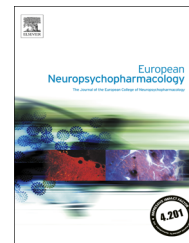




ELSEVIER

www.elsevier.com/locate/euroneuro



Long-term use of psychedelic drugs is associated with differences in brain structure and personality in humans[☆]



José Carlos Bouso^{a,b}, Fernanda Palhano-Fontes^c,
Antoni Rodríguez-Fornells^{d,e,f}, Sidarta Ribeiro^c, Rafael Sanches^g,
José Alexandre S. Crippa^g, Jaime E.C. Hallak^g,
Draulio B. de Araujo^c, Jordi Riba^{a,h,i,*}

^aHuman Neuropsychopharmacology Group, Sant Pau Institute of Biomedical Research (IIB-Sant Pau), C/Sant Antoni Maria Claret, 167, 08025 Barcelona, Spain

^bInternational Center for Ethnobotanical Education, Research & Service, Barcelona, Spain

^cBrain Institute/Hospital Universitario Onofre Lopes, Federal University of Rio Grande do Norte, Natal, Brazil

^dCognition and Brain Plasticity Group, IDIBELL (Bellvitge Biomedical Research Institute), L'Hospitalet de Llobregat, Barcelona 08097, Spain

^eDepartment of Basic Psychology, University of Barcelona, Barcelona 08035, Spain

^fCatalan Institution for Research and Advanced Studies, ICREA, Barcelona, Spain

^gNeuroscience and Behaviour Department, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil

^hCentre d'Investigació de Medicaments, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

ⁱDepartament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Barcelona, Spain

Received 14 August 2014; received in revised form 30 December 2014; accepted 10 January 2015

KEYWORDS

Ayahuasca;
Psychedelics;
N,N-dimethyltryptamine;
Cortical thickness;
Personality

Abstract

Psychedelic agents have a long history of use by humans for their capacity to induce profound modifications in perception, emotion and cognitive processes. Despite increasing knowledge of the neural mechanisms involved in the acute effects of these drugs, the impact of sustained psychedelic use on the human brain remains largely unknown. Molecular pharmacology studies have shown that psychedelic 5-hydroxytryptamine (5HT)_{2A} agonists stimulate neurotrophic and

[☆]Cortical thickness and psychedelic drugs.

*Corresponding author at: Human Neuropsychopharmacology Group, Sant Pau Institute of Biomedical Research (IIB-Sant Pau), C/Sant Antoni Maria Claret, 167, 08025 Barcelona, Spain.

Tel.: +34 93 556 5518; fax: +34 93 553 7855.

E-mail address: jriba@santpau.cat (J. Riba).

transcription factors associated with synaptic plasticity. These data suggest that psychedelics could potentially induce structural changes in brain tissue. Here we looked for differences in cortical thickness (CT) in regular users of psychedelics. We obtained magnetic resonance imaging (MRI) images of the brains of 22 regular users of ayahuasca (a preparation whose active principle is the psychedelic 5HT_{2A} agonist *N,N*-dimethyltryptamine (DMT)) and 22 controls matched for age, sex, years of education, verbal IQ and fluid IQ. Ayahuasca users showed significant CT differences in midline structures of the brain, with thinning in the posterior cingulate cortex (PCC), a key node of the default mode network. CT values in the PCC were inversely correlated with the intensity and duration of prior use of ayahuasca and with scores on self-transcendence, a personality trait measuring religiousness, transpersonal feelings and spirituality. Although direct causation cannot be established, these data suggest that regular use of psychedelic drugs could potentially lead to structural changes in brain areas supporting attentional processes, self-referential thought, and internal mentation. These changes could underlie the previously reported personality changes in long-term users and highlight the involvement of the PCC in the effects of psychedelics.

© 2015 Elsevier B.V. and ECNP. All rights reserved.

1. Introduction

Psychedelics, have been used since ancient times by geographically distant human groups for their capacity to induce profound modifications in the ordinary state of consciousness and to generate spiritually meaningful experiences (Schultes, 1979). The ancient practice of ritual psychedelic use not only survived well into the twentieth century, but has expanded beyond indigenous use following contact of previously isolated groups with foreigners (Tupper, 2008).

One of the most interesting contemporary adaptations of psychedelic use is the syncretism observed in Brazilian religious groups that consume ayahuasca, an infusion of the plants *Banisteriopsis caapi* and *Psychotria viridis*. These ayahuasca religions have expanded in the last decades, and it is estimated that around 20,000 people in 23 countries currently take ayahuasca regularly within a ritual context. Typically, participants attend ayahuasca-using rituals once every other week for many years (Grob et al., 1996).

Regarding the active principles present in ayahuasca, one of the plants, *P. viridis*, contains *N,N*-dimethyltryptamine (DMT). DMT is structurally related to serotonin and acts as a 5-HT_{2A} receptor agonist (González-Maeso and Sealton, 2009). DMT elicits intense, short-acting psychedelic effects when administered intravenously (Strassman et al., 1994) but is rapidly degraded by monoamine-oxidase (MAO) when orally ingested. Interestingly, DMT is rendered orally active by the MAO-inhibiting β -carboline alkaloids found in the other plant, *B. caapi*, used in ayahuasca (Riba et al., 2001).

Molecular pharmacology studies have shown that psychedelic 5-HT_{2A} agonists stimulate expression of immediate early genes that encode transcription factors, such as *c-fos* (Frankel and Cunningham, 2002), *egr-1* and *egr-2* (González-Maeso et al., 2007). They also increase the expression of the brain-derived neurotrophic factor (Gewirtz et al., 2002). Activation of these transcription factors has been associated with synaptic plasticity (O'Donovan et al., 1999), and cognitive processes such as memory (Jones et al., 2001) and attention (DeSteno and Schmauss, 2008).

Despite increasing research into the acute effects of psychedelics and the growing interest for their potential

use as therapeutic agents (Grob et al., 2011), little is known about the impact of sustained psychedelic use on the human brain. Based on the available molecular data mentioned above, we postulated that repeated exposure to psychedelics would correlate with changes in brain structure. To test this hypothesis we investigated brain cortical thickness (CT) in chronic psychedelic drug users who had minimal exposure to other drugs and their matched controls.

2. Experimental procedures

2.1. Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee at Hospital de Sant Pau (Barcelona, Spain). All participants provided written informed consent to participate in the study.

2.2. Participants

A group of 22 Spanish ayahuasca users and 22 controls were selected for the study. Ayahuasca users were Santo Daime church members who regularly participated in the rituals and were contacted directly by the researchers. Inclusion criteria were: (a) use of ayahuasca at least 50 times in the previous two years; (b) no personal history of psychiatric or neurological disorders; (c) lifetime use of cannabis on twenty occasions or less; (d) lifetime use of other drugs on ten occasions or less; and (e) no use of ayahuasca or other drugs for two weeks before scan, verified by urine toxicology test. The use of ayahuasca of 50 times in two years is a frequency of use of once every other week, which is typical for the Santo Daime church. To rule out a history of psychiatric and neurological disorders, users and controls were interviewed by a clinical psychologist (JCB). Study participants were specifically questioned if they had suffered from depression, psychotic disorders, drug dependence, loss of consciousness, or seizures at any time in their lives. Additionally, at the time of scanning, the structural MRI images were assessed by a neuroradiologist to rule out any CNS anomalies. The two participant groups were matched for sex, age, years of education, and verbal and fluid intelligence quotient (IQ). Each group comprised six male and 16 female participants. The verbal IQ test used was a Spanish version of the NART (Nelson and O'Connell, 1978), known as TAP—"Test de Acentuación de Palabras" ("Word Accentuation Test") (DelSer et al., 1997). The fluid IQ test

used was a computerized version of the Matrix Reasoning from the Wechsler Adult Intelligence Scale (WAIS)-III (Wechsler, 1981).

Table 1 shows sociodemographic data. Between-group comparisons using independent samples Student's *t*-tests and χ^2 did not show any significant differences for any of the matching variables used. Neither were differences found between groups regarding employment, marital status, or tobacco use. However, the number of individuals using tobacco and alcohol was larger in the control group and they also showed a higher frequency of use. Despite the differences in absolute numbers, the χ^2 tests only showed statistical trends.

Ayahuasca users had taken ayahuasca an average of 123 times (range: 50-352). They had been using ayahuasca for an average of 5.3 years (range: 2-13) and the age of initial use was 35.6 years (range: 5-55). Two of the participants had started taking ayahuasca at ages 5 years and 10 years, respectively. None of the participants in either group was currently using cannabis but some had consumed it in the past. As indicated above, maximum lifetime use of twenty times was established as an inclusion criterion and exposure to other drugs of abuse was limited to a maximum of ten times. Four participants in the ayahuasca sample and three in the control group had taken cocaine on less than ten occasions in their lifetime. Four participants in the control group reported having used psychedelics other than ayahuasca once in their lifetime. The drugs used were LSD (one participant) and *Psilocybe* mushrooms (three participants). Urine samples collected on the experimental day were negative for alcohol, benzodiazepines, amphetamines, cannabis, opiates and cocaine for all participants.

2.3. Acquisition and analyses of images

Structural images of the brain were acquired on a 3-T scanner (Magnetom Trio; Siemens, Munich, Germany) using a T1-weighted MPRAGE sequence with the following parameters: 240 sagittal slices; matrix size, 256 × 256; voxel resolution, 1 mm³; TR, 2300 ms; TE=1 ms. CT was estimated using FreeSurfer v5.0.0 in a

Mac-Pro OS X 10.8.2, 2 × 2.26 GHz, Quad-Core Intel Xeon. Images were resampled into common space using a spherical coordinate system, and spatially smoothed with a Gaussian filter (FWHM=10 mm).

A general linear model (GLM) was applied to estimate statistical differences at each voxel across the entire cortical surface. CT was set as the dependent variable and the group (ayahuasca users and controls) as the discrete factor. To control for possible global differences, the average CT of entire hemispheres was used as a covariate in the GLM model. Results were mapped onto the inflated white-matter surface of the average reconstruction of the brain. Differences between groups were calculated using two-tailed Student's *t*-tests at a statistical threshold of $p < 0.002$ uncorrected and a spatial threshold of ≥ 20 voxels. A parcellation atlas was used to identify the brain structures showing significant differences (Destrieux et al., 1998; Fischl et al., 2004). Statistical maps were color-coded. Brain structures that exhibited significant cortical thinning were represented in "cold" colors (blue-cyan), whereas regions with significant cortical thickening were represented in "hot" colors (red-yellow).

2.4. Assessment of personality, psychopathology and neuropsychology

Personality was assessed using a Spanish version of the Temperament and Character Inventory-Revised (TCI-R) questionnaire. The TCI-R is a 240-item questionnaire based on the psychobiological model of personality developed by Cloninger et al. (1993). The four primary dimensions of temperament are: harm avoidance (HA), novelty seeking, reward dependence, and persistence. The three primary dimensions of character are: self-directedness, cooperativeness and self-transcendence (ST).

Psychopathological assessment was carried out using the Symptom Check-List-90-Revised (SCL-90-R) questionnaire. This is a self-report questionnaire comprising 90 Likert-type items distributed in nine symptomatic dimensions: somatization; obsessive-compulsive; interpersonal

Table 1 Sociodemographic data as means (standard deviation) for age, years of education, WAIS matrices score (fluid IQ) and TAP score (verbal IQ). The statistic and *p* value columns show the results for the between-group comparisons (ayahuasca vs. controls) using Student's *t* tests (age, years of education, WAIS and TAP score) and χ^2 tests (all other variables).

	Ayahuasca users	Controls	<i>t</i> / χ^2	<i>p</i> Value
Matching variables				
<i>n</i> (Men/women)	6/16	6/16	0.00	NS
Age (years)	40.9 (12.6)	41.5 (11.8)	-0.15	NS
Years of education	13.0 (3.3)	13.1 (3.1)	-0.14	NS
WAIS matrices score	15.7 (3.5)	15.7 (3.6)	0.00	NS
TAP score	25.9 (3.5)	25.0 (3.7)	0.80	NS
Additional sociodemographic variables				
<i>Employment</i>				
employed/unemployed/student	20/0/2	20/0/2	0.00	NS
<i>Marital status</i>				
not married/married	18/4	16/6	0.52	NS
Tobacco and alcohol use				
<i>n</i> Current smokers	2	4	0.77	NS
<i>n</i> Current alcohol drinkers	15	20	3.49	0.062
<i>Pattern of alcohol use</i>				
>1 Drink/day (wine/beer)	0	1	6.58	0.087
1 Drink/day (wine/beer)	0	4		
<1 Drink/day (wine/beer)	1	4		
<1 Drink/week (wine/beer)	14	11		

NS: Not significant.

sensitivity; depression; anxiety; hostility; phobic anxiety; paranoid ideation; psychoticism. The scale also provides three global psychopathological indices: General Severity Index, Positive Symptoms Distress Index and Positive Symptoms Total. For all scales, higher scores imply worse symptomatology (Derogatis, 1994). A Spanish version of the questionnaire was used.

Three classic neuropsychological tests were administered by computer: (a) two-back test to assess working memory (Kircher, 1958); (b) Wisconsin Card-sorting Test (WCST) to assess executive function (including planning, set shifting and inhibition of impulsive responses (Heaton et al., 2001)); and (c) switching task assessing set-shifting (Zimmermann and Fimm, 1994).

The two-back task involved presentation of a sequence of letters. Participants had to identify if the letter was presented two steps back and make a yes/no decision. The series used comprised 100 items, 30% of which were targets.

In the WCST, a pack of 48 cards was presented sequentially on a computer screen. The participants had to classify them according to shape, color or number depending on the active rule. Responses were given by pressing a button. The rule changed after a fixed number of correct classifications. The following variables were assessed: total number of correct responses; total number of errors; total number of perseverances; reaction time for correct responses.

The switching task comprised 100 trials and was based on that described by Zimmermann and Fimm (1994). In each trial, a letter-digit or digit-letter pair is presented on the screen. From one trial to the next, the target item switches from letter to digit and vice versa in the following pattern: letter-digit-letter-digit... or digit-letter-digit-letter... until completion of the 100 trials. Participants have to indicate by button press where on the screen (left or right) the target item is located. Each trial requires a switch of the attention focus from letter to digit and vice versa. In some trials, the change of target type and change in response hand coincide (easy switch condition), and in other trials they do not (hard non-switch condition). Switching of the response hand (easy switch condition) is associated with shorter reaction times (Zimmermann and Fimm, 1994). The variables assessed were percentages of correct non-switch responses, erroneous non-switch responses, correct switch responses, and erroneous switch responses.

Personality, psychopathology and neuropsychological performance data were tested for normality using the Kolmogorov-Smirnov test. This test showed that personality scores (TCI) were normally distributed, whereas psychopathology (SCL-90-R) and neuropsychology (two-back, WCST and task-switching) were not. Thus, for TCI data, mean and standard deviations (SD) were calculated for each subscale and group, and differences between groups were assessed using the Student's *t*-test for independent samples. For the SCL-90-R questionnaire and the neuropsychological tests, medians and ranges were calculated and differences between groups assessed using the non-parametric Mann-Whitney test. Correlations were calculated using Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for non-normally distributed data. Results were considered statistically significant for $p < 0.05$.

3. Results

3.1. Structural MRI and CT

Fig. 1 shows the CT statistical maps for the comparison between ayahuasca users and their matched controls. Table 2 shows all clusters in which CT was found to be significantly different between groups. Thinning was observed in the ayahuasca-using group in six cortical areas: the middle frontal gyrus, the inferior frontal gyrus, the precuneus, the superior frontal gyrus, the posterior cingulate cortex (PCC), and the superior occipital gyrus. On the contrary, thickening was found in the precentral gyrus and in the anterior

cingulate cortex (ACC). The table shows cluster information including Talairach coordinates, Brodmann area, number of voxels and maximum *t* values.

3.2. Personality, psychopathology and neuropsychology

Mean scores on the main facets of the TCI-R are shown in Table 3 together with the results of the statistical comparison between groups. A detailed analysis of the various subscales comprising each of the seven dimensions of temperament and character was conducted only if the main scale showed significant differences between groups. Ayahuasca users scored lower than controls in terms of HA [$t(42) = -2.08, p = 0.044$]. This effect was driven by the lower scores in the “anticipatory worry” subscale [$t(42) = -2.98, p = 0.005$]. Scores on all other subscales were not significantly different. In addition to HA, ayahuasca users scored significantly higher on ST [$t(42) = 5.16, p < 0.001$]. Further analyses showed that all three subscales comprising this character dimension were significantly higher in the ayahuasca group than in the control group: Self-forgetfulness [$t(42) = 3.93, p < 0.001$], Transpersonal identification [$t(42) = 3.52, p = 0.001$], and Spiritual acceptance [$t(42) = 5.94, p < 0.001$].

Results from the psychopathology assessment are shown in Table 4. No significant differences were found for any of the symptomatic dimensions of the SCL-90-R using the non-parametric Mann-Whitney test.

Neuropsychological results are shown in Table 5. Ayahuasca users scored significantly better than controls in several variables derived from the three administered tests. In the two-back test, only the percentage of false alarms and correct rejections did not differ, comparisons for all other variables

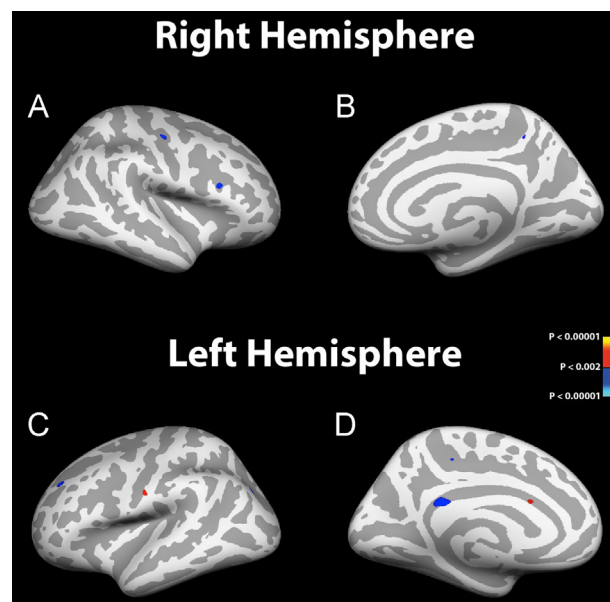


Fig. 1 Statistical maps of cortical thickness (CT) differences between ayahuasca-users and controls displayed onto an inflated cortex. Regions with significantly lower CT in the ayahuasca group are shown in “cold” colors (blue-cyan), and regions with significantly higher CT appear as “hot” colors (red-yellow). Results shown at $p < 0.002$ uncorrected and an spatial extension of 20 voxels.

Table 2 Brain areas showing statistically significant differences in cortical thickness between the ayahuasca-using group and the controls at $p < 0.002$ uncorrected and a spatial extension of 20 voxels. BA: Brodmann area.

Cortical area	BA	Talairach (x, y, z)	Number of voxels	Maximum <i>t</i> value
Ayahuasca < controls				
Middle frontal gyrus	6	(34, -12, 41)	86	-3.538
Inferior frontal gyrus	45	(42, 20, 17)	63	-3.421
Precuneus	7	(9, -42, 46)	23	-3.296
Superior frontal gyrus	9	(-23, 36, 29)	72	-3.538
Posterior cingulate cortex	23	(-4, -38, 23)	166	-3.475
Superior occipital gyrus	19	(-32, -71, 25)	40	-3.314
Ayahuasca > controls				
Precentral gyrus	4	(-57, -9, 25)	53	3.296
Anterior cingulate cortex	24	(-4, 17, 20)	20	3.084

Table 3 Statistical analyses of the six main dimensions of the temperament and character inventory (TCI) questionnaire and of the subscales of the dimensions showing significant differences between groups. Between-group comparisons were carried out using Student's *t* tests. Data are the mean (SD).

TCI	Ayahuasca users	Controls	<i>t</i> (df=42)	<i>p</i>
Novelty seeking	105.2 (10.9)	100.2 (11.8)	1.45	NS
Harm avoidance	86.3 (13.4)	95.3 (15.3)	-2.08	0.044
Reward dependence	107.9 (12.1)	103.1 (13.6)	1.23	NS
Persistence	108.2 (13.6)	104.5 (11.5)	0.99	NS
Self-directedness	157.0 (16.1)	147.3 (22.4)	1.65	NS
Cooperativeness	146.1 (13.1)	141.9 (13.9)	1.03	NS
Self-transcendence	92.8 (14.0)	70.0 (15.3)	5.16	<0.001
Subscales of harm avoidance				
Anticipatory worry	23.3 (4.9)	28.4 (6.2)	-2.98	0.005
Fear of uncertainty	23.0 (4.2)	24.2 (4.7)	-0.92	NS
Shyness with strangers	19.1 (4.0)	21.4 (6.1)	-1.49	NS
Fatigability and asthenia	20.9 (4.6)	21.3 (3.9)	-0.32	NS
Subscales of self-transcendence				
Self-forgetfulness	33.0 (6.0)	26.0 (5.8)	3.93	<0.001
Transpersonal identification	27.0 (5.8)	21.1 (5.4)	3.52	0.001
Spiritual acceptance	32.8 (4.3)	22.9 (6.5)	5.94	<0.001

NS: Not significant.

were significant. In the WCST, ayahuasca users showed a trend to a higher number of correct responses and lower errors. None of the other variables differed between groups. Finally, in the task-switching test, the percentage of correct responses was significantly higher and the percentage of errors was lower in the ayahuasca-using group for non-switch trials. No other significant differences were found.

3.3. Correlation analyses

Correlation analyses were conducted between mean CT values within the significant clusters and lifetime use of ayahuasca. Lifetime use of ayahuasca was inversely correlated to CT in the PCC ($r = -0.444$; $p = 0.038$) (Fig. 2a). Years of use of ayahuasca also showed a significant negative correlation with PCC ($r = -0.492$, $p = 0.020$) and age of initial use showed a statistical trend ($r = 0.390$; $p = 0.073$) with earlier use being associated with lower CT (Fig. 2b and c, respectively).

ST scores were correlated with CT in the PCC for each participant group independently (ayahuasca users: $r = -0.479$;

$p = 0.024$; controls: $r = -0.571$; $p = 0.005$). Independent correlations were also found for the transpersonal identification subscale (ayahuasca users: $r = -0.545$; $p = 0.009$; controls $r = -0.606$; $p = 0.003$) (Fig. 3b). No other significant correlations were found.

4. Discussion

We wished to investigate the impact of regular use of a psychedelic drug on brain structure, personality, psychopathology and neuropsychological function in humans. Our results showed differences in CT between users and controls. These differences were most prominent in medial parts of the brain, specifically an increase in CT in the anterior cingulate cortex and a decrease in CT in the posterior cingulate cortex. Personality assessment showed that the two samples also differed with respect to scores on ST, a character dimension of the TCI-R that measures certain aspects of spirituality (Cloninger et al., 1993) and is closely related to openness (McCrae, 2009).

Table 4 Median and ranges for scores on each of the subscales and indices of the SCL-90-R. Between-groups comparisons were carried out using non-parametric Mann-Whitney tests.

SCL-90-R	Ayahuasca users	Controls	z	p
Somatization	0.50 (0.00-1.17)	0.34 (0.00-1.83)	-0.92	NS
Obsessive-compulsive	0.35 (0.00-2.90)	0.75 (0.00-2.00)	-1.12	NS
Interpersonal sensitivity	0.22 (0.00-2.44)	0.33 (0.00-1.78)	-0.20	NS
Depression	0.15 (0.00-2.23)	0.35 (0.00-1.77)	-1.49	NS
Anxiety	0.25 (0.00-1.60)	0.25 (0.00-1.50)	-0.18	NS
Hostility	0.17 (0.00-2.33)	0.09 (0.00-1.00)	-0.65	NS
Phobic anxiety	0.14 (0.00-1.29)	0.00 (0.00-1.71)	-1.23	NS
Paranoid ideation	0.33 (0.00-1.83)	0.25 (0.00-1.00)	-0.85	NS
Psychoticism	0.20 (0.00-1.30)	0.20 (0.00-0.80)	-0.35	NS
General severity index	0.33 (0.00-1.87)	0.35 (0.03-1.41)	-0.35	NS
Positive symptoms distress index	22.00 (00-70.00)	23.00 (3.00-60.00)	-0.20	NS
Positive symptoms total	1.30 (0.00-2.40)	1.33 (1.00-2.12)	-0.72	NS

NS: Not significant.

Table 5 Medians and ranges for scores on the administered neuropsychological tests. Between-groups comparisons were carried out using non-parametric Mann-Whitney tests. Reaction times (RT) are given in milliseconds.

	Ayahuasca users	Controls	z	p
Two-back				
Hits	77.28 (36.36-93.94)	57.58 (12.12-81.82)	-2.58	0.010
False alarms	4.48 (0.00-56.72)	6.72 (0.00-61.19)	-0.94	NS
Misses	18.18 (3.03-63.64)	37.88 (15.15-87.88)	-2.50	0.013
Correct rejections	95.52 (40.30-100.00)	93.28 (37.31-98.51)	-1.05	NS
RT hits	564 (332-896)	644 (461-961)	-2.09	0.037
A-prime	0.92 (0.67-0.98)	0.86 (0.70-0.95)	-2.23	0.026
D-prime	2.25 (0.53-3.43)	1.63 (0.62-3.08)	-2.36	0.018
WCST				
Total correct	35.50 (17.00-45.00)	32.00 (9.00-42.00)	-1.92	0.055
Total errors	3.50 (0.00-25.00)	6.00 (0.00-37.00)	-1.74	0.081
Total perseverances	2.00 (0.00-17.00)	1.00 (0.00-17.00)	-0.09	NS
RT correct	4058 (2660-8489)	4401 (2252-12,645)	-0.75	NS
Task-switching				
% Correct non-switch	99.07 (86.96-100.00)	96.19 (60.00-100.00)	-2.00	0.046
% Error non-switch	0.93 (0.00-13.04)	3.81 (0.00-40.00)	-2.00	0.046
% Correct switch	100.00 (90.48-100.00)	98.13 (74.36-100.00)	-0.88	NS
% Error switch	0.00 (0.00-9.52)	1.87 (0.00-25.64)	-0.88	NS
RT Correct non-switch	1604 (1036-3193)	1659 (1023-3546)	-0.94	NS
RT Correct switch	1432 (980-4259)	1632 (765-2543)	-1.50	NS

WCST, Wisconsin Card-sorting test; NS: not significant.

The association between changes in brain structure and psychedelic use is supported by the correlations found between lifetime use of ayahuasca and CT in the PCC. Greater exposure to ayahuasca was associated with a higher degree of thinning of the PCC. Despite these structural differences, we did not observe increased psychopathology or worse neuropsychological performance in the ayahuasca-using group. These results are in accordance with studies of long-term users that have found little impact in terms of neuropsychological toxicity (Grob et al., 1996). Indeed, those studies point to a decrease in prior maladaptive behaviors such as drug abuse (Fábregas et al., 2010) and to a change in life attitudes and views as characterized by increased spirituality (Bouso et al., 2012).

The medial prefrontal cortex/ACC and PCC have been associated with the acute effects of ayahuasca and other psychedelic agents. Using single-photon emission tomography, regional cerebral blood flow after acute administration of ayahuasca was found to be increased in the medial aspect of the frontal cortex, including the ACC (Riba et al., 2006). These effects were in accordance with data from studies on psilocybin use employing positron emission tomography (Vollenweider et al., 1997). Analyses of electroencephalography sources showed changes in current density in the ACC, but even more so in the PCC (Riba et al., 2004). These electrophysiological findings have been replicated in a magnetoencephalography study of psilocybin use (Muthukumaraswamy et al., 2013). Interestingly, a recent functional magnetic resonance imaging

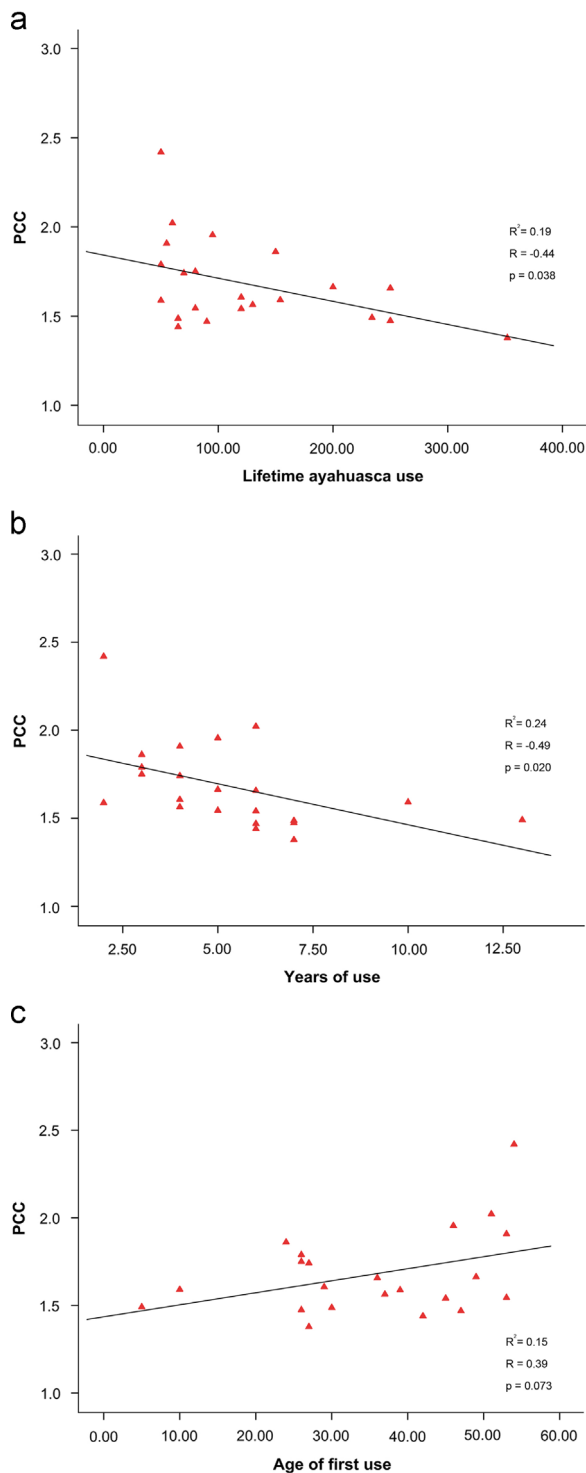
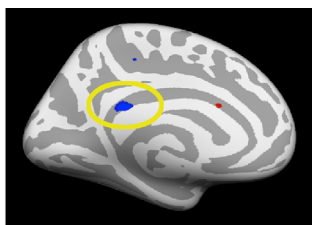


Fig. 2 Scatter plots showing the correlations between individual CT values in the posterior cingulate cortex (PCC) and ayahuasca use: (a) lifetime use; (b) years of use; and (c) age of first use. Talairach coordinates of the cluster center: $x = -4$, $y = -38$ and $z = 23$.

study of psilocybin effects found changes in the blood oxygen level-dependent response in the anterior and posterior cingulate cortices and in the functional coupling of these two regions (Carhart-Harris et al., 2012).

From a mechanistic perspective, the structural differences observed in the present study could reflect a direct drug-induced modulatory action or an adaptive response. Supporting the direct-action hypothesis is the fact that activation of 5-HT_{2A} receptors stimulates the expression of immediate early genes (e.g., c-fos) in the medial prefrontal and anterior cingulate cortices (Frankel and Cunningham, 2002). It also increases the expression of brain-derived neurotrophic factor (Gewirtz et al., 2002), which modulates the efficacy and plasticity of synapses (Soulé et al., 2006). Research by González-Maeso and coworkers has shown that psychedelic

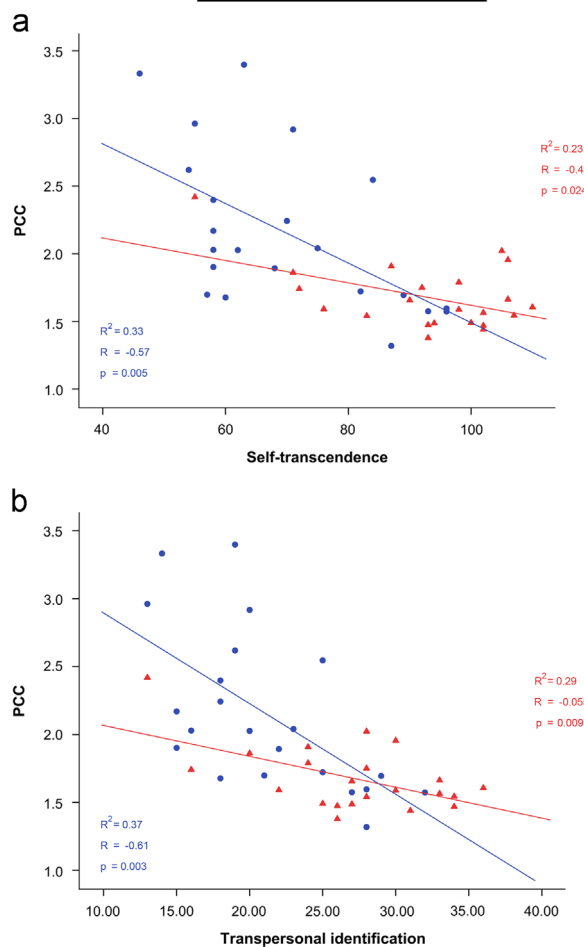
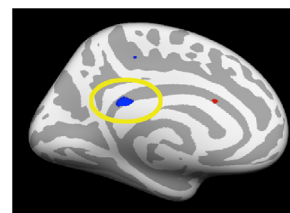


Fig. 3 Scatter plots showing the correlations between cortical thickness in the PCC and personality scores (TCI): (a) with self-transcendence; (b) with the transpersonal identification sub-scale. Blue dots indicate values for controls and red triangles values for ayahuasca users. Talairach coordinates of the cluster: $x = -4$, $y = -38$ and $z = 23$.

5-HT_{2A} agonists induce expression of the transcription factors *egr-1* and *egr-2* (Moreno et al., 2013). These transcription factors play a prominent part in synaptic plasticity (O'Donovan et al., 1999), and their upregulation and downregulation modulates short- and long-term memory (Jones et al., 2001; Poirier et al., 2008) and attention (DeSteno and Schmauss, 2008). Interestingly, Nichols and coworkers reported that chronic administration of lysergic acid diethylamide leads to altered expression of the genes of dopaminergic and serotonergic receptors long after cessation of drug use (Marona-Lewicka et al., 2011). Thus, it is plausible that the direct pharmacological action of DMT accounts for the observed structural differences after repeated exposure to ayahuasca.

The greater CT observed in anterior brain regions involved with attention and executive control (Raichle, 2011) could explain intriguing findings from experienced users of psychedelic agents. In a study involving 127 drug-free, long-term users of ayahuasca and 115 controls, long-term users scored significantly better on several neuropsychological tests, including the Stroop Test, the WCST and the letter-number sequencing task of WAIS-III (Bouso et al., 2012), indicating that ayahuasca use is not associated with impairment of executive function, and even suggests cognitive enhancement. Another study assessed the impact of prior drug experience on performance during the peak effects of drugs. Authors administered a test battery to two subgroups of users during the acute effects of an ayahuasca dose. They found impaired neuropsychological performance in the "occasional user" subgroup, but not in the "experienced" subgroup (who had taken ayahuasca an average of 180 times) (Bouso et al., 2013). They also reported a correlation between performance in the Tower of London task and lifetime use of ayahuasca. The authors concluded that greater exposure to ayahuasca was associated with less (rather than greater) incapacitation after intake. The present study identified lower CT in the PCC and increases in the ACC, structures involved in the default mode network (DMN) and attention/cognitive control, respectively. These two networks show anti-correlated activity (Fox et al., 2005); crucially, this feature is presumed to be lost under the effects of psychedelics (Carhart-Harris et al., 2012). In the long-term, psychedelic users show opposing structural differences. The observed structural differences at these levels could explain the preservation of neuropsychological function in ayahuasca users.

With regard to personality, we found that ayahuasca users scored higher on ST. This finding is consistent with results in Brazilian users of ayahuasca (Bouso et al., 2012) and work involving acute administration of psychedelic in laboratory settings (Griffiths et al., 2011). Interestingly, greater scores on ST were associated with increased thinning of the PCC. Thus, differences in this character dimension may have a neural basis and be the result of repeated intake of drugs. ST accounts for the tendency towards religiousness and spirituality. ST is believed to be a relatively stable facet, but chronic use of psychedelics was reported to induce personality changes as early as the 1960s and 1970s (Pahnke, 1969; Savage et al., 1966). Researchers described an increased frequency of unusual beliefs and sensations in regular users. It was postulated that psychedelics could cause a profound psychological impression that could lead to changes in attitudes and interests, from less materialistic values to greater open-mindedness and even to mystic-like feelings. These changes were considered positive by some authors (Pahnke,

1969), but others viewed them with concern at a time when use and abuse of these drugs by young adults was more widespread (Blacker et al., 1968). Interestingly, recent research by MacLean et al. (2011) has shown that a single dose of psilocybin can lead to increases in the trait of openness, an aspect of personality that is closely related to ST.

Our observation of a relationship between spirituality and the PCC is of particular note. This brain region is the focus of increasing attention because of its prominent role within the DMN. The network encompassing the ventral precuneus, retrosplenial and posterior cingulate cortices shows high blood flow and energy use under resting conditions (Raichle et al., 2001), and demonstrates extensive structural and functional connections to the other regions comprising the DMN (Horn et al., 2013). This network has been associated with internal mentation and the intimate sense of "self" (Cavanna and Trimble, 2006). The PCC is active during spontaneous stimulus-independent mind-wandering and in processes in which attention is directed internally, such as retrieving episodic memories, imagining and planning (Spreng, 2012). Carhart-Harris et al. (2012) observed deactivation of the PCC after psilocybin administration. They proposed that deactivation of the PCC and the DMN underlies the psychedelic experience, a state that is characterized typically by increased attention to the inner world, i.e., endogenous thoughts and feelings (Riba et al., 2001). The PCC has been proposed to work as a key "hub" regulating information flow around the brain, and its connectivity shows abnormalities in diseases such as schizophrenia (Calhoun et al., 2011), in which the boundaries between internal and external processes become blurred. Interestingly, Carhart-Harris and coworkers reported decreases in functional connectivity between the medial prefrontal cortex and the PCC after psilocybin administration. The results of the present study support involvement of these two areas in the effects of psychedelics.

To conclude, we found that regular use of a psychedelic agent was associated with structural differences in the medial aspects of the frontal and parietal cortices. These differences were associated with prior drug exposure and with greater ST, a personality trait reflecting religiousness and spirituality. Given the cross-sectional nature of the present study, causation cannot be established. However, our data suggest that regular use of psychedelic drugs could potentially lead to changes in brain tissue. Neural changes in brain areas associated with attention, internal thought processes and the sense of self could underlie previously described personality changes following long-term psychedelic use.

Funding

This work was funded by grant 2006/074 from the "Plan Nacional Sobre Drogas" (PNSD) of the Spanish Government. The PNSD had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

JCB conducted the experimental sessions.
FP-F and DBA analyzed the MR data.
ARF designed the neuropsychological test battery.

ARF, SR, RS, JAC and JECH contributed to data interpretation. JR designed the study, wrote the protocol and drafted the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Saül Martínez-Horta for his help in figure preparation, Amanda Feilding for her critical reading of the paper and Arshad Makhdum from the Beckley Foundation for editing the manuscript.

References

- Blacker, K.H., Jones, R.T., Stone, G.C., Pfefferbaum, D., 1968. Chronic users of LSD: the "acidheads". *Am. J. Psychiatry* 125, 97-107.
- Bouso, J.C., Fábregas, J.M., Antonijoan, R.M., Rodríguez-Fornells, A., Riba, J., 2013. Acute effects of ayahuasca on neuropsychological performance: differences in executive function between experienced and occasional users. *Psychopharmacology (Berl.)* 230, 415-424. <http://dx.doi.org/10.1007/s00213-013-3167-9>.
- Bouso, J.C., González, D., Fondevila, S., Cutchet, M., Fernández, X., Ribeiro Barbosa, P.C., Alcázar-Córcoles, M.Á., Araújo, W.S., Barbanoj, M.J., Fábregas, J.M., Riba, J., 2012. Personality, psychopathology, life attitudes and neuropsychological performance among ritual users of Ayahuasca: a longitudinal study. *PLoS One* 7, e42421. <http://dx.doi.org/10.1371/journal.pone.0042421>.
- Calhoun, V.D., Sui, J., Kiehl, K., Turner, J., Allen, E., Pearson, G., 2011. Exploring the psychosis functional connectome: aberrant intrinsic networks in schizophrenia and bipolar disorder. *Front. Psychiatry* 2, 75. <http://dx.doi.org/10.3389/fpsy.2011.00075>.
- Carhart-Harris, R.L., Erritzoe, D., Williams, T., Stone, J.M., Reed, L.J., Colasanti, A., Tyacke, R.J., Leech, R., Malizia, A.L., Murphy, K., Hobden, P., Evans, J., Feilding, A., Wise, R.G., Nutt, D.J., 2012. Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2138-2143. <http://dx.doi.org/10.1073/pnas.1119598109>.
- Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 129, 564-583. <http://dx.doi.org/10.1093/brain/awl004>.
- Cloninger, C.R., Svrakic, D.M., Przybeck, T.R., 1993. A psychobiological model of temperament and character. *Arch. Gen. Psychiatry* 50, 975-990.
- DelSer, T., González-Montalvo, J.I., Martínez-Espinosa, S., Delgado-Villalpalos, C., Bermejo, F., 1997. Estimation of premorbid intelligence in Spanish people with the Word Accentuation Test and its application to the diagnosis of dementia. *Brain Cogn.* 33, 343-356. <http://dx.doi.org/10.1006/brcg.1997.0877>.
- Derogatis, L.R., 1994. Symptom Checklist-90-R. Administration, Scoring and Procedures Manual. National Computer System, Minneapolis.
- DeSteno, D.A., Schmauss, C., 2008. Induction of early growth response gene 2 expression in the forebrain of mice performing an attention-set-shifting task. *Neuroscience* 152, 417-428. <http://dx.doi.org/10.1016/j.neuroscience.2008.01.012>.
- Destrieux, C., Halgren, E., Dale, A., Fischl, B., Sereno, M., 1998. Variability of the human brain studied on the flattened cortical surface. *Soc. Neurosci. Abstr.* 24, 1164.
- Fábregas, J.M., González, D., Fondevila, S., Cutchet, M., Fernández, X., Barbosa, P.C.R., Alcázar-Córcoles, M.Á., Barbanoj, M.J., Riba, J., Bouso, J.C., 2010. Assessment of addiction severity among ritual users of ayahuasca. *Drug Alcohol Depend.* 111, 257-261. <http://dx.doi.org/10.1016/j.drugalcdep.2010.03.024>.
- Fischl, B., van der Kouwe, A., Destrieux, C., Halgren, E., Ségonne, F., Salat, D.H., Busa, E., Seidman, L.J., Goldstein, J., Kennedy, D., Caviness, V., Makris, N., Rosen, B., Dale, A.M., 2004. Automatically parcellating the human cerebral cortex. *Cereb. Cortex (New York, NY)* 14, 11-22.
- Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9673-9678. <http://dx.doi.org/10.1073/pnas.0504136102>.
- Frankel, P.S., Cunningham, K.A., 2002. The hallucinogen d-lysergic acid diethylamide (d-LSD) induces the immediate-early gene *c-Fos* in rat forebrain. *Brain Res.* 958, 251-260.
- Gewirtz, J.C., Chen, A.C., Terwilliger, R., Duman, R.C., Marek, G.J., 2002. Modulation of DOI-induced increases in cortical BDNF expression by group II mGlu receptors. *Pharmacol. Biochem. Behav.* 73, 317-326.
- González-Maeso, J., Sealton, S.C., 2009. Agonist-trafficking and hallucinogens. *Curr. Med. Chem.* 16, 1017-1027.
- González-Maeso, J., Weisstaub, N.V., Zhou, M., Chan, P., Ivic, L., Ang, R., Lira, A., Bradley-Moore, M., Ge, Y., Zhou, Q., Sealton, S.C., Gingrich, J.A., 2007. Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* 53, 439-452. <http://dx.doi.org/10.1016/j.neuron.2007.01.008>.
- Griffiths, R.R., Johnson, M.W., Richards, W.A., Richards, B.D., McCann, U., Jesse, R., 2011. Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. *Psychopharmacology (Berl.)* 218, 649-665. <http://dx.doi.org/10.1007/s00213-011-2358-5>.
- Grob, C.S., Danforth, A.L., Chopra, G.S., Hagerty, M., McKay, C.R., Halberstadt, A.L., Greer, G.R., 2011. Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. *Arch. Gen. Psychiatry* 68, 71-78. <http://dx.doi.org/10.1001/archgenpsychiatry.2010.116>.
- Grob, C.S., McKenna, D.J., Callaway, J.C., Brito, G.S., Neves, E.S., Oberlaender, G., Saide, O.L., Labigalini, E., Tacla, C., Miranda, C.T., Strassman, R.J., Boone, K.B., 1996. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J. Nerv. Ment. Dis.* 184, 86-94.
- Heaton, R.K., Chelune, G.J., Talley, J.L., Kay, G.G., Curtis, G., 2001. Test de Clasificación de Tarjetas de Wisconsin. TEA Ediciones, S.A., Madrid.
- Horn, A., Ostwald, D., Reisert, M., Blankenburg, F., 2013. The structural-functional connectome and the default mode network of the human brain. *NeuroImage*. <http://dx.doi.org/10.1016/j.neuroimage.2013.09.069>.
- Jones, M.W., Errington, M.L., French, P.J., Fine, A., Bliss, T.V., Garel, S., Charnay, P., Bozon, B., Laroche, S., Davis, S., 2001. A requirement for the immediate early gene *Zif268* in the expression of late LTP and long-term memories. *Nat. Neurosci.* 4, 289-296. <http://dx.doi.org/10.1038/85138>.
- Kircher, W.K., 1958. Age differences in short-term retention of rapidly changing information. *J. Exp. Psychol.* 55, 352-358.
- MacLean, K.A., Johnson, M.W., Griffiths, R.R., 2011. Mystical experiences occasioned by the hallucinogen psilocybin lead to increases in the personality domain of openness. *J. Psychopharmacol. (Oxford, England)* 25, 1453-1461. <http://dx.doi.org/10.1177/0269881111420188>.
- Marona-Lewicka, D., Nichols, C.D., Nichols, D.E., 2011. An animal model of schizophrenia based on chronic LSD administration: old idea, new results. *Neuropharmacology* 61, 503-512. <http://dx.doi.org/10.1016/j.neuropharm.2011.02.006>.
- McCrae, R.R., 2009. The five-factor model of personality traits: consensus and controversy. In: Corr, P.J., Matthews, G. (Eds.),

- Cambridge Handbook of Personality Psychology. Cambridge University Press, Cambridge.
- Moreno, J.L., Holloway, T., Rayannavar, V., Sealton, S.C., González-Maeso, J., 2013. Chronic treatment with LY341495 decreases 5-HT(2A) receptor binding and hallucinogenic effects of LSD in mice. *Neurosci. Lett.* 536, 69-73. <http://dx.doi.org/10.1016/j.neulet.2012.12.053>.
- Muthukumaraswamy, S.D., Carhart-Harris, R.L., Moran, R.J., Brookes, M.J., Williams, T.M., Erntzoe, D., Sessa, B., Papadopoulos, A., Bolstridge, M., Singh, K.D., Feilding, A., Friston, K.J., Nutt, D.J., 2013. Broadband cortical desynchronization underlies the human psychedelic state. *J. Neurosci.* 33, 15171-15183. <http://dx.doi.org/10.1523/JNEUROSCI.2063-13.2013>.
- Nelson, H.E., O'Connell, A., 1978. Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. *Cortex* 14, 234-244.
- O'Donovan, K.J., Tourtellotte, W.G., Millbrandt, J., Baraban, J.M., 1999. The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci.* 22, 167-173.
- Pahnke, W.N., 1969. Psychedelic drugs and mystical experience. *Int. Psychiatry Clin.* 5, 149-162.
- Poirier, R., Cheval, H., Mailhes, C., Garel, S., Charnay, P., Davis, S., Laroche, S., 2008. Distinct functions of egr gene family members in cognitive processes. *Front. Neurosci.* 2, 47-55. <http://dx.doi.org/10.3389/neuro.01.002.2008>.
- Raichle, M.E., 2011. The restless brain. *Brain Connect.* 1, 3-12. <http://dx.doi.org/10.1089/brain.2011.0019>.
- Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. *Proc. Natl. Acad. Sci. U.S.A.* 98, 676-682. <http://dx.doi.org/10.1073/pnas.98.2.676>.
- Riba, J., Anderer, P., Jané, F., Saletu, B., Barbanoj, M.J., 2004. Effects of the South American psychoactive beverage ayahuasca on regional brain electrical activity in humans: a functional neuroimaging study using low-resolution electromagnetic tomography. *Neuropsychobiology* 50, 89-101. <http://dx.doi.org/10.1159/000077946>.
- Riba, J., Rodríguez-Fornells, A., Urbano, G., Morte, A., Antonijoan, R., Montero, M., Callaway, J.C., Barbanoj, M.J., 2001. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology (Berl.)* 154, 85-95.
- Riba, J., Romero, S., Grasa, E., Mena, E., Carrió, I., Barbanoj, M.J., 2006. Increased frontal and paralimbic activation following ayahuasca, the pan-Amazonian inebriant. *Psychopharmacology (Berl.)* 186, 93-98. <http://dx.doi.org/10.1007/s00213-006-0358-7>.
- Savage, C., Fadiman, J., Mogar, R., Allen, M.H., 1966. The effects of psychedelic (LSD) therapy on values, personality, and behavior. *Int. J. Neuropsychiatry* 2, 241-254.
- Schultes, R.E., 1979. *Plants of the Gods: Origins of Hallucinogenic Use*. McGraw-Hill, New York.
- Soulé, J., Messaoudi, E., Bramham, C.R., 2006. Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. *Biochem. Soc. Trans.* 34, 600-604. <http://dx.doi.org/10.1042/BST0340600>.
- Spreng, R.N., 2012. The fallacy of a "task-negative" network. *Front. Psychol.* 3, 145. <http://dx.doi.org/10.3389/fpsyg.2012.00145>.
- Strassman, R.J., Qualls, C.R., Uhlenhuth, E.H., Kellner, R., 1994. Dose-response study of *N,N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Arch. Gen. Psychiatry* 51, 98-108.
- Tupper, K.W., 2008. The globalization of ayahuasca: harm reduction or benefit maximization? *Int. J. Drug Policy* 19, 297-303. <http://dx.doi.org/10.1016/j.drugpo.2006.11.001>.
- Vollenweider, F.X., Leenders, K.L., Scharfetter, C., Maguire, P., Stadelmann, O., Angst, J., 1997. Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. *Neuropsychopharmacology* 16, 357-372. [http://dx.doi.org/10.1016/S0893-133X\(96\)00246-1](http://dx.doi.org/10.1016/S0893-133X(96)00246-1).
- Wechsler, D., 1981. *Wechsler Adult Intelligence Scale-III (WAIS-III)*. The Psychological Corporation, San Antonio, TX.
- Zimmermann, P., Fimm, B., 1994. *Testbatterie zur Aufmerksamkeitsprüfung (TAP)*. Psytest, Herzogenrath.