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Supplementary information

Increased global integration in the brain after psilocybin therapy for depression

In the format provided by the authors and unedited

Supplementary information

fMRI head motion

To investigate the potentially confounding effect of head motion in this study, the mean framewise displacement (FD) and the percentage of scrubbed volumes were extracted from each scan and patient and then compared between sessions, groups and against brain network modularity. The means and standard deviations of FD and scrubbed volumes across patients and scans are displayed in table 1.

In study 1, there was no evidence of a significant difference in FD (paired t-test – t_{15}=1.33, CI=-0.01 to 0.06, p=0.20) or the percentage of volumes scrubbed (t_{15}=0.60, CI=-0.03 to 0.05, p=0.56) between the two scanning sessions. Moreover, there was no significant correlation between head motion and network modularity for FD or percentage of volumes scrubbed in either session (Table 2).

In study 2, FD and percentage of volumes scrubbed were compared in separate two-way rm-anova models with session as the within-subject factor (baseline, post-treatment) and study arm as the between-subject factor (psilocybin, escitalopram). For FD, there was no significant effect of arm ($F_{1,41}$=1.83, p=0.18), session ($F_{1,41}$<0.01, p=0.98) or session * arm interaction ($F_{1,41}$=0.09, p=0.77). Using the same model with percentage of volumes scrubbed as the dependent variable, there was no significant effect of arm ($F_{1,41}$=1.19, p=0.28), session ($F_{1,41}$=0.02, p=0.88) or session * arm interaction ($F_{1,41}$<0.01, p=0.99). Moreover, there was no significant correlation between head motion and network modularity for FD or percentage of volumes scrubbed in either session or study arm (see Table 2).

Taken together, these analyses of head motion suggest that head motion was stable across session and that changes in network modularity are unlikely to have been confounded by noise introduced by fMRI head motion.

Baseline BDI & network cartography

Evidence of the abnormal functional network organisation previously reported in patients with depression was tested for here. Baseline Beck Depression Inventory (BDI) scores were pooled across trials and Pearson correlated with the baseline functional network cartography metrics and FDR-corrected for multiple comparisons (Figure 1). As expected, DMN network recruitment positively correlated with BDI (r=0.381, p=0.003), replicating prior observations that heightened FC of the DMN is associated with worse depression severity (Hamilton et al., 2011). Moreover, the between-network integration of the DMN with the executive network (EN) or the salience network (SN) negatively correlated with depression severity (DMN-EN: r=-0.394, p=0.003; DMN-SN: r=-0.438, p=0.002). This replicated prior observations that hypoconnectivity between the DMN and the lateral frontoparietal networks correlates with worse depression symptoms (Lydon-Staley et al., 2019).

Changes in network recruitment and between-network integration the day-after psilocybin therapy

An exploratory analysis of the network recruitment and between-network integration metrics was conducted to identify changes in network organisation following psilocybin therapy (Figure 2). In the open-label trial, where the post-treatment scan occurred the day-after psilocybin therapy, the two-tailed paired t-tests showed significant effects that did not survive correction for multiple comparisons. However, the uncorrected pattern implied a

decrease in DMN network recruitment and an increase in between-DMN integration with several other functional networks. The equivalent analysis for the double-blind randomised control trial (DB-RCT), where the post-treatment scan occurred 3 weeks after psilocybin therapy, did not render any significant differences in network recruitment or between-network integration.

Mass-univariate analysis of functional connectivity

The network modularity represents a composite score that was derived from a functional connectivity (FC) matrix. A mass-univariate analysis of these matrices was also conducted to test for between session differences and the relationship between FC changes and patient's treatment response. These analyses were conducted at the network and node level. Consistent with the main analyses, positive correlation weights were retained and fisher transformed to z-scores. For the network-level comparisons, node weights were mean-averaged by network identity.

For the open-label trial (study 1), network-level paired two-tailed t-tests showed FC was significantly increased between multiple networks the day after psilocybin-therapy (Figure 2a). Reflecting the main functional cartography analyses, FC between the Default mode network (DMN) increased with lateral frontoparietal networks, such as the dorsal attention network (DA). Moreover, connectivity increased between the lateral frontoparietal networks such as between the DA, executive network (EN) and salience networks (SN).

A network-level analysis was conducted between individual differences in FC change between scans and patient BDI (6 month change from baseline). All significant correlations were negative (Supplementary Figure 2b) . Mirroring the main modularity analysis, these correlations indicate that increases in between-network connectivity the day after psilocybin therapy correlate with improved long-term patient outcomes. The same analyses repeated at the level of the individual node for the between-session and individual differences effects are displayed in Supplementary Figure 2c/d.

For the double-blind randomised control trial (DB-RCT, study 2), the post-treatment scan was conducted 3 weeks after psilocybin therapy (compared to the day after in study 1). Repeating the mass-univariate analyses at the network-level showed no significant between-session differences for the escitalopram-arm. A significant decrease in within-network FC for the limbic network was observed in the psilocybin-arm (Figure 3a/b). The individual differences analyses indicate that an increase in FC between the LI and the visual (VS) and somatomotor (SM) networks correlated with improve patient outcomes in the psilocybin-arm (Supplementary Figure 3b). For the node-level analyses (Supplementary Figure 3c/d), a similar pattern of results was observed between the psilocybin-arm and the open-label trial. Changes in a wide-spread set of between-network connections negatively correlated with patient outcomes. This, again, indicates that increased between-network integration accompanies improvements in patient well-being.

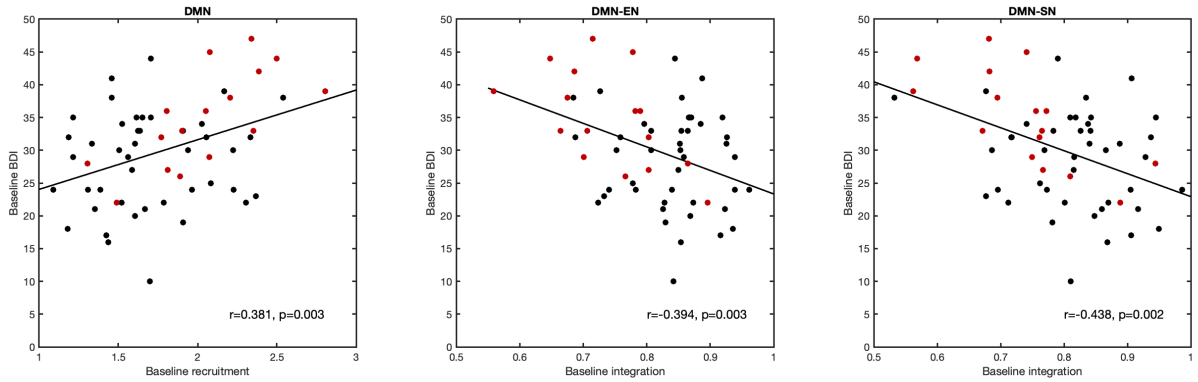
Self-reported in-scanner sleep

After each scan in study 2, patients completed visual analogue scales (VAS) before exiting the scanner. These asked patients to self-report "The amount of time spent asleep in the last scanning period?" and "How sleepy did you feel in the last scanning period?". The time asleep VAS ranged from "Not at all" to "All the time" and the feeling sleepy VAS ranged from "Not at all" to "The most imaginable". VAS ratings ranged from 1-20, with 1 equal to "Not at all" in both cases.

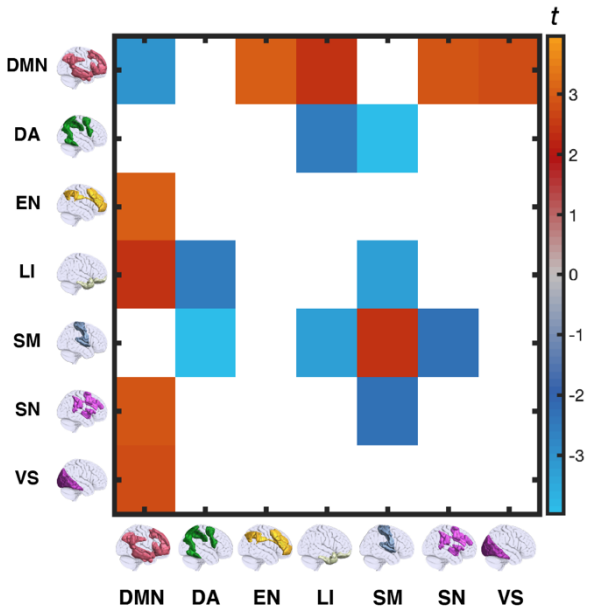
The mean average VAS scores across study arms and scanning sessions were low for time asleep (mean=1.99, SD=3.01) and feeling sleepy (mean=6.79, SD=4.43). Generalised linear mixed effect models were fitted to the responses from each question to test for the fixed effects of treatment-arm (psilocybin, escitalopram) and scanning session (Baseline, post-treatment) with individual patient as a random effect. Neither VAS response showed a significant effect. For time asleep, there was no effect of treatment-arm ($F(1,80)=0.120$, $P=0.730$), scanning

session ($F(1,80)=0.702$, $P=0.405$) and there was no significant treatment-arm * scanning session interaction ($F(1,80)=0.149$, $P=0.701$). For feeling sleepy, there was no effect of treatment-arm ($F(1,80)=2.016$, $P=0.160$), scanning session ($F(1,80)=2.166$, $P=0.145$) and there was no significant treatment-arm * scanning session interaction ($F(1,80)=0.369$, $P=0.545$). Together, this analysis suggests in-scanner sleep/sleepiness is unlikely to be an issue or a confounding factor in this study.

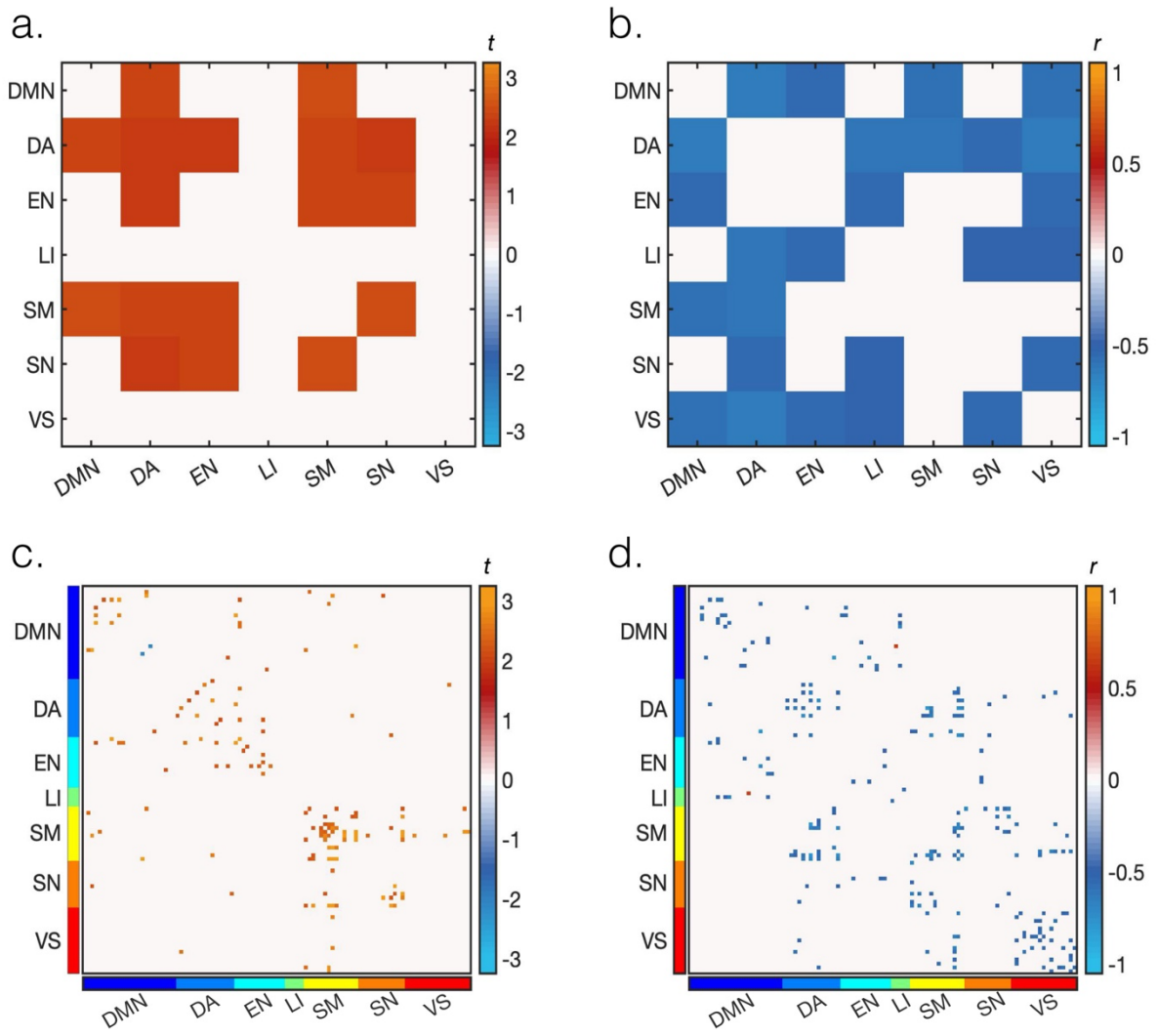
Figures



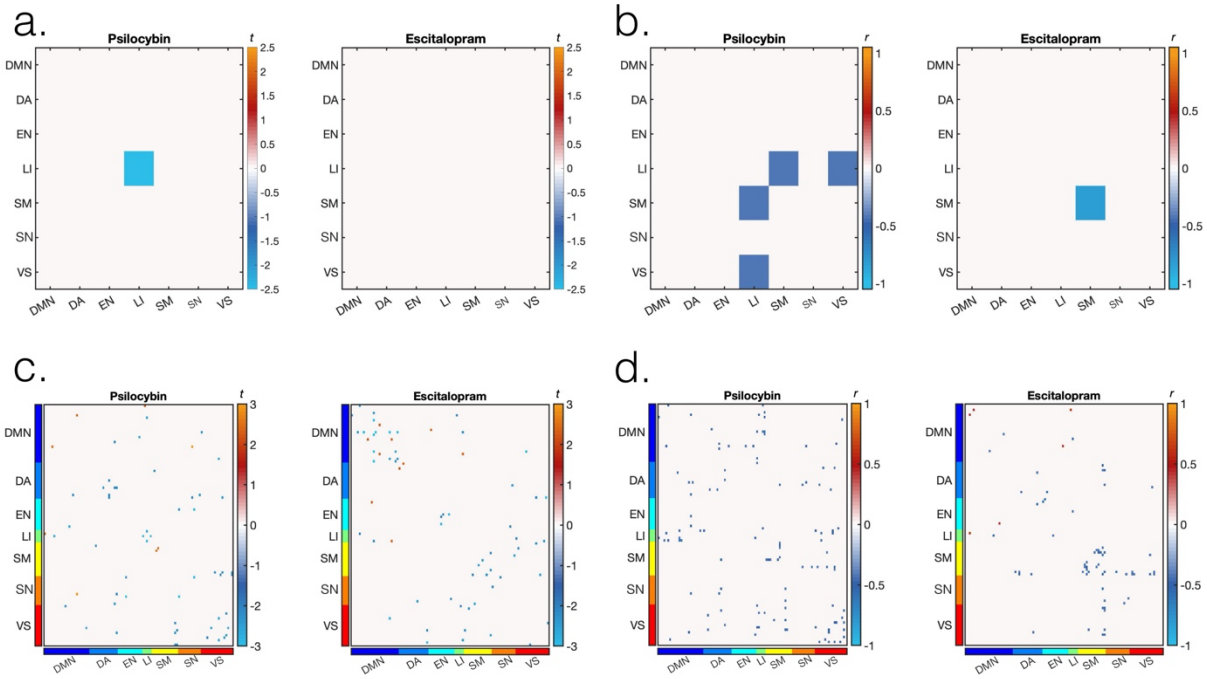
Supplementary Figure 1. **Baseline BDI relationships with network cartography.** Beck Depression Inventory (BDI) severity at baseline correlates (FDR-corrected) with the baseline network recruitment of the default mode network (DMN) (r_{57}=0.381, p=0.003) and the baseline between-network integration of the DMN with the executive network (EN) (r_{57}=0.394, p=0.003) and with salience network (SN) (r_{57}=0.438, p=0.002). Patients with treatment-resistant depression are rendered in red. P-values are two-tailed and have been FDR-corrected for multiple-comparisons.



Supplementary Figure 2. **Open-label trial exploratory analysis of network cartography**. Paired t-statistics of the change in network recruitment (diagonal) and between-network integration (off-diagonal). This exploratory two-tailed analysis uses an uncorrected $P<.05$ threshold (Red=increase, Blue=Decrease). Comparisons do not survive multiple comparisons correction. DMN=Default Mode Network, DA=Dorsal attention, EN=Executive Network, LI=Limbic, SM=Somatomotor, SN=Salience Network, VS=Visual.



Supplementary Figure 3. **Open-label trial: Mass-univariate analysis of Pearson correlation functional connectivity (FC).** a) Significant differences in network FC between the scanning sessions (baseline, post-treatment). Warm colours indicate an FC increase following psilocybin therapy. b) Significant Pearson correlations between changes in network-level FC and Beck Depression Inventory (BDI) score (6 month minus baseline). Cool colours from this individual differences analysis indicate an FC increase that was accompanied by a decrease in BDI. Analyses at the level of individual nodes were repeated to test for between-session effects in FC (c) and correlations between changes in FC and BDI score (d). All analyses are two-tailed and thresholded at an uncorrected $p<0.05$ level. These effects do not survive FDR-correction. DMN=Default Mode Network, DA=Dorsal Attention, EN=Executive Network, LI=Limbic, SM=SomatoMotor, SN=Salience Network, VS=Visual.



Supplementary Figure 4. **DB-RCT: Mass-univariate analysis of Pearson correlation functional connectivity (FC)**. Analysis performed in Figure 2 repeated for the DB-RCT but separated by treatment arm (psilocybin, escitalopram). a) Significant differences in network FC between the scanning sessions (baseline, post-treatment). Cool colours indicate a decrease in FC following the intervention. b) Significant Pearson correlations between changes in network-level FC and Beck Depression Inventory (BDI) score (6 weeks minus baseline). Cool colours from this individual differences analysis indicate an FC increase that was accompanied by a decrease in BDI. Analyses at the level of individual nodes were repeated to test for between-session effects in FC (c) and correlations between changes in FC and BDI score (d). All analyses are two-tailed and thresholded at an uncorrected $p<0.05$ level. These effects do not survive FDR-correction. DMN=Default Mode Network, DA=Dorsal Attention, EN=Executive Network, LI=Limbic, SM=SomatoMotor, SN=Salience Network, VS=Visual.

Table 1. Head motion descriptive statistics. Mean and standard deviation (SD) of framewise displacement (FD) and the percentage of volumes scrubbed (0.5mm FD threshold) for each study, scanning session and group.

		Psilocybin		Escitalopram	
		Mean	SD	Mean	SD
Study 1	*Framewise Displacement*				
	Baseline	0.185	0.087	-	-
	Post treatment	0.162	0.083	-	-
	Scrubbed volumes (%)				
	Baseline	4.333	5.100	-	-
	Post treatment	3.333	5.200	-	-
Study 2	*Framewise Displacement*				
	Baseline	0.181	0.168	0.132	0.070
	Post treatment	0.176	0.126	0.137	0.082
	Scrubbed volumes (%)				
	Baseline	7.773	18.905	3.343	6.602
	Post treatment	7.523	17.892	3.105	9.563

Table 2. Pearson correlation coefficients between individual patients' fMRI brain network modularity and the head motion estimates, framewise displacement (FD) and the percentage of scrubbed volumes (0.5mm FD). Analyses are two-tailed. No correction for multiple comparisons has been applied.

		Psilocybin		Escitalopram	
		r	p	*r*	p
Study 1	*Framewise Displacement*				
	Baseline	-0.290	0.277	-	-
	Post treatment	-0.199	0.461	-	-
	Scrubbed volumes (%)				
	Baseline	-0.265	0.321	-	-
	Post treatment	-0.111	0.683	-	-
Study 2	*Framewise Displacement*				
	Baseline	-0.212	0.344	-0.420	0.058
	Post treatment	-0.299	0.176	-0.323	0.154
	Scrubbed volumes (%)				
	Baseline	-0.250	0.262	-0.257	0.261
	Post treatment	-0.263	0.237	-0.280	0.219

References

Hamilton, J. P. *et al.* Default-Mode and Task-Positive Network Activity in Major Depressive Disorder: Implications for Adaptive and Maladaptive Rumination. *Biol. Psychiatry* **70**, 327–333 (2011).

Lydon-Staley, D. M. *et al.* Repetitive negative thinking in daily life and functional connectivity among default mode, fronto-parietal, and salience networks. *Transl. Psychiatry* **9**, 234 (2019).