPL was statistically significant for $k \in \{4, \ldots, 9\}$, whereas for SDI, this association was statistically significant for $k \in \{4, \ldots, 11, 13\}$. In other words, the higher the PPL and SDI, the larger the hazard ratio, i.e., the less average continuous time spent in frontoparietal state 1. For $k \geq 8$, frontoparietal state 2 also showed a negative association with both PPL ($k = 11$: HR = 1.022, 95% CI$_{\text{unadj}} = [1.009; 1.036]$; $\text{FWER}\text{-BH} = 0.011$) and SDI ($k = 11$: HR = 1.047, 95% CI$_{\text{unadj}} = [1.025; 1.069]$; $\text{FWER}\text{-BH} < 0.001$). Overall, frontoparietal state 2 was significantly inversely associated to PPL for $k \in \{10, \ldots, 13\}$ and to SDI for $k \in \{10, \ldots, 15, 17\}$. For $k \geq 4$ we observed that dwell time of the fully connected state was positively associated with both PPL ($k = 7$: HR = 0.982, 95% CI$_{\text{unadj}} = [0.971; 0.994]$; $\text{FWER}\text{-BH} = 0.022$) for $k = \{6, \ldots, 9\}$ and SDI ($k = 7$: HR = 0.970, 95% CI$_{\text{unadj}} = [0.953; 0.987]$; $\text{FWER}\text{-BH} = 0.005$) for $k \in \{4, \ldots, 9, 12, 13, 20\}$.

3.2. Stability of highlighted states

To identify the three brain states across $k$, we defined template centroids ($k = 7$ for frontoparietal state 1 and the fully connected state, $k = 11$ for frontoparietal state 2, see Supplementary Figures S6–8). For every $k$, the brain state most closely matching each of these three templates were marked. In Supplementary Figure S9, between-$k$ correlation coefficients indicate that the three states had very similar centroids across the range of $k$. The between-$k$ similarity can also be confirmed visually in Supplementary Figures S6–S8. Frontoparietal state 2 appeared
Fig. 2. Brain states estimated using LEiDA and diametrical clustering with $k = 7$ with spatial connectivity representation (A), coherence maps defined as the outer product of the cluster centroid (B), and surface rendering of the cluster centroid (C). Only edges above the 75th percentile of absolute edge strengths are shown. In (A), all positive edges are shown in red and negative edges in blue, while nodes are colored according to the sign of their element in the respective cluster centroid.

Fig. 3. Summary statistics linking brain state fractional occurrence and dwell time with plasma psilocin level (PPL) and subjective drug intensity (SDI). (A): Linear mixed-effects models of the association between PPL and brain state fractional occurrence. (B): Cox proportional hazards frailty models of the association between PPL and brain state dwell time. (C): Linear mixed-effects models of the association between SDI and brain state fractional occurrence. (D): Cox proportional hazards frailty models of the association between SDI and brain state dwell time. Horizontal red line denotes family-wise error rate (FWER) threshold for statistical significance. Fractional occurrence p-values were corrected using 100,000 permutations and max-T correction applied within-k (see Methods). Where an observed statistic exceeded all permuted values, the p-value was set to $10^{-5}$ (i.e., $-\log_{10}(p) = 5$). Dwell time p-values were corrected using Bonferroni-Holm applied within-k. For every k, points were identified as one of the three brain states by matching all the corresponding centroids to the templates ($k = 7$ for frontoparietal state 1 and the fully connected state, $k = 11$ for frontoparietal state 2).
Initially at $k=8$ and qualitatively became more associated with PPL and SDI than frontoparietal state 1 (see Fig. 3). Likewise, estimated fractional occurrence slopes for the association between frontoparietal state 1 and PPL and SDI approximately halved at the transition from $k=7$ to $k=8$ (Supplementary Table S3), suggesting that frontoparietal state 2 was incorporated within frontoparietal state 1 for $k < 8$.

### 3.3. Diametrical clustering stability and comparison to k-means

Like most clustering algorithms, diametrical clustering is initialized randomly. To quantify the variation in brain state centroid location between initializations, we ran diametrical clustering 1000 times with five replications and extracted the two frontoparietal states and the fully con-
nected state. Supplementary Figure S10 shows the histogram of Fisher’s r-to-z scores of the Pearson correlation coefficients across all initialization pairs, including a fitted Gaussian curve. Generally, we see high clustering stability regardless of initialization. The average correlation coefficient between initializations is numerically higher for the fully connected state, followed by frontoparietal states 1 and 2. As expected, stability decreases with increasing k.

We compared brain state-specific differences in centroid locations between those obtained using the diatrical clustering method presented here and Euclidean k-means used in previous LEiDA-studies. Notably, at k = 7, the “fully connected state” is not identified when using the Euclidean k-means approach for clustering (Supplementary Figure S11). Although there are clear similarities across the brain states paired between the two clustering methods, the magnitudes of these similarities are variable. To understand this variability more comprehensively, we ran both diatrical clustering and Euclidean k-means without replications using 1000 initializations and computed the correlation coefficient for all 1000 × 1000 combinations between the two methods for each of the extracted centroids for frontoparietal state 1, frontoparietal state 2, and the fully connected state. The mean, μk, and standard deviation, σk, of these correlation coefficients are described in Supplementary Table S12. Although often highly correlated, these results show variability in the correlation between these two clustering methods, suggesting they do not always produce convergent results.

Finally, we evaluated the effect of motion on our results by evaluating the association between brain state fractional occurrence and per-scan-averaged framewise displacement of the motion correction translation and rotation time series using a linear mixed effects model with subject as the random effect (see Supplementary Figure S13 for p-values). Notably, the fully connected state was significantly associated with motion for many of the evaluated k. Both frontoparietal states showed a significant association with motion for a few k.

4. Discussion

Here we evaluated acute psilocybin effects on dynamic functional brain connectivity in healthy individuals. Most prominently, the higher the subjective experience intensity and plasma psilocin level, the lower the fractional occurrence of two discrete frontoparietal-like brain states. Similarly, the dwell time of these brain states was inversely related to plasma psilocin level and subjective drug intensity. We observed an increase in the fractional occurrence and dwell time of a “fully-connected” brain state where all elements have the same sign, although the statistical associations for this state were weaker. Together, these findings provide a novel mapping of drug availability and perceptual intensity of a clinically relevant psilocybin-induced psychedelic experience onto distributed whole-brain functional connectivity dynamics. We propose an alternative method for clustering LEiDA-tvFC estimates that more faithfully respects the spherical manifold and sign ambiguity of orthonormal eigenvectors (Dhillon et al., 2003; Sra and Karp, 2013). Taken together, these findings implicate dynamic neural processes underlying the acute psychedelic effects of psilocybin, an important contribution to understanding the effects of this rapidly emerging clinical therapeutic.

The highlighted frontoparietal states 1 and 2 were both characterized by phase coherence between areas commonly assigned to a network described as, e.g., the “frontoparietal” network, “central executive”, “executive control”, or “dorsal attention” network (Witt et al., 2021). Similarly, these brain states expressed phase coherence between regions in the cingulum, ventromedial prefrontal cortex, and some regions around the parieto-occipital fissure, sporting some overlap with default mode network regions (see Fig. 4 and Supplementary Figure S4). The regions with strong “negative” loadings were remarkably similar between the two states. The two states mostly differed in the centroid loadings for elements in the temporal lobe and the Rolandic operculum. A previous study applying LEiDA to model dynamic functional connectivity following psilocybin administration reported a similar brain state for models in the range k ∈ {5, …, 10} (Lord et al., 2019). Despite methodological differences between the studies, e.g., we administered psilocybin orally, measured PPL, scanned participants multiple times after administration, and applied diatrical clustering; it is encouraging that our findings offer convergent evidence that decreased frontoparietal connectivity is a critical neural characteristic of the psilocybin-induced drug experience. Notably, the networks with which these frontoparietal states more prominently aligned are so-called “higher-order” or “transmodal” networks, which are thought to support complex cognitive processes, e.g., abstract stimulus representation and manipulation (Margulies et al., 2016). Although speculative, this may reflect the profoundly affected thoughts generated as well as interpretation of thoughts and external stimuli that characterize psilocybin and other psychedelic compounds. We show here, for the first time, that these changes are proportionally related to PPL and SDI across the duration of the psychedelic experience. Contributing to our mechanistic understanding of the neurobiological mechanisms that shape psilocybin effects, our findings implicate a systems-level neural correlate (frontoparietal state prevalence) to the relation between available psilocin, which we have previously shown to be associated with 5-HT2AR occupancy and subjective intensity of the psychedelic experience (Madsen et al., 2019).

Consistent with the observed effects on fractional occurrence, we observed some evidence that dwell time, i.e., average time spent in the state before switching, of the frontoparietal states were similarly negatively associated with PPL and SDI. However, this effect was statistically significant for only a subset of the evaluated number of brain states, k. Notably, the integral of the subject-specific survival function for which hazard ratios were estimated is proportional to fractional occurrence. This means that dwell time models not merely the (instaneous) probability of being in a given state but also the exponential decrease of that probability over consecutive time points. We infer that the numerically consistent associations with fractional occurrence and dwell time reflect a psilocybin-induced “bias shift” away from the observed frontoparietal brain states. Previous studies examining brain state switching mechanisms have typically evaluated transition probability matrices and specific state-to-state transition probabilities conditioned only on the current state. Although dwell time is related to the diagonal elements of the transition matrix, modeling it as a hazard ratio informs state survival across a broader time window, giving a more complete perspective on brain state dynamics. Modeling dwell time using survival analysis does not model all state-to-state transition probabilities individually. However, many of these transitions occur only rarely, and the set of statistical tests squares with the number of brain states, k, both of which constrain associated statistical estimates. In this way, we view the Cox proportional hazards model as a valuable trade-off for evaluating state dwell time and switching probability.

Previous studies applying LEiDA have reported alterations in a “fully connected” brain state, characterized by all elements having the same sign (Cabral et al., 2017; Escrichs et al., 2021; Farinha et al., 2022; Figueroa et al., 2019; Larabi et al., 2020; Lord et al., 2019; Stark et al., 2021; Vohrinyk et al., 2020). Here we also observed this fully connected state and report an increase in fractional occurrence significantly associated with SDI, but not PPL. Interestingly, however, Supplementary Figure S11 shows that we would not have identified this fully connected state if we applied LEiDA using the Euclidean k-means clustering method for k = 7 described previously. The observed slope estimates for the fully connected state are similar, and opposite to those for the two frontoparietal states for k ≥ 8, and approximately half that of frontoparietal state 1 for k < 8. Similarly, dwell time for the fully connected state was significantly positively associated with both PPL and SDI. Here, hazard ratio estimates were similar for all three highlighted brain states regardless of k. These results indicate that while psilocin induces a decrease in the fractional occurrence and dwell time of frontoparietal connectivity dynamics, only approximately half of the corresponding increase in brain activity can be explained by a shift toward the fully connected state. We observed that the FO of the fully connected state was associated with
motion (Supplementary Figure S13). The sometimes very significant association between the fully connected state and motion suggests that perhaps this state encapsulates some noise-related signal. Interestingly, this is related to the ongoing discussion of the global signal potentially being partly explained by noise factors such as motion. As fractional occurrences must sum to one across all states, these findings suggest additional increases are spread across other states below the statistical significance threshold, given the current data.

Here we have presented the application of diametrical clustering, which we view as a fundamentally more appropriate clustering method than k-means based on Euclidean distance because eigenvectors are, in practice, normalized to unit length. As such, the 21,600 points to be clustered exist on a \((P - 1)\)-dimensional spherical manifold, with \(P = 90\) being the number of regions in the specified AAL atlas. The cluster centroids should be estimated respecting this geometry, which is not the case with Euclidean k-means (Supplementary Figure S1). Additionally, diametrical clustering acknowledges the antipodal symmetry along both directions of a given eigenvector. The classical LEIDA approach seemingly addresses this axial symmetry by flipping the 90-dimensional leading eigenvector for every time point \(\tau\), if the number of positive elements exceeds the number of negative elements, which we show can lead to sub-optimal clustering (see Supplementary Figure S1). By acknowledging that the points are distributed on an antipodally symmetric unit hypersphere using diametrical clustering, we obviate the need for arbitrary eigenvector sign flips. Supplementary Table S12 highlights that although these two strategies can and do produce convergent centroids in some circumstances, there are instances where the two methods diverge (e.g., see State 6 in Supplementary Figure S11). We view diametrical clustering as a technically more appropriate method for clustering eigenvectors since it explicitly models vectors with unit length and arbitrary sign. Therefore, we suggest it is used in future studies investigating tVPC using LEIDA.

In this study, we did not address the question of the optimal number of brain states (i.e., cluster centroids). Rather, we explored a range of \(k\), 2 to 20, consistent with previous studies (Cabral et al., 2017; Figueroa et al., 2019). We believe such an approach to be less arbitrary than selecting a single \(k\) based on heuristics such as elbow criteria. However, the number of statistical tests increases substantially. Here we corrected p-value thresholds for multiple comparisons only within-k and presented results for brain states that showed significant associations with PPL or SDI across multiple contiguous values, leading us to believe that such effects are less likely to be random. In a hard class assignment model like diametrical clustering and other k-means models where cluster variance is disregarded, the addition of states will generally shift the location of all other states, thus changing the state-allocation of some samples. Thus, brain state centroids and, correspondingly, statistical tests will vary slightly over the range of \(k\). An encouraging sign of the robustness of our observations is that cluster centroids were robust to initialization (Supplementary Figure S10) and stable across \(k\) (Supplementary Figure S9).

Opportunities remain for developing the methodology surrounding the clustering of dynamic BOLD time series. Like other k-means methods, diametrical clustering applies a hard class assignment. Probabilistic estimates of cluster assignment for points on a spherical manifold can be estimated using the Watson mixture model, and non-circular cluster outlines can be estimated using the Bingham distribution (Bingham, 1974; Sra and Karp, 2013; Watson, 1965). Beyond cluster centroids, directional archetypal analysis may be used to determine representative corners (archetypes) of the data cloud and thus modeling brain traversal between archetypes (Olsen et al., 2022). These methods are not commonly used, and their development may assist in clustering dynamic functional connectivity data structures and more objectively estimating how many brain states to include. Finally, retaining only the first eigenvector from the eigenvalue decomposition of the phase coherence matrix may remove meaningful information. The rank of a matrix with cosine entries is always two since the angle difference identity allows us to construct two linearly independent vectors, cosine and sine of the input vector, respectively, that fully characterize all information in the input matrix. The instantaneous leading eigenvector constructed as part of the LEIDA pipeline is thus some linear combination of those two trigonometric identities. We observed that, on average, 58% of the variance was explained by the first eigenvector. Future methodological studies should consider whether modeling a multivariate Hilbert phase series without explicitly computing coherence maps and their eigenvectors is possible.

We have previously reported a negative association between static functional connectivity within a priori defined resting-state networks, as well as clusters of brain regions showing increased global functional connectivity as a function of PPL and SDI using rs-fMRI data evaluated here (Madsen et al., 2021). Although the orientation of those and the current findings are conceptually convergent, there are differences. The brain states resolved here by our clustering method are not easily translated to canonical resting-state networks. The frontoparietal states observed here share some regional overlap with default mode network elements (blue nodes; precuneus, posterior cingulate cortex, and to some extent ventromedial prefrontal cortex) and executive control network (red nodes; lateral anterior prefrontal cortex, posterior parietal cortex), but notable regions are absent, such as the angular gyrus from the default mode network. Further, some areas related to visual processing are encompassed by the frontoparietal states (blue nodes; lingual gyrus, calcarine sulcus, cuneus). Together, our studies present complementary perspectives on the associations between resting-state connectivity and PPL and SDI.

The current study examined only acute psilocybin effects on dynamic functional connectivity, while previous studies indicate that psilocybin induces lasting changes in clinical symptoms, mood, and core personality traits. Four studies have examined long-term psilocybin effects on functional brain imaging, all primarily examining static connectivity measures (Barrett et al., 2020; Carhart-Harris et al., 2017; Doss et al., 2021; McCulloch et al., 2021), see (McCulloch et al., 2022) for a recent review. Two of these studies analyzed dynamic conditional correlation (Engle, 2002) as a variance measure of edge-specific correlation coefficients (Barrett et al., 2020; Doss et al., 2021). Further evaluation of lasting modulation of connectivity dynamics will provide complementary insight into the neurobiological mechanisms underlying lasting behavioral and clinical effects of psilocybin.

Our model estimates that a plasma psilocin level of 20 pg/L, corresponding to 70% neocortex 5-HT2AR occupancy (Madsen et al., 2019), results in a more than 50% decrease in the fractional occurrence of frontoparietal state 1 (for \(k = 7\)). Although this indicates a pronounced change in this brain state, the fact that two participants showed low fractional occurrence values at baseline indicates individual variability in these connectivity motifs that needs to be understood more thoroughly. The absence of a brain state identifiable only before or after drug administration suggests that even marginal changes in connectivity dynamics may encompass profound perceptual alterations induced by psychedelics. It is likely that alternative methods for measuring or quantifying functional connectivity dynamics or brain function will provide complementary insights into the neural mechanisms underlying psychedelics. For example, a magnetencephalography study reported pronounced alterations in resting-state network activity following psilocybin administration (Muthukumaraswamy et al., 2013). Findings across the field to date suggest that relevant acute neural effects of psychedelics remain to be fully explored.

Our study is not without its limitations. Our study was without a placebo condition, instead evaluating pre- and post-drug scans. This limits our ability to control for some confounds, e.g., the effect of laying in the scanner for a long time. Pulse and breathing rate data were not available, and therefore, we could not directly regress physiological noise from our data. To overcome this limitation, we attempted to model noise sources via the anatomical component correction algorithm (Belzadi et al., 2007). Head motion was more prevalent during brain scans following psilocybin administration (Madsen et al., 2021).
This is an inherent challenge to scanning participants during peak periods of the psychedelic experience. We performed image realignment and regressed out motion parameters and their first derivatives. Additionally, we excluded two full scan sessions, where motion artifacts were pervasive. Significant motion during brain scans acquired at peak psychedelic effects is to be expected; we observed that the FO of the fully connected state, and at some k the frontoparietal states was associated with motion. Notably, the fact that only the highlighted states were affected during periods of motion, and not all states, suggests that periods of motion, perhaps corresponding to peak psychedelic effects, coincides with periods of altered fractional occurrence of states. Notably, as discussed above, “frontoparietal” is an incomplete description of the brain states observed. This incompleteness reflects the challenge in naming brain states identified through data-driven analytic strategies such as LEiDA, which need not necessarily conform to canonical networks. Conversely, this “trans-network” structure may more fully reflect that psilocybin effects relatedly extend across canonical networks. Furthermore, despite intriguing convergent evidence of psilocybin effects on connectivity dynamics, our sample size is small (N = 15). Clustering in a high-dimensional space exposes risk to the “curse of dimensionality”, where most points in space are equally far away from each other, which can hinder the performance of clustering strategies. Here we modeled 21,600 points, approximately 12x as many points as used in a previous, related study (Lord et al., 2019). Our convergent findings and the stability of our centroids (Supplementary Figures S9–10) support the validity of our findings. Nevertheless, replication in this emergent field is critical, and thus, our results would greatly benefit from replication in other data sets (McCulloch et al., 2022). Finally, subject-specific brain states characteristics are difficult to estimate with such low temporal resolution (sample rate = 0.5 Hz) and may be better explored using faster imaging strategies.

In conclusion, we report that acute psilocybin-induced modulation of brain connectivity dynamics is significantly associated with PPL and SDI. These findings implicate distributed functional motifs in the acute and possibly lasting effects of this drug. Methodologically, we propose an alternative method for clustering eigenvectors that more closely reflects their spherical manifold and sign ambiguity. We also highlight a number of important and relevant analyses of data-driven brain states, including survival analysis of dwell time and assessment of clustering stability.

Funding

This work was supported by Innovation Fund Denmark (grant number 4108–000048), Independent Research Fund Denmark (grant number 6110–0518B), Ester M. og Konrad Kristian Sigurdsdøns Dyrevaernsfond (grant number 850–22–55,166–17-LNG). MKM was supported through a stipend from Rigshospitalets Research Council (grant number R130-A5324). BO was supported by the European Union’s Horizon 2020 research and innovation program under the Marie-Skłodowska-Curie grant agreement No 746,850. Funding agencies did not impact the study and played no role in manuscript preparation and submission.

Declaration of Competing Interest

GMK: H. Lundbeck A/S (research collaboration), Novo Nordisk/Novozymes/Chr. Hansen (stockholder), Janssen Pharmaceutica NV (research collaboration), Sage Therapeutics (advisory board). GMK is currently the president of the European College of Neuropsychopharmacology (ECNP). All other authors declare no conflicts of interest.

Credit authorship contribution statement

Anders S. Olsen: Methodology, Software, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. Brice Ozenne: Methodology, Software, Formal analysis. Martin K. Madsen: Conceptualization, Investigation, Data curation, Writing – review & editing, Project administration, Funding acquisition. Dea S. Stenbæk: Conceptualization, Investigation, Writing – review & editing, Project administration. Sophia Armand: Investigation, Writing – review & editing. Morten Merup: Methodology, Writing – review & editing. Melanie Ganz: Methodology, Writing – review & editing. Gitte M. Knudsen: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. Patrick M. Fisher: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

We have shared the data/code availability statement in the “attach files” step.

Acknowledgements

We gratefully acknowledge the work of MRI assistants: Maja Rou Marstrand-Joergensen and Albin Arvidsson for assisting with data collection; Agnete Dyssgaard and Arafat Nasser for biobank management; Oliver Overgaard Hansen and Vibeke Dam for assistance at psilocybin interventions; Lone Freyr, Gerda Thomsen, Svitlana Olsen, Peter Jensen and Dorthe Givard for technical/administrative assistance; the BAFA laboratory, University of Chemistry and Technology and the National Institute of Mental Health (Prague, CZ) for the production of psilocybin; Glostrup Apotek (Glostrup, DK) for encapsulation (GMP); and Sys Stybe Johansen and Kristian Linnet from the University of Copenhagen Department of Forensic Medicine (Copenhagen, DK) for quantification of plasma psilocin levels.

We have published the MATLAB (The MathWorks, inc.) and R code used to generate the results presented in this study at (https://github.com/anders-s-olsen/psilocybin_dynamic_FC). The datasets generated and/or analyzed during the current study can be made available upon completion of a formal data sharing agreement.

Clinical trial registration number: NCT03289949, first registered 14/09/2017.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.119716.

References


