

Supplementary Information

Head mean framewise displacement in mm		
Placebo	First tf-fMRI scan	Second tf-fMRI scan
1	0.09	0.08
2	0.12	0.16
3	0.11	0.15
4	0.10	0.08
5	0.17	0.24
6	0.11	0.12
7	0.14	0.13
8	0.11	0.10
9	0.23	0.28
10	0.08	0.09
11	0.08	0.10
12	0.10	0.09
13	0.14	0.18
14	0.20	0.17
15	0.25	0.20
16	0.19	0.14
17	0.06	0.06
18	0.12	0.11
19	0.11	0.10
20	0.15	0.12
21	0.13	0.17
Ayahuasca	First tf-fMRI scan	Second tf-fMRI scan
1	0.20	0.17

2	0.12	0.14
3	0.13	0.14
4	0.15	0.11
5	0.13	0.11
6	0.16	0.18
7	0.18	0.15
8	0.10	0.10
9	0.14	0.16
10	0.07	0.06
11	0.15	0.20
12	0.12	0.11
13	0.14	0.18
14	0.10	0.12
15	0.18	0.16
16	0.19	0.17
17	0.17	0.29
18	0.11	0.14
19	0.18	0.14
20	0.12	0.15
21	0.13	0.15
22	0.15	0.21

Supplementary Table S1. Individual motion parameters. Head mean framewise displacement (in mm) before and after the session for all participants included in the analyses.

	Coordinates (x, y, z)	Anatomical label	Cluster size (voxels)	t	p
Saliency network					
One-sample t-test baseline maps	-4, 30, 26	Left Anterior Cingulate Cortex	6465	31.78	< 0.0001
	36, 12, -6	Right Insula	957	13.47	< 0.0001
	26, 48, 30	Right Middle Frontal Gyrus	895	12.77	< 0.0001
	-40, 14, -6	Left Insula	624	12.03	< 0.0001
	-4, -18, 38	Left Middle Cingulate Cortex	654	11.63	< 0.0001
Default mode network					
One-sample t-test baseline maps	-2, -40, 34	Left Precuneus	6187	37.37	< 0.0001
	-44, -64, 34	Left Angular Gyrus	1485	15.56	< 0.0001
	44, -62, 40	Right Angular Gyrus	1582	14.86	< 0.0001
	4, 46, 18	Right Frontal Superior Gyrus	3395	14.83	< 0.0001
	-18, 42, 42	Left Frontal Superior Gyrus	307	12.13	< 0.0001
Visual network					
One-sample t-test baseline maps	-16, -100, 0	Left Middle Occipital	14559	25.88	< 0.0001
	16, -60, -2	Right Lingual Gyrus	56	8.00	< 0.0001
Sensorimotor network					

One-sample t-test baseline maps	20, -24, 72	Right Precentral Gyrus	9490	26.71	< 0.0001
	-56, -4, 20	Left Postcentral Gyrus	77	8.20	< 0.0001
	40, -24, 18	Right Rolandic Operculum	69	7.82	< 0.0001

Supplementary Table S2. Related to brain network maps in Figures 1; one-sample t-tests using the seed-based connectivity maps. Voxel-wise statistics. If not specified otherwise, extent probability threshold of $p < 0.01$ FWE corrected, voxel-wise threshold of $p < 0.001$ FWE corrected for multiple comparisons, only the first five major clusters are reported. Anatomical labels derived from the AAL atlas (<http://www.gin.cnrs.fr/en/tools/aal-aal2/>) (Tzourio-Mazoyer et al., 2002).

	Coordinates (x, y, z)	Anatomical label	Cluster size (voxels)	t	p
	Saliency network				
Two-sample t-test change maps ayahuasca > placebo	0, 50, 24	Left Frontal Superior Gyrus	248	4.14	< 0.0001
	Default mode network				
Two-sample t-test change maps ayahuasca < placebo	0, -68, 20	Left Precuneus	262	3.73	< 0.0001

Supplementary Table S3. Related to brain network maps in Figures 2; two-sample t-tests using the seed-based connectivity change maps. Voxel-wise statistics. Joint extent and cluster probability thresholds of $p < 0.01$ for the saliency network and $p < 0.05$ for the default mode network. Anatomical labels derived from the AAL atlas (<http://www.gin.cnrs.fr/en/tools/aal-aal2/>) (Tzourio-Mazoyer et al., 2002).

	Coordinates (x, y, z)	Anatomical label	Cluster size (voxels)	t	p
	Saliency network				
Two-sample t-test change maps ayahuasca > placebo	-2, 52, 24	Left Frontal Superior Gyrus	288	4.65	< 0.0001
	Default mode network				
Two-sample t-test change maps ayahuasca < placebo	0, -68, 20	Left Precuneus	206	3.62	< 0.0001

Supplementary Table S4. Related to brain network maps in Supplementary Figures S2; two-sample t-tests using the seed-based connectivity change maps corrected for the difference in head mean framewise displacement between scans. Voxel-wise statistics. Joint extent and cluster probability thresholds of $p < 0.01$ for the saliency network and $p < 0.05$ for the default mode network. Anatomical labels derived from the AAL atlas (<http://www.gin.cnrs.fr/en/tools/aal-aal2/>) (Tzourio-Mazoyer et al., 2002).

	Coordinates (x, y, z)	Anatomic al label	Cluster size (voxels)	t	p
	Salience network				
Change maps ayahuasca > placebo	0, 50,24	Left Frontal Superior Gyrus	90	4.21	0.009
	Default mode network				
Change maps ayahuasca < placebo	0, -68, 20	Left Precuneus	582	3.78	0.003

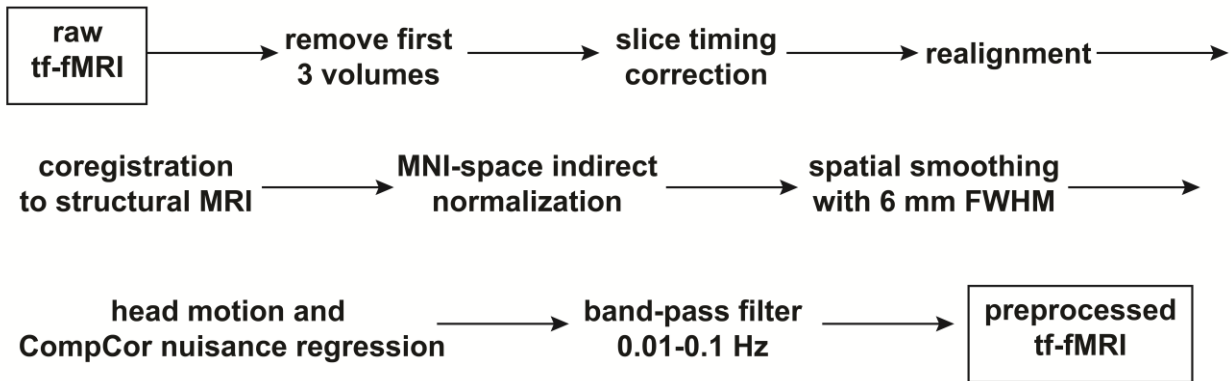
Supplementary Table S5. Related to brain network maps in Figures S3; nonparametric permutation-based statistical inference. Voxel-wise statistics, $p < 0.05$ threshold-free cluster enhancement. Anatomical labels derived from the AAL atlas (<http://www.gin.cnrs.fr/en/tools/aal-aal2/>) (Tzourio-Mazoyer et al., 2002).

	Coordinates (x, y, z)	Anatomic al label	Cluster size (voxels)	t	p
Salience network					
One-sample t-test baseline maps^A	-36, 8,-2	Left Insula	1868	16.47	< 0.0001
	-30, 42, 22	Left Middle Frontal Gyrus	1796	16.10	< 0.0001
	-4,14,40	Left Anterior Cingulate Cortex	2994	15.55	< 0.0001
	32, 44, 18	Right Middle Frontal Gyrus	1428	15.31	< 0.0001
	56, 14, -4	Right Insula	1194	13.25	< 0.0001
Two-sample t-test change maps ayahuasca > placebo	0, 50, 12	Left Anterior Cingulate Cortex	291	3.40	< 0.0001
Default mode network					
One-sample t-test baseline maps^A	2, -42, 24	Left Precuneus	5714	18.64	< 0.0001
	46, -58, 40	Right Angular Gyrus	872	15.62	< 0.0001
	-44, -62, 44	Left Angular Gyrus	995	12.84	< 0.0001
Two-sample t-test change maps ayahuasca < placebo	-10, -52, 18	Left Precuneus	234	3.16	0.001

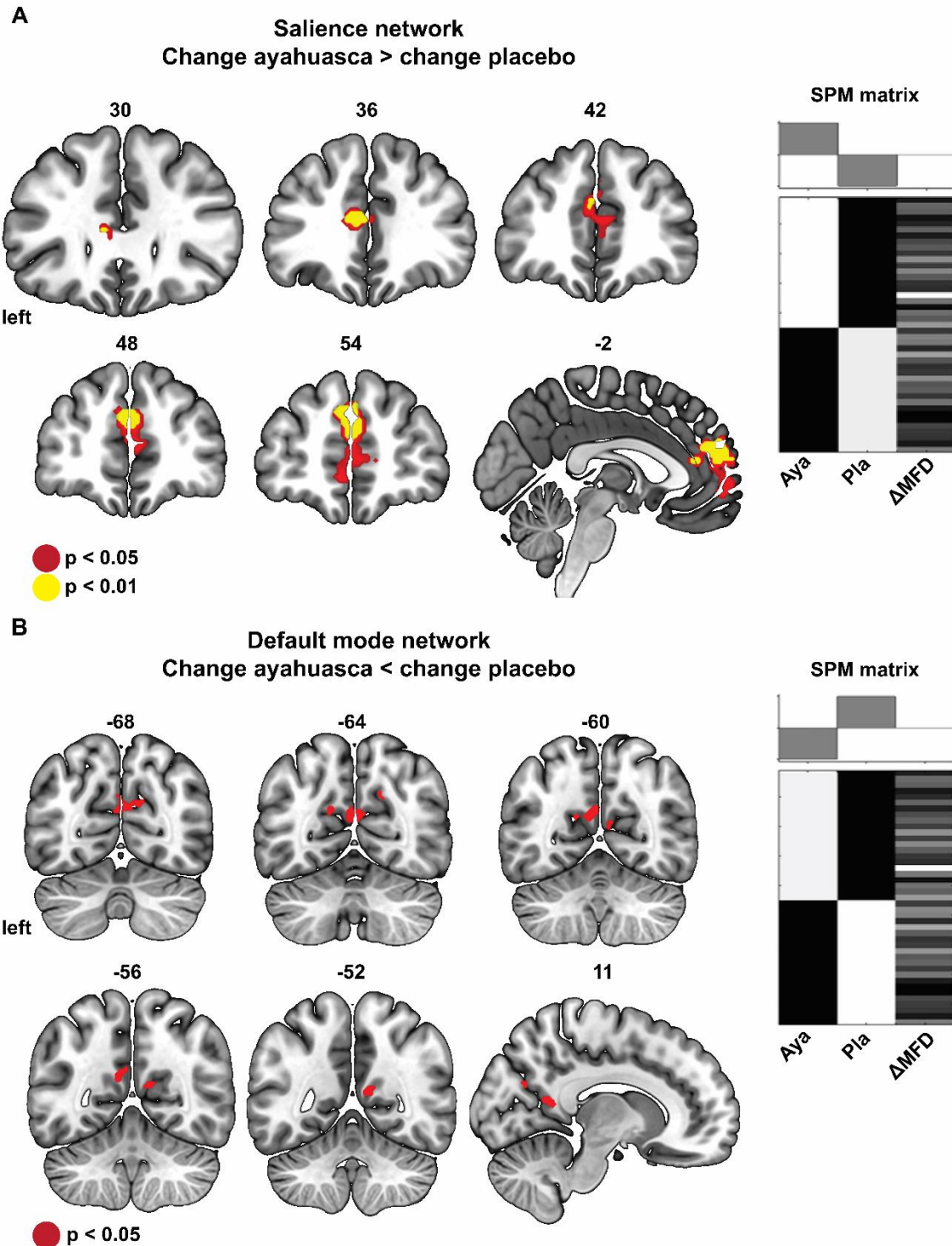
Supplementary Table S6. Related to Supplementary Figures S4; findings using independent component analysis (ICA). Voxel-wise statistics. ^AExtent probability threshold of $p < 0.01$ FWE corrected, voxel-wise threshold of $p < 0.001$ FWE corrected for multiple comparisons, only the first five major clusters are reported. For two-sample t-tests, joint and cluster extent probability thresholds of $p < 0.05$ for the salience network and $p < 0.1$ for the default mode network are reported. Anatomical labels derived from the AAL atlas (<http://www.gin.cnrs.fr/en/tools/aal-aal2/>) (Tzourio-Mazoyer et al., 2002).

	Within salience network connectivity		Within default mode network connectivity		Between salience and default mode network connectivity	
HRS subscales	Ayahuasca	Placebo	Ayahuasca	Placebo	Ayahuasca	Placebo
Somesthesia	0.54*	0.01	-0.06	-0.01	0.21	0.24
Affect	0.39 ⁺	0.27	-0.02	-0.18	0.47[^]	-0.06
Perception	0.31	0.20	-0.01	-0.30	0.10	0.40 ⁺
Cognition	0.29	0.09	-0.20	-0.26	0.42 ⁺	0.13
Volition	0.33	0.41 ⁺	-0.59**	-0.43 ⁺	0.35	-0.05
Intensity	0.27	0.03	-0.29	-0.17	0.02	0.13

Supplementary Table S7. Partial correlation coefficients associating subacute functional connectivity changes with subscales of the HRS, corrected for the difference in head mean framewise displacement between the second and first scan. Significant findings are highlighted in bold. ** $p < 0.005$; * $p < 0.01$; [^] $p < 0.03$; ⁺ $p < 0.1$

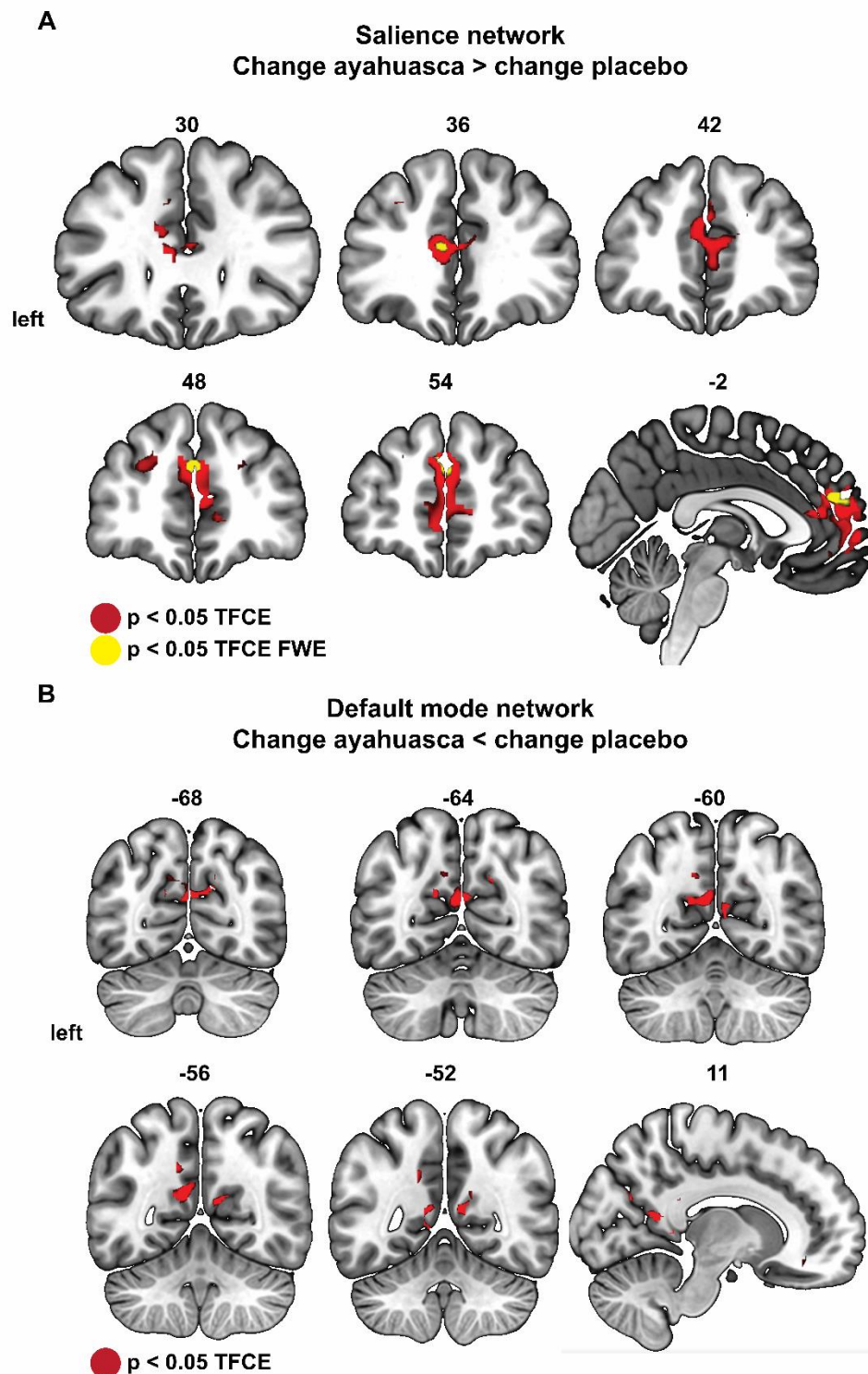


Supplementary Figure S1. Preprocessing pipeline of tf-fMRI data. tf-fMRI = task-free fMRI data.



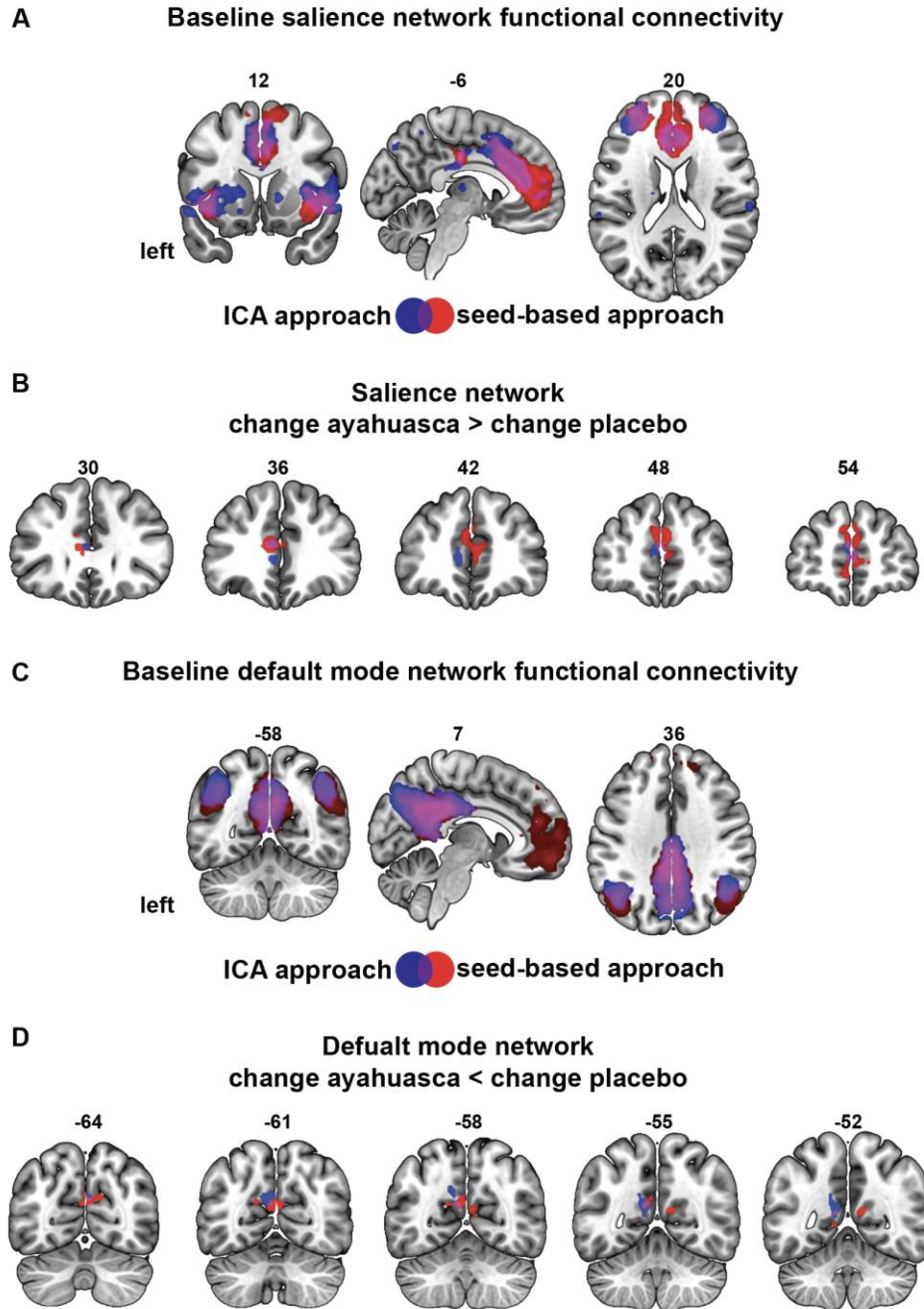
Supplementary Figure S2. Intra-network functional connectivity changes corrected for head movement. (A) Significant saliience network functional connectivity increases within the anterior cingulate cortex for the ayahuasca compared to the placebo group. Joint extent and cluster probability thresholds of $p < 0.05$ (red) and $p < 0.01$ (yellow). Left is on the left. **(B)** Significant default mode network functional connectivity decreases within the posterior cingulate cortex for the ayahuasca compared to the placebo group. Joint cluster and extent probability

thresholds of $p < 0.05$ (red). Left is on the left. Voxel-wise two-sample t-tests corrected for the difference in head mean framewise displacement between scans. Legend for SPM matrix: Aya = ayahuasca group; Pla = placebo group; Δ MFD = difference in head mean framewise displacement between scans.



Supplementary Figure S3. Non-parametric permutation inference. (A) Significant salience network functional connectivity increases in the anterior cingulate cortex for the ayahuasca compared to the placebo group. **(B)** Significant default mode network functional connectivity

decreases in the posterior cingulate cortex for the ayahuasca compared to the placebo group. FWE = family wise error corrected for multiple comparisons, TFCE = threshold-free cluster enhancement.



Supplementary Figure S4. ICA-based analyses. (A) Overlap (purple) of salience network maps derived with the ICA-based (in blue) and seed-based (in red) approaches. One-sample t-test over all participants at baseline ($t = 6.5$; extent probability threshold of $p < 0.01$ FWE corrected, voxel-wise threshold of $p < 0.001$ FWE corrected for multiple comparisons). **(B)** Significant salience network functional connectivity increases in the ayahuasca compared to the placebo group, overlap (purple) of ICA-based (blue) and seed-based (red) approaches. $t = 1.3$; joint cluster and extent probability thresholds of $p < 0.05$ for both seed-based and ICA-based two-sample t-test. **(C)** Overlap (purple) of default mode network maps derived with the ICA-based (in blue) and seed-based approach (in red). One-sample t-test over all participants at

baseline ($t = 6.5$; extent probability threshold of $p < 0.01$ FWE corrected, voxel-wise threshold of $p < 0.001$ FWE corrected for multiple comparisons). **(D)** Significant default mode network functional connectivity decreases in the ayahuasca compared to the placebo group, overlap (purple) of ICA-based (blue) and seed-based (red) approaches. $t = 1.3$, joint cluster and extent probability thresholds of $p < 0.05$ for seed-based two-sample t-test; $t = 1.3$, joint cluster and extent probability thresholds and $p < 0.1$ for ICA-based two-sample t-test. Left is on the left. ICA = independent component analysis.