

Title

Increased global integration in the brain after
psilocybin therapy for depression.

Authors list

Richard E. Daws^{*2,7}, Christopher Timmermann^{1,2}, Bruna Giribaldi¹, James D.
Sexton¹, Matthew B. Wall^{3,4,5}, David Erritzoe¹, Leor Roseman¹, David Nutt¹ and
Robin Carhart-Harris^{1,6}.

Affiliations

¹Centre for Psychedelic Research, Imperial College London, UK.

²The Computational, Cognitive and Clinical Neuroimaging Laboratory (C³NL), Imperial College London, UK.

³Invicro London, Hammersmith Hospital, UK.

⁴Faculty of Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, UK.

⁵Clinical Psychopharmacology Unit, University College London, UK.

⁶Psychedelics Division, Neuroscape, Dept. of Neurology, University of California, San Francisco, USA.

⁷Centre for Neuroimaging Science, Kings College London, London, UK.

Corresponding author

Richard E. Daws richard.daws@kcl.ac.uk

Abstract

Psilocybin therapy shows antidepressant potential, but its therapeutic actions are not well understood. We assessed the sub-acute impact of psilocybin on brain function in two clinical trials of depression. The first was an open-label trial of orally administered psilocybin (10mg and 25mg, 7 days apart) in treatment-resistant depression (TRD). fMRI was recorded at baseline and one day after the 25mg dose. Beck's depression inventory (BDI) was the primary outcome measure (MR/J00460X/1). The second trial was a double-blind phase 2 randomised control trial (DB-RCT) comparing psilocybin therapy with escitalopram. Major depressive disorder (MDD) patients received either: 2 x 25mg oral psilocybin, 3 weeks apart, plus 6 weeks of daily placebo ('psilocybin-arm'); or 2 x 1mg oral psilocybin, 3 weeks apart, plus 6 weeks of daily escitalopram [10-20mg] ('escitalopram-arm'). fMRI was recorded at baseline and 3 weeks after the 2nd psilocybin dose (NCT03429075). In both trials, the antidepressant response to psilocybin was rapid, sustained and correlated with decreases in functional MRI (fMRI) brain network modularity, implying that psilocybin's antidepressant action may depend on a global increase in brain network integration. Network cartography analyses indicated that 5-HT_{2A} receptor-rich higher-order functional networks became more functionally inter-connected and flexible post psilocybin. The antidepressant response to escitalopram was milder and no changes in brain network organisation were observed. Consistent efficacy-related brain changes, correlating with robust antidepressant effects across two studies, suggest an antidepressant mechanism for psilocybin therapy: Global increases in brain network integration.

1 Main text

2 Depression is a highly prevalent mental health condition ¹, the incidence of which
3 has increased during the Covid-19 pandemic ², e.g., as reflected in increased
4 prescriptions of antidepressant medications ³. However, even the best performing
5 antidepressant drugs show modest efficacy, non-negligible side effects,
6 discontinuation problems, and high relapse rates ⁴⁻⁷, highlighting the need for new
7 improved treatments ⁸.

9 Patients with a diagnosis of depression often exhibit a negative cognitive bias,
10 characterised by pessimism, poor cognitive flexibility, rigid thought patterns and
11 negative fixations regarding 'self' and the future ^{9,10}. A number of authors have
12 directly or indirectly taken inspiration from dynamical systems theory to describe
13 depressive episodes as 'attractor states', i.e., stereotyped cognitive states with
14 'gravitational pull' ¹¹.

16 Neuroimaging research has consistently found examples of abnormal brain
17 functioning in depression, resonant with its phenomenology ¹²⁻¹⁴. A hierarchically
18 supraordinate intrinsic brain network ¹⁵, the default mode network (DMN), is
19 associated with introspection and self-referential thinking ¹⁶. These cognitive
20 functions are often overactive in depression ⁹ and several studies have linked
21 excessive engagement of DMN functioning with depressive symptomatology ¹².

23 In addition to the DMN, other higher-order brain networks such as the executive (EN)
24 and salience networks (SN) have been implicated in depression ^{14,17}. These

networks are associated with ‘cognitive control’ and internal versus external attention switching ^{18,1920}. Such attentional switching is often impaired in depression ²¹.

Tellingly, the serotonin 2A (5-HT_{2A}) receptor subtype, which is the key proteomic binding-site of ‘classic’ serotonergic psychedelic drugs, such as psilocybin ²², is most densely expressed in a broad pattern of cortex that closely resembles a conjunction map of the DMN, EN and SN ²³, corresponding to the transmodal portion of the brain’s principal hierarchical gradient ¹⁵.

In the last 15 years, at least six separate clinical trials have reported impressive improvements in depressive symptoms with psilocybin therapy ²⁴. Included among these studies are: 1) an open-label trial in treatment-resistant depression ²⁵, and 2) a double-blind randomised controlled trial (DB-RCT) with an active comparator, the selective serotonin reuptake inhibitor (SSRI) and conventional antidepressant, escitalopram ²⁶. These two trials, which included pre- and post-treatment functional magnetic resonance imaging (fMRI), are the focus of the present paper’s analyses.

The therapeutic action of psilocybin and related psychedelics is incompletely understood. However, one model proposes that psychedelics cause a 5-HT_{2A} receptor induced dysregulation of spontaneous population-level neuronal activity, linked to a temporary ‘disintegration’ of intrinsic functional brain networks ²⁷ and a hypothesised decrease in the precision-weighting of predictive models encoded (at least in part) by the integrity of functional modules ²⁸. One important corollary of modular ‘disintegration’ appears to be the broadening of the brain’s functional repertoire of states – commensurate with a broader or flatter global energy landscape ²⁹.

1

2 Here we hypothesize that the well-replicated finding of brain network disintegration
3 and desegregation under psychedelics ^{30,31} will be apparent sub-acutely, in post-
4 treatment resting-state fMRI data. We also hypothesise that this effect, consistent
5 with a flatter energy landscape, will relate to improved depression outcomes, and will
6 not be observed after a course of the selective serotonin reuptake inhibitor (SSRI),
7 escitalopram.

Results

Open-label trial

Rapid antidepressant effect of psilocybin therapy

Patients with treatment-resistant depression (TRD) participated in a single-arm, open-label psilocybin therapy clinical trial (Figure 1a). Baseline clinical assessment and resting-state fMRI were followed by fixed-order 'low' (10mg) and 'high' (25mg) psilocybin therapy dosing days (DD) that were separated by one week. A second clinical assessment and fMRI scan was conducted one day after DD2. Remote assessments of clinical status were conducted 1 week, 3 months and 6 months after DD2. Further details are available in the methods section and in ²⁵.

Of the 19 patients recruited, 3 were excluded due to excessive fMRI head-motion (Figure 2a). We first confirmed an antidepressant effect of psilocybin in this imaging sample of 16 patients (mean age=42.75, SD=10.15, 4 female) using the Beck Depression Inventory (BDI-1A). This patient-rated measure was preregistered for the original investigation ([gtr.ukri.org: MR/J00460X/1](https://gtr.ukri.org/MR/J00460X/1)). The BDI captures a broad range of symptoms and places particular emphasis on the cognitive features of depression ³², which may be an important target of psilocybin therapy.

Baseline BDI scores indicated severe depression (mean BDI=34.81, SD=7.38). In line with our prior report ²⁵, rapid, substantial and sustained reductions in depression severity were observed post-treatment (Figure 3a/b). Relative to baseline, significant BDI reductions were observed at 1 week (mean difference, -21.0 points; $t_{15}=7.11$,

95% CI -27.30 to -14.71, $P < .001$, $d = 1.78$) and still evident at 6 months (mean difference, -14.19 points; $t_{15} = 4.26$, 95% CI -21.29 to -7.09, $P < .001$, $d = 1.07$).

Decreased brain modularity one day after psilocybin therapy

To test our primary hypothesis, preprocessed fMRI data were used to estimate normalised network modularity from Pearson correlation functional connectivity matrices of the cortex (see Methods). Confirming our primary hypothesis, brain network modularity was significantly reduced (Figure 4a) one day after psilocybin therapy (mean difference, -0.29; $t_{15} = 2.87$, 95% CI 0.07 to 0.50, $P = .012$, $d = 0.72$).

This result implies an increase in functional connectivity between the brain's main intrinsic networks.

Decreased modularity predicts improved clinical outcomes

We hypothesised that decreased brain network modularity would relate to the sustained improvements in depression severity that follow psilocybin therapy. To test this, we calculated Pearson correlations between the post-treatment brain modularity and BDI scores from the 3 post-treatment timepoints (1 week, 3 months, 6 months).

After false discovery rate (FDR) correction for multiple comparisons, a strong significant Pearson correlation was observed at the 6 months primary endpoint ($r_{14} = 0.64$, 95% CI 0.29 to 0.84, $P = .023$, Figure 4b). Directionally consistent relationships were seen at 3 months ($r_{14} = 0.46$, 95% CI 0.03 to 0.74, $P = .114$) and 1 week ($r_{14} = 0.29$, 95% CI -0.16 to 0.64, $P = .284$), but these did not survive correction.

Pre- vs post-treatment change in modularity significantly correlated with change in BDI score at 6 months, relative to baseline ($r_{14} = 0.54$, 95% CI 0.14 to 0.78, $P = .033$, Figure 4c). These results imply that decreased brain modularity one day after

psilocybin therapy relates to long-term improvements in depression symptom severity.

DMN changes in within and between FC one day after treatment

Previous research has implicated depressive symptomology with hyperconnectivity of the DMN¹² and hypoconnectivity of the DMN with other higher-order 'cognitive' networks, including the EN and SN^{14,17}. We, therefore, tested for evidence of these abnormalities being attenuated post-treatment using functional cartography (see methods). Consistent with our prior hypothesis, significant reductions in DMN network recruitment (mean difference, -0.54; $t_{15}=-2.99$, 95% CI -0.92 to -0.15, $P=.009$, $d=0.75$, Figure 4d), and increased between-network integration between the DMN and EN (mean difference, 0.53; $t_{15}=3.01$, 95% CI 0.15 to 0.90, $P=.01$, $d=0.75$) and between the DMN and SN (mean difference, 0.55; $t_{15}=2.89$, 95% CI 0.14 to 0.95, $P=.01$, $d=0.72$) (FDR-corrected) were observed one day post psilocybin therapy. An exploratory analysis of the changes in network recruitment and between-network integration of other brain networks is available in the supplementary information (Supplementary Figure 2).

Taken together, these findings indicate a clinically relevant decrease in brain network modularity following psilocybin therapy for TRD. A network cartography analysis suggests that this global change in network organisation may be underpinned by a specific decrease in within-DMN connectivity and increase in DMN connectivity with other higher-order networks including the EN and SN.

Double-blind randomised controlled trial

Psilocybin therapy versus escitalopram for depression

The design of this DB-RCT (Figure 1b) gave a novel opportunity to compare - not just the safety and efficacy - but also the mechanisms-of-action of psilocybin therapy to those of a conventional antidepressant drug, escitalopram. Patients with major depressive disorder (MDD) were randomly allocated to a 'psilocybin-arm' or 'escitalopram-arm' (Figure 1b). Baseline clinical assessment and resting-state fMRI was followed by DD1 where patients received either 25mg psilocybin (psilocybin-arm) or a presumed inactive 1mg psilocybin dose (escitalopram-arm). All patients were informed that they would receive psilocybin but were blind to the dosage. DD2 occurred three weeks after DD1 and was a duplicate dosage. Beginning one day post DD1, patients took daily capsules for 6 weeks and 1 day in total. For both conditions, one capsule per day was ingested for the first 3 weeks and two capsules per day were ingested thereafter. Capsule content was either inert placebo (microcrystalline cellulose, psilocybin-arm) or 10mg escitalopram in the escitalopram-arm, i.e., 10mg daily for the first 3 weeks and 2 x 10mg = 20mg daily for the final 22 days. Further details are available in the methods section and in ²⁶.

Of the 59 MDD patients recruited, 29 were randomly allocated to the escitalopram-arm. Of those, four discontinued due to adverse reactions to escitalopram, one was lost due to the Covid-19 UK lockdown, and three were excluded due to excessive fMRI head motion (Figure 2b). The remaining 21 patients (mean age=40.9, SD=10.1, 6 female) were included in the escitalopram imaging sample. Thirty patients were randomly allocated to the psilocybin-arm. Of those, one was excluded for choosing not to take the daily (placebo) capsules, two did not attend the post-

treatment session due to the Covid-19 UK lockdown and five were excluded due to excessive fMRI head motion. The remaining 22 patients (mean age=44.5, SD=11.0, 8 female) were included in the psilocybin imaging sample (Figure 2b).

The BDI was a secondary outcome measure for this DB-RCT (ClinicalTrials.gov identifier: NCT03429075); however, this measure proved to be an especially sensitive index of post psilocybin reductions in symptom severity across the trials²⁶. Between-trials, baseline BDI (Figure 3c) was significantly greater in the open-label TRD trial compared with the DB-RCT MDD trial (mean difference, 6.53 points; $t_{57}=3.01$, 95% CI 2.18 to 10.88, $P=.013$, $d=0.83$). This difference is likely due to TRD being an inclusion criteria in the open-label trial, but not in this DB-RCT.

As described in our previous report²⁶, BDI-measured reductions in depressive symptom severity were significantly greater under psilocybin than escitalopram, indicating superior efficacy of psilocybin therapy vs. escitalopram (Figure 3d). Moreover, we confirmed the statistical significance of this contrast within the smaller neuroimaging sample included in the present analyses after testing for an arm x timepoint ANOVA interaction on BDI scores ($F_{3,123}$, 4.47; $P=0.005$). FDR-corrected pairwise comparisons relative to baseline were significantly different at 2 weeks (mean difference, -8.73; $t_{41}=-3.66$, 95% CI -13.55 to -3.91, $P=0.002$, $d=0.98$), 4 weeks (mean difference, -7.79; $t_{41}=-2.69$, 95% CI -13.62 to -1.95, $P=0.013$, $d=0.77$) and at 6 weeks (mean difference, -8.78; $t_{41}=-2.61$, 95% CI = -15.58 to -1.97, $P=0.013$, $d=0.75$), all favouring the psilocybin-arm.

Decreased brain modularity for psilocybin but not escitalopram

Reconfirming our primary hypothesis (Figure 5a-b) and replicating analyses on the open-label trial data, brain network modularity was significantly reduced at the trial's primary endpoint, 3 weeks after psilocybin therapy (mean difference, -0.39; $t_{21}=-2.20$, 95% CI -0.75 to -0.02, $P=0.039$, $d=0.47$). Moreover, for the psilocybin condition, post-treatment decreases in brain network modularity significantly correlated with improvements in depression symptom severity at this primary endpoint ($r_{20}=0.42$, $P=.025$, one-tailed).

Importantly, there was no significant interaction between treatment-arm and scanning session on network modularity ($F_{1,41}=2.719$, $p=0.107$). However, there was evidence that the reduction in network modularity and its relationship to depression severity may be specific to the psilocybin-arm. Namely, in the escitalopram group (Figure 5d-e), network modularity did not change from baseline (mean difference, 0.01; $t_{20}=0.07$, 95% CI -0.35 to 0.33, $P=0.95$, $d=0.02$) and there was no significant correlation between changes in modularity and changes in BDI scores ($r_{19}=0.08$; $P=0.361$, one-tailed).

Response to psilocybin correlates with network flexibility

The specific changes in network recruitment observed one day after psilocybin therapy in the open-label trial were not replicated at 3 weeks in this DB-RCT (Supplementary Information). However, the faster fMRI scanning protocol adopted in the DB-RCT generated twice as much temporal data per scanning session (see methods). This, therefore, provided the rare opportunity to examine changes in the dynamic flexibility of brain networks following psilocybin therapy.

1 The metric known as 'dynamic flexibility' indexes how often brain regions change
2 their community allegiance over time - during the course of an fMRI scan ^{33,34} (see
3 methods). Reduced functional dynamics have been previously associated with
4 depression symptomology ¹⁴. In an exploratory analysis, post psilocybin therapy
5 changes in network flexibility were correlated with changes in BDI score (Figure 5c).
6 After FDR-correction, increased EN dynamic flexibility strongly correlated with
7 greater symptom improvement at the 6-week primary endpoint for the psilocybin-arm
8 ($r_{20}=-0.76$, 95% CI -0.90 to -0.50, $P=0.001$). Strong correlations that survived FDR-
9 correction were also observed when combining regions from the EN with other
10 lateral frontoparietal networks such as the SN and dorsal attention (DA) network
11 (Figure 5c). Critically, there were no significant correlations between changes in BDI
12 scores and changes in dynamic flexibility in the escitalopram-arm (Figure 5f).

Discussion

In light of growing evidence for the antidepressant efficacy of psilocybin therapy ²⁶, the present findings advance our understanding of its possible underlying brain mechanisms. Across two trials, decreased brain modularity was observed and correlated with improvements in depressive symptomatology. Moreover, this antidepressant action may be specific to psilocybin therapy, as no changes in modularity were observed with the conventional SSRI antidepressant, escitalopram.

Research into the acute brain action of psychedelics has revealed well-replicated changes in global brain function that are somewhat consistent with those observed here, i.e., an increased repertoire of inter-regional and between-network FC ^{29–31}. A previous analysis of ours had suggested some contrasting changes in the architecture of spontaneous brain function one day following psilocybin treatment for depression relative to what has been observed during the acute psychedelic state itself; i.e., spatially expanded DMN FC (one day post-treatment for TRD) versus acute DMN ‘disintegration’ ²⁵. However, others have reported evidence of increased inter-network FC 1 week and 1-month post-psilocybin ³⁵, as well as 1-day post-ayahuasca, including increases in DMN-SN FC in healthy volunteers ³⁶. These findings are consistent with the present study, but here we show robust and reliable evidence that increases to global brain network integration accompanies the antidepressant efficacy of psilocybin therapy.

The present modularity metrics may be more sensitive indices of the antidepressant action of psilocybin than previously applied time-averaged within and between-

network FC analyses²⁵. Indeed, they may bear relevance to other FC metrics applied to acute-state psychedelic fMRI data^{29,30} where a general picture of increased global FC and a broadened dynamic state-space has emerged²⁸. In this context, the results could be understood as a ‘carryover’ effect resembling brain dynamics associated with the acute action of psychedelics, albeit at an attenuated level and in a specific population (i.e., depressed patients). To show robustness to analytical method, we also carried out more traditional mass-univariate analyses – and these yielded consistent findings (Supplementary Figures 3/4). However, an advantage of network modularity is its capacity to elegantly summarise global changes in the brain’s functional network organisation³⁷.

Previous research on resting-state activity in depression has found heightened network modularity correlating with symptom severity^{17,38}. Additional work implies heightened within-DMN FC and elevated FC between limbic regions such as the amygdala and high-level cortical regions correlates with ruminative symptoms in depression^{39,12}. Taken together, a model emerges of abnormally modular spontaneous brain function in depression that is effectively remediated by psilocybin therapy. According to various findings, the FC energy landscape or state-space in depression can be described as abnormally constricted, paralleling the narrow, internally focused, ruminative quality of mood and cognition in the disorder¹¹. In contrast, psilocybin appears to increase the brain’s ability to visit a broader state-space, both acutely *and* post psilocybin therapy in depressed patients, as shown here. Moreover, this ‘liberating’ action of psilocybin is paralleled by subjective reports of ‘emotional release’^{40,41} as well as sub-acute increases in behavioural optimism⁹, cognitive flexibility⁴², and psychological flexibility post psychedelic⁴³. Indeed,

heightened emotional responsiveness may be specific to psilocybin therapy versus SSRIs ²⁶.

It is plausible that this putative liberating effect of psilocybin on cortical activity occurs via its direct agonist action on cortical 5-HT_{2A} receptors, dysregulating activity in regions rich in their expression. We surmise that chronic escitalopram does not have the same effect on brain modularity due to its more generalised action on the serotonin system and predominant action on inhibitory postsynaptic 5-HT_{1A} receptors, which are richly expressed in limbic circuitry ^{27,44}.

Beyond the global decrease in network modularity post psilocybin, we observed functional changes in default mode (DMN), executive (EN) and salience (SN) network dynamics that are consistent with neurobiological models of depression ⁴⁵. These high-order transmodal networks house the highest density of 5-HT_{2A} receptors, the principal action-site for serotonergic psychedelics ^{22,23}. High-order networks are implicated in the acute action of psychedelics, where they show reduced modularity and increased communication with regions ordinarily outside of their community limits ^{29–31}.

The EN and SN have been associated with tasks requiring cognitive flexibility such as learning and task-switching ^{18,19,46,47}; impaired functioning of these networks have been reported in depression ^{14,17}, and other disorders exhibiting cognitive inflexibility such as autism spectrum disorder ⁴⁹ and obsessive-compulsive disorder ⁵⁰. Our results suggest that decreased modularity, or increased flexibility of these networks following psilocybin therapy, is a key component of its therapeutic mechanism of

1 action. We did not formally assess cognitive flexibility in the clinical trials reported
2 here but we did observe improvements in general cognitive functioning post
3 psilocybin treatment in the DB-RCT, as well as treatment-specific improvements in
4 ‘emotional avoidance’ – i.e., an inversion of the related construct ‘psychological
5 flexibility’ (see ²⁶).

6
7 It should be noted that psychological processes that do not reliably relate to brain
8 modularity changes may have played a role in the main clinical outcomes of this
9 study – and an inability to discount such factors – precludes the making of confident
10 inferences that drug alone was the main causal determinant of the imaging
11 outcomes – or indeed, that decreased modularity is sufficient for response to
12 psilocybin therapy. Nevertheless, the changes observed in the neuroimaging data
13 were consistent with prior brain imaging research regarding the acute action of
14 psychedelics and are plausible in light of evidence of elevated modularity and
15 abnormal functioning of higher-order networks in depression ^{12,17,38,39,48}.

16
17 Successful Phase 3 DB-RCTs will be required to achieve licensing for psilocybin
18 therapy but pragmatic trials may better address questions regarding treatment
19 practicability, specificity and optimization ⁵¹. Given the emerging research into
20 psychedelic therapy, it is important for large scale trials to establish the
21 generalisability, reliability, and specificity of psilocybin’s antidepressant response.
22 For example, it is likely that efficacy will depend on symptom severity, depression
23 sub-types and co-morbidities – as well as other key pharmacological and
24 extrapharmacological factors ⁵². For brain imaging studies, we would recommend
25 network modularity analyses like those employed here. fMRI datasets are complex,

1 burdensome and susceptible to noise, contributing to the challenge of detecting
2 reliable biomarkers. Simplifying composite measures, such as network modularity,
3 combined with a research domain, symptoms-based approach to psychological
4 phenomena, may be a particularly productive way forward ^{37,51}.

5
6 It should be noted that the present study's findings do not support baseline
7 modularity as a predictor of response to psilocybin therapy. Patients with a range of
8 baseline modularity values showed modularity reductions post treatment with
9 psilocybin. However, the present results do suggest that the early phase modularity
10 change is predictive of the long-term treatment response to psilocybin therapy.

11
12 It is important to consider the potentially confounding effects of head motion when
13 interpreting fMRI data. Here, a robust fMRI preprocessing pipeline was employed
14 alongside strict head motion criteria for patient inclusion. To examine robustness to
15 motion, an analysis of head motion was conducted and is available in the
16 Supplementary Information: fMRI head motion. These analyses bolster the present
17 findings, as there was no evidence that head motion differed between sessions,
18 treatment-arms or correlated with network modularity. The fMRI data were collected
19 with an eyes-closed protocol – which has some advantages; however, it would be
20 compelling if these findings were replicated in data acquired with an eyes-open
21 protocol. In-scanner sleep can be more likely during eyes-closed fMRI – which is a
22 particular disadvantage of it ⁵³. In-scanner sleep cannot be ruled out here.
23 However, the head motion analyses make it unlikely that sleep was a confound.
24 Furthermore, self-reported visual analogue scales (VAS) of time spent asleep and
25 'sleepiness' were acquired immediately after all scan runs in study 2. An analysis of

these data is available in the Supplementary Information (Self-reported in-scanner sleep). Critically, ratings of in-scanner sleep or sleepiness were low and did not differ between treatment-arms or scanning session.

This study's primary hypothesis was confirmed and replicated despite substantial differences between the design of the two trials. Baseline depression severity was significantly greater in the open-label trial. Furthermore, the open-label trial post-treatment fMRI scan was only one day after DD2 and was recorded with a 12-channel head coil and a two second repetition time (TR). In contrast, the DB-RCT post-treatment scan was conducted 3 weeks after DD2 and was recorded with a 32-channel head coil and a 1.25 second TR.

Acknowledging these differences between the trial designs serves to strengthen the validity of the main findings, as they were robustly replicated. However, the lack of replication in the finer-grained cartography analyses limits the network-specific inferences. A supplementary analysis (Supplementary information: Baseline BDI & network cartography) did confirm that baseline depression severity correlated with within-DMN connectivity and between-network DMN-EN and DMN-SN connectivity, as has been previously reported^{12,17,38,39,48}; however, in the DB-RCT, we did not replicate the entirety of the changes in network cartography that were observed in the open-label trial. Given that the observed network effects directly follow predictions from the depression literature, it may be that they are more pronounced in more severe cases - such as those included in the open-label TRD trial. Alternatively, it may be that these effects are only detectable in a short-term, sub-acute phase after psilocybin therapy and not, for example, 3 weeks later. Parsing

1 the relative contribution of baseline severity and time-since-treatment will be an
2 important feature of future clinical trials with neuroimaging – ideally with larger
3 samples and repeated scanning sessions.

4
5 Dynamic analyses can be challenging to conduct. To be sufficiently powered,
6 timeseries need to be of sufficient length to be split into multiple time-windows that
7 are themselves sufficiently long to compute reliable FC measures, and prior research
8 guided our selection of parameters^{54,55}. Collecting sufficient fMRI data in patient
9 cohorts can be challenging but given the appeal of dynamic analyses, efforts are
10 underway to facilitate and improve them⁵⁴. It should be noted that a sufficiently
11 broad window of time was used to estimate Pearson correlation FC; however,
12 ongoing work is required to better understand how to capture the most functionally
13 meaningful dynamic data.

14
15 With these caveats entered, it should be emphasised that the inferences from both
16 cartography analyses converged on the brain's higher-order networks. In particular,
17 the DB-RCT analyses showed robust correlations ($r \sim 0.8$) between increased higher-
18 order network flexibility and psilocybin's treatment response and this converges with
19 the open-label trial as well as prior research¹⁴.

20
21 In summary, depression is a major public health problem associated with huge
22 burden and cost. Here, we identify a robust, reliable and potentially specific
23 biomarker of response to psilocybin therapy for depression. Our results may help to
24 explain why psilocybin therapy holds promise as a novel treatment option in
25 psychiatry.

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Author Contributions Statement

This study was designed and planned by R.C.H. and D.N. and conducted by B.G., M.B.W, D.E. and L.R. This specific analysis was designed by R.E.D. and C.T. The analysis was conducted and visualised by R.E.D. The manuscript was drafted by R.E.D., C.T. and R.C.H. All authors contributed to the interpretation of the study results and revised and approved the manuscript for intellectual content. The corresponding author (R.E.D.) attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Competing Interests Statement

R.C.H. reports receiving consulting fees from Enttheon Biomedical and Beckley Psytech; Dr. Giribaldi, receiving consulting fees from SmallPharma; D.E., receiving consulting fees from Field Trip and Mydecine; D.N., receiving consulting fees from Algernon and H. Lundbeck and Beckley Psytech, advisory board fees from COMPASS Pathways, and lecture fees from Takeda and Otsuka and Janssen plus owning stock in Alcarelle, Awakn and Psyched Wellness. No other potential conflict of interest relevant to this article was reported.

Figures

Figure 1. Trial design schematics. a) Open-label trial. Eligible patients attended a baseline clinical assessment and resting-state fMRI visit. This was followed by two orally administered psilocybin therapy dosing days (DD) separated by 1 week, which differed in dose strength (10mg on DD1, 25mg on DD2). The post-treatment fMRI scan occurred one day after DD2. Remote clinical assessment continued for 6 months. b) DB-RCT. Patients attended a baseline clinical assessment and resting-state fMRI visit and were randomly assigned to the psilocybin-arm (top-branch) or escitalopram arm (bottom-branch). The psilocybin-arm involved 2 x 25mg psilocybin therapy DD's with 3 weeks of daily placebo capsules following each DD. The escitalopram-arm involved 2 x 1mg psilocybin therapy DD's with 3 weeks of 10mg daily escitalopram following DD1 and 20mg of escitalopram following DD2. Both groups attended a post-treatment clinical assessment and fMRI visit 3 weeks and one day after DD2.

1

2 Figure 2. Recruitment flow diagrams. a) The open-label trial (a) was conducted during
3 2015/16. b) The DB-RCT was conducted during 2019/20.

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Figure 3. **Beck depression inventory (BDI) scores.** a) Open-label trial boxplots of treatment-resistant depression (TRD) patient's BDI scores across timepoints. b) Qualitative raster plots of individual patient's BDI for each timepoint (columns). c) Baseline BDI of the TRD patients in the open-label trial was significantly greater than the major depressive disorder patients (MDD) in the DB-RCT ($t_{57}=3.01$, 95% CI 2.18 to 10.88, $P=.013$, $d=0.83$, two-tailed). d) DB-RCT BDI scores from each study arm and timepoint. Qualitative raster plots of individual patient's BDI for each timepoint for the psilocybin-arm (e) and escitalopram-arm (f). The boxplot central marks represent the group median, the box edges represent the 25th-75th percentiles and the whiskers extend to the data range. Independent samples of $n=16$ TRD were used in (a) and alongside $n=43$ MDD in (c), of which $n=21$ in the escitalopram-arm and $n=22$ in the psilocybin-arm are displayed in (d). The rows from each qualitative raster plot were ordered by the sum BDI score across timepoints. (BL=Baseline, w=week, m=month).

Figure 4. **Open-label trial: Treatment-resistant depression patient responses to psilocybin therapy relate to increases in brain network integration.** a) Brain modularity (Q - normalised) significantly reduced, indicating a global increase in brain network integration following psilocybin therapy in patients with TRD ($t_{15}=2.87$, 95% CI 0.07 to 0.50, $P=.012$, $d=0.72$, two-tailed). The solid and dotted lines represent the mean and median, respectively. Patient's data are connected by solid lines and rendered in colour if modularity decreased. b) Absolute post-treatment scan modularity correlated with absolute Beck depression inventory (BDI) scores at the 6 months primary endpoint ($r_{14}=0.64$, 95% CI 0.29 to 0.84, $P=.023$, two-tailed, FDR-corrected). c) Post-treatment change in brain modularity significantly correlated with treatment response (BDI: Baseline – 6 months) ($r_{14}=0.54$, 95% CI 0.14 to 0.78, $P=.033$, two-tailed). d) DMN (red) recruitment decreased ($t_{15}=-2.99$, 95% CI -0.92 to -0.15, $P=.009$, $d=0.75$) and its between-network integration with the EN (gold) ($t_{15}=3.01$, 95% CI 0.15 to 0.90, $P=.01$, $d=0.75$) and SN (purple) ($t_{15}=2.89$, 95% CI 0.14 to 0.95, $P=.01$, $d=0.72$) increased following psilocybin therapy (All two-tailed and FDR-corrected). The boxplot central marks represent the group median, the box edges represent the 25th-75th percentiles and the whiskers extend to the data range. Independent samples of $n=16$ TRD were used in (a-d). DMN=Default Mode Network, EN=Executive Network, SN=Salience Network.

Figure 5. DB-RCT: Increased global brain network integration correlates with treatment response following psilocybin, but not following escitalopram. a) Significant decreases in brain network modularity (Q - normalised), indicating greater brain network integration following psilocybin therapy ($t_{21}=-2.20$, 95% CI -0.75 to -0.02, $P=0.039$, $d=0.47$, two-tailed). The solid and dotted lines on the distributions represent the mean and median, respectively. Individual patient data are represented and connected with solid lines between sessions that are rendered with colour if modularity decreased between sessions. b) Post-treatment change in brain modularity significantly correlated with the treatment response primary endpoint (BDI: Baseline – 6 weeks) ($r_{20}=0.42$, $P=.025$, one-tailed). c) Significant correlations between increased dynamic network flexibility and the psilocybin primary endpoint treatment response (BDI: Baseline - 6 weeks) are coloured (white = $P>.05$, * = survives FDR-correction). The EN exhibited the largest correlation ($r_{20}=-0.76$, 95% CI -0.90 to -0.50, $P=0.001$, two-tailed). The equivalent analyses of brain network modularity in the escitalopram-arm did not show significant session differences (d), relationship to individual treatment response (e) or network flexibility (f). $n=22$ independent psilocybin-arm samples are displayed in (a-c) and $n=21$ independent escitalopram-arm samples are displayed in (d-f). DN=Default Mode Network, DA=Dorsal attention, EN=Executive network, LI=Limbic, SM=Somatomotor, SN=Salience Network VS=Visual.

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Methods

Trial overviews

The trial designs (Figure 1) for and the main clinical outcomes of the open-label ³¹ (gtr.ukri.org: MR/J00460X/1) and DB-RCT ³² (clinicaltrials.gov: NCT03429075) have been previously published. Both trials were conducted at the NIHR Imperial Clinical Research Facility and received Imperial College London Sponsorship, NHS research and Imperial college Joint Research and Compliance Office (JRCO) ethical approval, Health Research Authority and MHRA approval. This work was done under a UK Home Office Schedule 1 Drug Licence. All participants provided written informed consent. Participants were not financially compensated.

Participants

For both trials, eligibility required a general practitioner (GP) confirmed diagnosis of unipolar MDD (16+ on the 21-item Hamilton Depression Rating scale [HAM-D]). The open-label trial had the additional criteria of treatment-resistant depression (TRD), as defined by no improvement despite multiple courses of antidepressant medication (mean = 4.6 ± 2.6 past medications, range = 2-11)³⁹. Patients were asked if they had prior experience of using psychedelics. In the open-label trial, 25% had prior experience. Similarly, in the DB-RCT, 31% of patients in the psilocybin-arm and 24% in the escitalopram-arm had prior experience.

Exclusion criteria were: Immediate family or personal history of psychosis, risky physical health condition (physician-assessed), history of serious suicide attempts,

positive pregnancy test and MRI contraindications. The DB-RCT had the additional exclusion criteria of selective serotonin reuptake inhibitor (SSRI) contraindications or previous escitalopram use. NB: Treatment resistance was neither an inclusion or exclusion criterion in the DB-RCT. All eligible patients undertook telephone screening interviews, provided written informed consent and their mental and physical medical histories were thoroughly evaluated.

Interventions

19 TRD patients were recruited to the open-label trial and attended a 1-day pre-treatment baseline session that included eyes-closed resting-state fMRI and clinical assessment (Figure 1a). This was followed by two psilocybin therapy dosing days (DD), separated by 1 week. A low-dose of psilocybin (10mg) was orally ingested on DD1 and followed by a high-dose dose (25mg) on DD2. The follow-up fMRI and clinical assessment occurred one day after DD2. Patients attended an on-site clinical assessment at 1-week post-DD2 and completed further clinical assessment electronically at 3 and 6 months. Of the 19 TRD patients, 16 were retained (mean age=42.75, SD=10.15, 4 female) for the present analysis after 3 were excluded due to excessive fMRI head motion (Figure 2a).

Of the 59 MDD patients recruited to the DB-RCT, a random number generator allocated 30 to the 'psilocybin-arm' and 29 to the 'escitalopram-arm' (Figure 1b). The final imaging samples for this investigation were n=22 for the psilocybin-arm (mean age=44.5, SD=11.0, 8 female) and n=21 for the escitalopram-arm (mean age=40.9, SD=10.1, 6 female) (Figure 2b). Patients attended a pre-treatment baseline eyes-closed resting-state fMRI. DD1 consisted of either 25mg psilocybin (psilocybin-arm)

or a presumed negligible 1mg psilocybin (escitalopram-arm) dose. All patients were informed that they would receive psilocybin but were blind to the dosage. DD2 occurred three weeks after DD1 and was a duplicate dosage. There was no dosage-crossover. Beginning one day post DD1, patients took daily capsules for 6 weeks and 1 day in total. For both conditions, one capsule per day was ingested for the first 3 weeks and two thereafter. Capsule content was either inert placebo (microcrystalline cellulose, psilocybin-arm) or escitalopram in the escitalopram-arm, 10mg for the first 3 weeks and 2 x 10mg = 20mg total thereafter.

Measuring depression severity

Beck Depression Inventory 1A (BDI-1A) scores were used to assess depression severity in both studies. This patient-rated measure captures a broader range of symptoms, with an additional focus on the cognitive features of depression, compared to other measures such as the QIDS-SR-16⁴⁰. BDI was preregistered as a primary outcome measure in the open-label trial ([gtr.ukri.org: MR/J00460X/1](https://gtr.ukri.org/MR/J00460X/1)) and was measured at baseline and 1 week, 3 months and 6 months post DD2. For the DB-RCT, BDI was measured at baseline and 2, 4 and 6 weeks post DD1. BDI was a secondary outcome measure for this DB-RCT (ClinicalTrials.gov: NCT03429075) and was used here to test for a replication of the effects observed in the open-label trial.

MRI acquisition

Brain imaging was performed on a 3 T Siemens Tim Trio at Invicro, London, UK. Anatomical images were acquired using the ADNI-GO (Alzheimer's Disease Neuroimaging Initiative, Grand Opportunity⁵⁶) recommended MPRAGE parameters

(1mm isotropic voxels, TR=2300ms, TE=2.98ms, 160 sagittal slices, 256 × 256 in-plane FOV, flip angle=9 degrees, bandwidth=240 Hz/pixel, GRAPPA acceleration=2).

In both studies, eyes-closed resting-state fMRI data were collected with T2*-weighted echo-planar images (EPI) with 3 mm isotropic voxels. In study 1, a 12 channel head coil was used to acquire 240 volumes in ~8 minutes: TR=2000ms, TE=31ms, 36 axial slices, flip angle=80 degrees, bandwidth=2298Hz/pixel, GRAPPA acceleration=2). In study 2, a 32-channel head coil was used to acquire 480 volumes in ~10 minutes: TR=1250ms, TE=30ms, 44 axial slices, flip angle=70 degrees, bandwidth=2232Hz/pixel, GRAPPA acceleration=2).

fMRI data preprocessing

Imaging data were preprocessed via a custom in-house pipeline composed of tools from the FMRIB Software Library (FSL) ⁵⁷, Analysis of Functional NeuroImages (AFNI) ⁵⁸, Freesurfer ⁵⁹ and Advanced Normalisation Tools (ANTs) ⁶⁰ packages. Patients were excluded if either fMRI scan contained >20% of volumes with a framewise displacement (FD) greater than 0.5 mm.

Specifically, the following pre-processing stages were performed: 1) removal of the first three volumes; 2) de-spiking (3dDespike, AFNI); 3) slice time correction (3dTshift, AFNI); 4) motion correction (3dvolreg, AFNI) by registering each volume to the volume most similar, in the least-squares sense, to all others; 5) brain extraction (BET, FSL); 6) rigid body registration to anatomical scans (BBR, FSL); 7) non-linear registration to 2 mm MNI brain (Symmetric Normalization (SyN), ANTs); 8) scrubbing

- using a framewise displacement (FD) threshold of 0.5 mm, scrubbed volumes were replaced with the mean of the neighbouring volumes. 9) 6 mm full width at half maximum (FWHM) gaussian spatial smoothing (3dBlurInMask, AFNI); 10) 0.01 to 0.08 Hz band-pass filtering (3dFourier, AFNI); 11) linear and quadratic de-trending (3dDetrend, AFNI); 12) Voxelwise nuisance regression with the 6 realignment motion regressors and 3 tissue signal regressors (ventricles (Freesurfer, eroded in 2 mm space), draining veins (DV) (FSL's CSF minus Freesurfer's Ventricles, eroded in 1 mm space) and local white matter (WM) (FSL's WM minus Freesurfer's subcortical grey matter (GM) structures, eroded in 2 mm space). Regarding local WM regression, AFNI's 3dLocalstat was used to calculate the mean local WM time-series for each voxel, using a 25 mm radius sphere centred on each voxel.

Functional connectivity (FC)

Following the preprocessing, a functional atlas was used to separate the cerebral cortex into 100 regions of interest (ROI) ⁶¹. FC between each pair of ROIs was calculated with a Pearson correlation coefficient between each pair of mean signal 'timecourses' (representing fluctuations in neural activity over time). This resulted in an $N \times N$ FC matrix, with each element representing the connectivity strength between a pair of ROIs. Positive values were retained and Fisher transformed to z-scores. This procedure was repeated independently for each patient and scan (baseline, post-treatment).

Brain network modularity

The community structure, or segregation between the brain's functional networks, was measured by summarising each FC matrix with a common Louvain-like community detection algorithm⁶² where the objective is to maximise the extent to which brain areas can be separated into non-overlapping communities or modules. The modularity quality function score, Q ⁶³, tends to be high when the brain exhibits a high segregation between its functional networks (e.g., strong clusters of FC within brain networks/communities with weak FC to the rest of the brain).

This approach has been commonly applied to fMRI data to characterise how brain function adapts in a number of contexts⁵⁵. Here, modularity, Q ⁶³, was defined in the standard way by:

$$Q = \frac{1}{2m} \sum_{ij} \left(A_{ij} - \gamma \frac{k_i k_j}{2m} \right) \delta(c_i, c_j), \quad (1)$$

Where A_{ij} represents the weight of FC (correlation) between ROI i and j , γ is the structural resolution free parameter (set to 1) and $\frac{k_i k_j}{2m}$ is the expected null FC defined with $k_i = \sum_j A_{ij}$ as the total FC across all connections with ROI i , c_i is the community to which ROI i is assigned, $\delta(c_i, c_j)$ is the Kronecker delta function and equals 1 if ROI i and j belong to the same community and 0 otherwise⁶⁴ and $m = \frac{1}{2} \sum_{ij} A_{ij}$ is the total FC of the network.

To allow valid comparisons between patients and scans, the modularity scores were generated 100 times and the partition with the largest modularity score was normalised by the mean modularity generated from 100 randomly rewired (shuffled)

FC matrices⁶⁵. This common procedure was applied to account for the non-deterministic and near-degenerate partitions (solutions with differing but similar optimality) generated by Louvain algorithms and to account for modularity scores relating to the total sum of FC within the network⁶⁶. This process was repeated independently for each patient and scan.

Functional cartography

The community detection procedure generates a community assignment to each ROI. We used these labels to determine the extent to which ROIs were recruited to the functional network that they typically ‘belong to’, as defined by healthy adults (e.g., DMN regions should reliably form communities with each other).

First, we created an allegiance matrix, P ,³³ which represented the probability that two regions i and j were assigned to the same community across the 100 iterations of the modularity algorithm, defined here as:

$$P_{ij} = \frac{1}{O} \sum_{o=1}^O a_{i,j}^{k,o} \quad (2)$$

where $O = 100$ as the number of partitions. For each partition, $a_{i,j}^{k,o}$ equals 1 if regions i and j belong to the same community.

Using the allegiance matrix, we then summarised how often ROIs either formed communities with ROIs from the same functional network (network recruitment) or formed communities with ROIs between different networks (between-network integration) across the partitions³³. These functional cartography measures were

then normalised against the mean values from 1000 randomly shuffled ROI-network assignments to account for differences in the number of regions in each network⁵⁵. Finally, network recruitment and between-network integration scores were averaged at the network level using 7 predefined cortical networks⁶¹.

Dynamic flexibility

The short TR used in the fMRI protocol of study 2 generated ~twice the number of timepoints and this afforded an additional analysis of dynamic flexibility. Multilayer modularity estimation³⁴ was conducted using a $N \times N \times T$ matrix of 30-volume sliding-windows (37.5 seconds of real-time) with 50% overlap. This window size is typical for estimating dynamic FC with fMRI⁵⁴. For each patient and scan, multilayer modularity, Q_{ML} , was estimated 100 times from each $N \times N \times T$ FC matrix by:

$$Q_{ML} = \frac{1}{2\mu} \sum_{ijlr} \left[\left(A_{ijl} - \gamma_l \frac{k_{il}k_{jl}}{2m_l} \right) \delta_{lr} + \delta_{ij} \omega_{jlr} \right] \delta(c_{il}, c_{jr}), \quad (3)$$

where $\mu = \frac{1}{2} \sum_{ijl} A_{ijl}$ is the total FC of the multilayer network, and $m_l = \frac{1}{2} \sum_{ijl} A_{ijl}$ is the total FC of layer l , A_{ijl} is the FC between ROI i and j at layer l , $\frac{k_{il}k_{jl}}{2m_l}$ is the expected null FC at layer l . The two free γ and ω structural and temporal resolution parameters are used to scale the number of communities and strength of inter-layer edges, respectively. As is typical for fMRI modularity analyses, both were set to 1^{33,55}.

The multilayer modularity estimation generates an $N \times T$ matrix where each element represents the community assignment of each ROI at each layer (time-window).

From this, the flexibility metric, f , can be simply calculated as the number of times an ROI changes its community allegiance, given the number of observations ³³:

$$f_i = 1 - \frac{1}{T-1} \sum_l^{T-1} \delta(c_{il}, c_{i,l+1}), \quad (4)$$

Flexibility scores close to 0 represent rigid ROIs whose community allegiance is stable across time, scores close to 1 represent flexible ROIs whose community allegiance regularly changes (highly flexible). Network level flexibility scores were defined by the average flexibility of ROIs assigned to the given network.

Code availability statement

All analyses and data visualisations were conducted in Matlab R2020a. Code for generating each data figure are available at <https://github.com/rdaws/psilodep>.

Data availability statement

All requests for raw and analyzed data and materials are promptly reviewed by RCH and DJN, CI and PI on the original work. Patient-related data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality.