Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users

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Abstract

Background. Recreational drugs are generally used to intentionally alter conscious experience. Long-lasting cannabis users frequently seek this effect as a means to relieve negative affect states. As with conventional anxiolytic drugs, however, changes in subjective feelings may be associated with memory impairment. We have tested whether the use of cannabis, as a psychoactive compound, is associated with alterations in spontaneous activity in brain networks relevant to self-awareness, and whether such potential changes are related to perceived anxiety and memory performance. **Methods**. Functional connectivity was assessed in the Default and Insula networks during resting state using fMRI in 28 heavy cannabis users and 29 control subjects. Imaging assessments were conducted during cannabis use in the unintoxicated state and repeated after one month of controlled abstinence. **Results.** Cannabis users showed increased functional connectivity in the core of the Default and Insula networks and selective enhancement of functional anticorrelation between both. Reduced functional connectivity was observed in areas overlapping with other brain networks. Observed alterations were associated with behavioral measurements in a direction suggesting anxiety score reduction and interference with memory performance. Alterations were also related to the amount of cannabis used and partially persisted after one month of abstinence. **Conclusions.** Chronic cannabis use was associated with significant effects on the tuning and coupling of brain networks relevant to self-awareness, which in turn are integrated into brain systems supporting the storage of personal experience and motivated behavior. The results suggest potential mechanisms for recreational drugs to interfere with higher-order network interactions generating conscious experience.

Introduction

Individuals may use recreational drugs altering conscious experience because of a variety of reasons including being adventurous and curious, and peer pressure. Nevertheless, the most common reason given for long-lasting cannabis use is relief from tension or to attenuate negative affect states such as anxiety (Buckner *et al*, 2007; Crippa *et al*, 2009; Ogborne *et al*, 2000; Reilly *et al*, 1998). Like other psychoactive drugs, however, cannabis has potential side effects. Apart from the possibility of generating drug dependence and the potentially deleterious effect in subjects at risk of developing psychosis (Large *et al*, 2011), continued cannabis use may impair cognition. Memory is the cognitive domain that has been most consistently reported as impaired in cannabis users (Solowij & Battisti, 2008), although such impairment tends to be mild when no other substances of abuse are implicated (Hall & Solowij, 1998; Solowij & Battisti, 2008).

Neuroimaging research is contributing uniquely to understand the biological bases of mental states. Functional magnetic resonance imaging (fMRI) of spontaneous brain activity permits the identification of a range of functional networks on the basis of region synchrony, defined as functional connectivity. Of relevance are recent studies suggesting the contribution of particular networks to conscious awareness of self. The "Default" network is perhaps the network most extensively investigated. Its main elements are the posterior cingulate cortex (PCC) and adjacent precuneus, angular gyri and medial frontal cortex (Buckner *et al*, 2008; Harrison *et al*, 2008). Default network contribution to self-referential mental processes is thought to be related to awareness of the (somatic) body and its relationship to the external environment (Buckner & Carroll, 2007; Buckner *et al*, 2008; Shulman *et al*, 1997; Small *et al*, 2003). In the temporal

dimension, the Default network may assist autobiographical memory retrieval, but may also modulate working memory processes (Bluhm *et al*, 2011; Buckner *et al*, 2008; Leech *et al*, 2011). On the other hand, the insula cortex and functionally connected regions are known to be relevant for interoceptive awareness (Caseras *et al*, 2011; Craig, 2009; Critchley *et al*, 2004). Activity in the Insula network is associated with conscious perception of the physiological conditions of the (visceral) body (e.g., cardiovascular, airway, gut and sexual sensations) that jointly give rise to an internal representation of oneself, and provide a foundation for subjective feeling states that color emotional experience (Craig, 2002, 2009; Critchley *et al*, 2004).

Although the Default and Insula networks underlie distinct aspects of self-awareness, there is relevant function overlap, which concerns the contribution of Default network anterior areas to the cognitive control of interoception (Bishop *et al*, 2004; Ochsner & Gross, 2005; Sylvester *et al*, 2012), and Insula network posterior areas to the somatic representation of the body (Augustine, 1996; Eickhoff *et al*, 2006; Ruben *et al*, 2001). Moreover, activity in these networks seems to be closely coordinated, as their fMRI signal fluctuations show a strong negative correlation during resting state, with periods of high activity in one network often corresponding to low activity in the other network (Fox *et al*, 2005; Harrison *et al*, 2011). These functionally "anticorrelated" networks, however, may synchronically deactivate during highly-demanding goal-directed behavior suggesting the attenuation of both somatic and visceral awareness when attention is focused on external targets (Harrison *et al*, 2011).

In the current study, we have assessed spontaneous activity in the Default and Insula networks in chronic cannabis users. Our hypothesis was that cannabis, as a psychoactive compound, would modulate activity in networks relevant to self-

awareness and that this effect would be related to both anxiety levels and cognitive performance. Specifically, we have investigated whether resting-state functional connectivity alterations exist in early onset and heavy cannabis users without comorbid psychiatric disorders compared with control subjects, and whether such potential alterations are associated with variations in anxiety and memory measurements. Resting-state fMRI was initially acquired during cannabis chronic use in the unintoxicated state. The assessment was repeated after one month of abstinence with the prediction that functional alterations may show long-lasting effects, as suggested in a recent review by our group (Batalla *et al*, 2013).

Methods

Participants

A total of 28 chronic cannabis user men (mean ± SD age, 21 ± 2 years) were assessed and compared with a reference control group of 29 men (age, 22 ± 3 years, ns). One cannabis user was excluded from an original sample of 29 subjects due to non-optimal data acquisition. All participants were followed-up during one month of controlled abstinence, and 27 cannabis users and 28 control subjects were available to repeat fMRI with identical procedures. Written informed consent was obtained from all participants. The study was approved by the local ethics committee (CEIC-IMAS, Barcelona) and was in compliance with the Declaration of Helsinki.

Participants were recruited via a web page and distribution of flyers and ads. To evaluate study eligibility, a comprehensive telephone screening was carried-out. When eligible, participants were assessed using a detailed medical history, physical examination, a structured psychiatric interview (PRISM; Torrens *et al*, 2004), blood biochemical analyses and urine toxicology analyses. To facilitate open disclosure, confidentiality was guaranteed within ethical and legal limits.

Inclusion to the cannabis group required participants to be male, aged between 18 and 30 years, with at least 10 years of education (mean ± SD, 14 ± 2 years), cannabis use onset before age 16, cannabis consumption (smoking) more than 14 times a week at the time of selection and during at least 2 years prior to the study, positive urine test for

cannabinoids and negative for opiates, cocaine, amphetamines and benzodiazepines (immunometric assay kits, Instant-View, ASD Inc, Poway, California). Exclusion criteria were: Diagnostic and Statistical Manual for Mental Disorders-Fourth Edition (DSM-IV; American Psychiatric Association, 2000) Axis I disorder, relevant medical or neurological disorders, learning disabilities, use of psychoactive medications, previous use of any other recreational drug for more than 5 occasions lifetime except alcohol and nicotine, lifetime criteria for alcohol abuse or dependence and relevant current alcohol consumption. Current alcohol intake was very low in both study groups, showing a mean ± SD of 5.3 ± 4 units a week in users and 3.1 ± 2.6 units a week in control subjects. On average, cannabis users smoked a mean ± SD of 5.9 ± 5.2 cigarettes a day and control subjects, 2.4 ± 5.9 cigarettes a day. Only three participants (two users and one control subject) smoked more than 10 cigarettes per day. All subjects were right-handed.

Control subjects were required to be male, aged between 18 and 30 years, with at least 10 years of education (15 ± 1 years), showing less than 15 lifetime experiences with cannabis (none in the past month) and negative urine drug screen. Exclusion criteria were identical to the cannabis group. Cannabis users and control subjects showed a mean difference of one year in education ($t= 2.2$, $p= 0.032$).

Participants were required to refrain from smoking and caffeine six hours, and alcohol and cannabis 12 hours before fMRI. The study consisted of two fMRI assessments. The second fMRI session was carried out in all available participants after a period of 28 days of controlled cannabis abstinence.

Behavioral assessment

Primary assessments were the State–Trait Anxiety Inventory (STAI; Spielberger *et al*, 1983) and Rey Auditory-Verbal Learning Test (RAVLT; Geffen *et al* 1990). During the administration of RAVLT, participants were read a list of 15 unrelated words and were asked to recall as many words as they could remember. The same list was repeated over five trials, followed by an interference trial with a new 15-word list, a short-delay free recall trial, and a long-delay free recall trial 20 min later. In this study, the following measurements were considered: "verbal span" (number of words recollected on the first trial), "verbal learning" (over trials; sum of words on trials 1 to 5 minus 5 times the words on trial 1), "recall" (number of recalled words after 20 minutes) and "forgetting rate" (words on trial 5 minus recalled words after 20 minutes).

Image acquisition and preprocessing

A 1.5 Tesla Signa Excite system (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array head coil and single-shot echoplanar imaging (EPI) software was used. The functional sequence consisted of gradient recalled acquisition in the steady state (time of repetition [TR], 2000 ms; time of echo [TE], 50 ms; pulse angle, 90º) within a field of view of 24 cm, with a 64 x 64-pixel matrix, and with a slice thickness of 4 mm (inter-slice gap, 1.5 mm). Twenty-two interleaved slices were prescribed parallel to the anterior-posterior commissure line covering the whole-brain. A 6-min continuous resting-state scan was acquired for each participant. Participants were instructed to relax, stay awake and lie still without moving, while keeping their eyes closed throughout. This scan generated 180 whole-

brain EPI volumes. The first four (additional) images in each run were discarded to allow magnetization to reach equilibrium.

fMRI data were preprocessed and analyzed using the Statistical Parametric Mapping 8 (SPM8) package, Wellcome Department of Imaging Neuroscience (http://www.fil.ion.ucl.ac.uk/spm/), running on Matlab 7.1 (The Mathworksinc. Natick, Mass). Functional images were realigned (motion corrected), resliced into 2mm isotropic voxels and spatially warped into the standardized (Montreal Neurological Institute, MNI) SPM template space. A Full Width at Half Maximum (FWHM) 8-mm Gaussian kernel was used to smooth the functional images. All image sequences were inspected for potential acquisition and normalization artifacts. No subjects were excluded because of poor quality images. In addition, we compared both study groups as for potential differences in translational motion, rotational motion, mean interscan displacement and total displacement and found no significant differences in any parameter.

Functional connectivity analysis.

Resting-state functional connectivity was assessed using a seed-based approach as detailed in previous studies (Harrison *et al*, 2009; Pujol *et al*, 2012). Functional connectivity maps of Default and Insula networks were generated using regions of interest ("seeds") located in the PCC and anterior insula, respectively. The PCC seed was placed at MNI coordinates x= 6, y= -44, z= 37, which corresponds to the limit between dorsal (anterior) and ventral (posterior) subdivisions of PCC (Leech *et al*, 2011, 2012; Vogt *et al*, 2006). The insula seed was placed at x= 36, y= 16, z= 2, which

corresponds to the anatomical center (orthocenter) of the anterior insula (Naidich *et al*, 2004). Although functional connectivity mapping was also carried out using left hemisphere seeds, only data obtained using the right hemisphere seeds are reported for the sake of brevity, as the analysis using left hemisphere seeds gave comparable results.

To generate the maps, the signal time course of a selected seed region was used as a regressor to be correlated with the signal time course of every voxel in the brain, and the obtained voxel-wise regression coefficients served to build first-level output (.con) images. For both locations, seeds were defined as 3.5-mm radial spheres (sampling approximately 25 voxels) using MarsBaR region-of-interest toolbox in MNI stereotaxic space (Brett *et al*, 2002). Signal values for the seeds were calculated as the average signal of the voxels included in the seed at each time point. In addition, we derived estimates of white matter, CSF, and global brain signal fluctuations to be included as confounding ("nuisance") variables in the analyses.

First-level images generated for each participant were then included in second-level (group) random-effects analyses. One-sample t-statistic maps were calculated to obtain Default and Insula network functional connectivity maps for each group, and two-sample t-tests were performed to map between-group differences. Voxel-wise analyses in SPM were also performed to map the correlation between resting-state functional connectivity and behavior ratings (anxiety and memory) and cannabis consumption (average joints per year).

Thresholding criteria. Spatial extent thresholds were determined by 1,000 Monte Carlo simulations using AlphaSim (Ward, 2000) as implemented in the SPM REST

toolbox (Song *et al*, 2011). For within-group effects, the input parameters to AlphaSim included an individual voxel threshold probability of 0.005, cluster connection radius of 5 mm, 8 mm FWHM smoothness, incorporating a whole-brain mask volume (256,299 voxels). The estimated minimum cluster size extent was 176 voxels in order to satisfy a family-wise error rate correction of $P_{FWE}< 0.05$. For between-group effects and correlation maps, the incorporated mask instead corresponded to the network maps identified in within-group effects (adding voxels from both cannabis user and control maps), corresponding to 50,427 voxels for the Default network and 64,017 voxels for the Insula network. The respective cluster sizes to satisfy a FWE rate correction of P <0.05 were 102 and 106 voxels. Based on these estimates, clusters greater than 176 voxels with $P < 0.005$ were considered significant (corrected $P < 0.05$) to identify functional connectivity networks in one-sample analyses and clusters greater than 106 voxels with $P < 0.005$ to identify between-group differences and correlation findings.

Hippocampus functional connectivity map. Owing to the relevance of the hippocampus in memory, an additional functional connectivity map was generated for this structure to further characterize the relationship between memory and brain spontaneous activity. The seed region of interest was placed at the midpoint of the hippocampus long axis, corresponding to MNI coordinates x= 26, y= -25, z= -14 (Kahn et al, 2008).The data were analyzed similarly to the main networks of interest as described above.

Statistical analysis of behavioral data. Student-t test was used to compare demographic and behavioral variables between groups, and ANCOVA was used instead when covariates were included in the comparison.

Results

Behavioral assessment. Anxiety and memory ratings were within normative values in both study groups (Table 1). Nevertheless, group comparison showed subtle differences that were significant for specific measurements. Cannabis users showed higher anxiety scores, reduced verbal memory span and delayed recall, and increased forgetting rate. After controlling for the effect of education, group differences in memory ratings remained significant for verbal memory span and delayed recall.

Functional connectivity maps. The PCC seed consistently identified the elements of the Default network in both groups. Functional connectivity maps included the PCC/precuneus, angular gyri, medial (and lateral) frontal cortex, anterior cingulate cortex and lateral temporal cortex. Compared with control subjects, however, cannabis users showed increased functional connectivity in the ventral part of the PCC and decreased functional connectivity in the dorsal PCC/precuneus junction (Figure 1 and Table S1).

The insula seed identified a network that included bilateral insula and opercula (extending to the lateral prefrontal cortex and supramarginal gyri), basal ganglia, anterior cingulate cortex and ventral brain structures involving the brainstem and right amygdala in both groups. Cannabis users showed increased functional connectivity relative to controls in the anterior portion of the left insula and supramarginal gyri bilaterally, and reduced functional connectivity in the anterior cingulate cortex and superior brainstem (Figure 1, Table S1).

Results from mapping the regions showing negative correlations with the seeds were also of interest. The PCC “anticorrelation” map included the Insula network, dorsal sensorimotor cortex, visual areas and cerebellum in both groups. This map

additionally included the amygdalae in cannabis users. Compared with control subjects, cannabis users showed stronger anticorrelation specifically with areas of the Insula network (Figure 2, Table S2). Reciprocally, the insula anticorrelation map included the Default network and part of neighboring networks. In cannabis users, the insula seed showed a stronger anticorrelation specifically with primary Default network areas (ventral PCC, frontal medial cortex and right angular gyrus).

Cannabis users, therefore, showed a pattern of increases and decreases in functional connectivity within the Default and Insula networks and enhanced anticorrelation between both. In a further analysis, we investigated to which extent the observed functional connectivity alterations (extracted at peak group differences) were able to account for group mean differences in behavioral ratings (i.e., comparing means using ANCOVA with functional connectivity measurements as covariates). Group differences in state anxiety showed a tendency to increase after controlling for ventral PCC functional connectivity ($F= 7.6$ and $P= 0.008$ before and $F= 11.6$ and $P= 0.001$ after removing the effect). This effect was more obvious when controlling for PCC-amygdala anticorrelation (state anxiety group differences; $F= 7.6$ and $P = 0.008$ before and $F= 14.6$ and $P= 0.0003$ after removing the effect). Conversely, we observed that group differences in verbal recall ($F= 7.4$ and $P= 0.009$) were no longer significant when the analysis was controlled for ventral PCC functional connectivity ($F= 3.5$ and $P= 0.066$) and PCC-insula anticorrelation ($F= 2.3$ and $P= 0.133$). Overall, this analysis indicates that the effect of functional connectivity changes was in the direction of reducing anxiety scores and interfering with memory.

Correlation of functional connectivity with anxiety ratings. A relationship between anxiety ratings and functional connectivity in SPM maps was found involving the right

insula (Figure 3, Table S3, and Supplementary Figure 1). Specifically, cannabis users showed stronger (than controls) negative correlation between insula functional connectivity and state anxiety (i.e., greater connectivity, less anxiety).

Correlation of functional connectivity with memory ratings. Memory ratings correlated with functional connectivity measurements in the Default network overlapping with areas showing group differences (i.e., in ventral PCC as a subthreshold but highly specific finding, and dorsal PCC/precuneus junction) (Figure 3, Table S3, and Supplementary Figure 1). Specifically, cannabis users showed stronger (than controls) negative correlation between ventral PCC functional connectivity and verbal recall (i.e., greater connectivity, worse recall), and stronger (than controls) positive correlation between dorsal PCC/precuneus functional connectivity and verbal recall (i.e., less connectivity, worse recall). The correlation pattern was marginally affected by controlling for years of education (e.g., dorsal PCC/precuneus showed F= 13.5 and P= 0.0003 before and F= 12.9 and P= 0.0004 after controlling for years of education).

Correlation with the amount of cannabis used. Average joints per year in cannabis users showed a positive correlation with the strength of functional connectivity in the PCC (subthreshold) and insula (Figure 4, Table S4).

Hippocampus seed analysis. The hippocampus seed identified a typical hippocampal connectivity map including medial temporal lobe structures extending to the retrosplenial region and upper brainstem (Figure 5, Table S5). Compared with control subjects, cannabis users showed an area of reduced functional connectivity in the right hippocampus, and stronger positive correlation between left parahippocampus functional connectivity and verbal recall (i.e., less connectivity, worse recall).

We investigated the extent to which hippocampus alterations were able to account for memory impairment in cannabis users. We observed no reduction in group differences for verbal recall before (F= 7.4 and P= 0.009) and after (F= 8.4 and P= 0.006) controlling for hippocampal functional connectivity (at peak group differences).

Long-term cannabis use effect on functional connectivity. After one month of abstinence, there was a general tendency for the magnitude of observed functional alterations to be reduced. Nevertheless, between-group differences persisted for connectivity increases and decreases in the Default network and connectivity increases in the Insula network (Figure 6).

Discussion

Chronic cannabis use was associated with functional connectivity alterations in brain networks relevant to self-awareness. Compared with control subjects, cannabis users showed a specific combination of increases and decreases in functional connectivity within the Default and Insula networks and selective enhancement of fMRI signal anticorrelation between both. These alterations were associated with behavioral measurements in a direction suggesting both anxiety score reduction and interference with memory performance. The functional changes were related to the amount of cannabis used and partially persisted after one month of abstinence.

Increased functional connectivity within the Default network involved the ventral (posterior) portion of the PCC. This region has a key role in the context of Default network function as its connectivity pattern (Leech *et al*, 2012) and prominent participation in different testing conditions suggest. In an early study, we found activation in this PCC area when normally developing adolescents responded to moral dilemmas and passively viewed their outcomes (Pujol *et al*, 2008). In a subsequent adult study, the ventral/posterior region was again the PCC portion more strongly connected during spontaneous rest, more activated during moral dilemma and more deactivated during a Stroop task (Harrison *et al*, 2008). Cannabis use, therefore, appears to be associated with increased connectivity in an area highly representative of the PCC (and Default network) function. By contrast, functional connectivity reduction was identified in an area that overlaps with a cognitive control network (see below).

The Default network, as a functional unit, is active in situations involving self-referential mental activity, as in moral dilemma solving, self-judgments, conceiving the viewpoint of others, autobiographical memory recall and prospective thinking (Buckner & Carrol, 2007; Greicius *et al*, 2003; Gusnard *et al*, 2001; Northoff *et al*, 2006). In these situations indeed, it is supposed that a representation of oneself is projected into active mental processes to generate the subjective perspective (see full argumentation in Buckner & Carroll, 2007). To converge with traditional conceptions of Default network function (Buckner *et al*, 2008; Mesulam, 1990; Small *et al*, 2003), it was argued that a representation of self can be generated upon awareness of the body in space (Shulman *et al*, 1997). Brain lesions involving the PCC and right angular gyrus typically interfere with awareness of both the body (somatoagnosia) and the extrapersonal space (neglect) (Mesulam, 1981, 1990).

Increased functional connectivity within the Insula network involved the anterior insula cortex. This brain area is a highly convergent node participating in a variety of functions covering a full range from emotion to cognition (Caseras *et al*, 2010, 2011; Cauda *et al*, 2012; Craig, 2009; Singer *et al*, 2009). At the level of basic brain operations, however, it is proposed that the anterior insula primarily underlies interoceptive or visceral awareness (Caseras *et al*, 2011; Critchley *et al*, 2004). This paralimbic cortex is highly coupled with the anterior cingulate cortex and amygdala, which together are likely critical for integrating interoceptive information into emotion (Craig, 2002; Critchley *et al*, 2004; Naqvi & Bechara, 2009). In a previous study, for example, we found that anxiety provocation and interoceptive (heartbeat) awareness activated similar portions of the anterior insula and anterior cingulate cortices (Caseras *et al*, 2011). In cannabis users, we have found increased functional connectivity within

the anterior insula, but decreased connectivity to the anterior cingulate cortex (and the thalamus/midbrain junction). Interestingly, both anterior cingulate cortex and thalamus/midbrain are areas of the Insula network overlapping with the Default network (Figure 1). The anatomy of the findings therefore suggests a markedly specific effect of chronic cannabis use on network tuning (and coupling) involving increased functional connectivity in core areas and reduced connectivity in areas bordering with other networks.

The increase in connectivity within the anterior insula seems to be associated with anxiety score reduction. This finding may give further support to a model of addiction which proposes that the ability of addictive drugs to enhance visceral sensations via insula activation is likely to modify an individual's affect state (and contribute to promote addiction), as these sensations themselves may be pleasurable and rewarding (Naqvi & Bechara, 2009). It is also important, however, that a relevant part of the Insula network overlaps with the classical dopamine rewarding system at the level of the medial frontal cortex, hippocampus and amygdale (Morales & Pickel, 2012; Swanson, 1982), and that cannabis-related alterations within the dopamine system have also been described (Iversen, 2003; Nestor *et al*, 2010; van Hell *et al*, 2010). Therefore, it appears that cannabis use could influence motivated behavior by a direct action on the classical rewarding system and indirectly via insula modulation.

The effect of psychoactive drugs on the sense of well-being is frequently associated with memory disturbances (Robbins *et al*, 2008; Solowij & Battisti, 2008). In our study, increased functional connectivity in the ventral PCC and reduced functional connectivity in the dorsal PCC/precuneus junction were both associated with impaired verbal recall. The dorsal and ventral parts of the PCC are histologically and

functionally distinct (Leech *et al*, 2011; Vogt, 2006). The dorsal PCC is a Default network area, but is also highly connected to a dorsal cognitive control network relevant to working memory (Leech *et al*, 2011, 2012). In the memory task used in our study, verbal recall scores notably depend on verbal span, which is a typical form of working memory (65% of verbal recall variance was explained by the combination of verbal span and forgetting rate in our data). Dorsal PCC alterations could indeed affect verbal recall by interfering with working memory processes. It has previously been observed that reduced PCC connectivity with the other Default network areas at rest predicts poorer performance during working memory tasks (Hampson *et al*, 2006).

The ventral PCC may fulfill a functional role more conventionally associated with the Default network. This PCC area is activated during memory operations requiring an internal focus of attention, such as autobiographical memory retrieval (Svoboda *et al*, 2006). In our study, increased ventral PCC functional connectivity at rest was associated with impaired verbal recall. This is a paradoxical finding, as lower (as opposed to higher) ventral PCC connectivity has been reported to predict poorer memory performance in older individuals (Wang *et al*, 2010). Studies using nicotine may shed light on how increased PCC connectivity could also interfere with verbal recall. In contrast with the effects of cannabis on memory, nicotine may improve accuracy for word recall (Heishman *et al*, 2010). When tested using fMRI, nicotine was associated with reduced PCC activity during rest (Newhouse *et al*, 2011; Tanabe *et al*, 2011). One hypothesis is that nicotine may enhance cognitive performance by suppressing Default network activity (Tanabe *et al*, 2011). By analogy, cannabis use could impair verbal memory due to defective suppression of Default network activity. Relevantly, memory success is critically related to the ability to switch from PCC

deactivation during encoding to PCC activation during retrieval (Daselaar *et al*, 2009; Kim *et al*, 2010). Moreover, the retrosplenial PCC is thought to be responsible for the ability to switch from first-person to observer perspectives during recall (Vann *et al*, 2009). Therefore, one hypothesis to be further tested is whether cannabis use interferes with memory performance by reducing PCC flexibility.

Memory impairment as a collateral effect of sedative drugs may result from direct action on the hippocampal system (Robbins *et al*, 2008). Cannabis receptors are notably present in the hippocampus and hippocampus alterations have been proposed to account for memory impairment in cannabis users (Iversen, 2003; Yücel *et al*, 2008). We found a reduction in functional connectivity within the hippocampus. In our analysis, however, group memory differences were better explained by changes in Default network connectivity than by hippocampal alterations. Nevertheless, the Default network and the hippocampus systems are closely related (Vann *et al*, 2009). The ventral PCC, for example, is reciprocally connected with the hippocampus and parahippocampal region and with the anterior thalamus, closing the classical Papez circuit relevant to episodic memory (Vann *et al*, 2009). Overall, the scenario suggests that cannabis abuse has potential to critically interfere with the integration of self-referential processes into the storage of personal experiences.

This study was limited in that cannabis users and control subjects had a mean education level difference of one year. Although participants in both groups were selected to have a minimum of 10 years education, the difference could potentially affect memory performance. To partially circumvent this limitation, we specifically covaried for years of education in our analyses. Secondly, while the study design has allowed us to establish significant associations between chronic cannabis use and brain

functional changes, is not appropriate for making direct statements regarding the causal role of cannabis. Nonetheless, the observed correlations between amount of cannabis use and functional connectivity suggest such relationships may exist. The current findings may also express a relatively long-lasting effect on brain functional connectivity, as the pattern of alterations persisted after one month of abstinence. On the other hand, these alterations had a tendency to be less pronounced in the follow-up assessment, which begs the question of their potential reversibility.

In conclusion, we have identified specific patterns of altered functional connectivity associated with chronic cannabis use that appear to involve the tuning and coupling of brain networks relevant to self-awareness. The Default and Insula networks, in turn, show anatomical overlap and strong functional connection with brain networks devoted to cognitive control, storage of personal experiences and motivated behavior. The results suggest potential mechanisms for recreational drugs to interfere with higher-order network interactions generating conscious experience.

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Figure legends

Figure 1. Default network and Insula network functional connectivity maps, and significant group differences. CU, cannabis users; C, control subjects. The right hemisphere corresponds to the right side of axial views. Bottom right CU>Cview corresponds to MNI x=-36.

Figure 2. Functional connectivity maps (negative correlations). PCC, Posterior Cingulate Cortex, and significant group differences.CU, cannabis users; C, control subjects.The right hemisphere corresponds to the right side of axial views.

Figure 3. Correlation between functional connectivity and behavior ratings in the Insula (top) and Default network maps (bottom). Significant interactions between group and correlation pattern are reported. Cannabis users showed a stronger (than controls) negative association between insula functional connectivity and state anxiety, and stronger negative (ventral PCC) and positive (dorsal PCC/precuneus) associations between functional connectivity and verbal recall. Top view corresponds to MNI x= 38.

Figure 4. Correlations between functional connectivity measurements and amount of cannabis used. In cannabis users, strength of functional connectivity correlated positively with average joints per year in primary regions of the Default and Insula networks (subthreshold cluster extent in the case of PCC).The right hemisphere corresponds to the right side of the axial view.

Figure 5. Hippocampus functional connectivity maps, significant group differences (bottom left) and correlation between functional connectivity and the memory performance (bottom right). Significant interaction between group and correlation

pattern is reported for verbal recall. CU, cannabis users; C, control subjects.The right hemisphere corresponds to the right side of the axial and coronal views.

Figure 6. Persistent effects on functional connectivity after one month of cannabis abstinence. Group differences in the Default (top) and Insula (bottom) networks are reported. CU, cannabis users; C, control subjects. Bottom right view corresponds to MNI x=-36.

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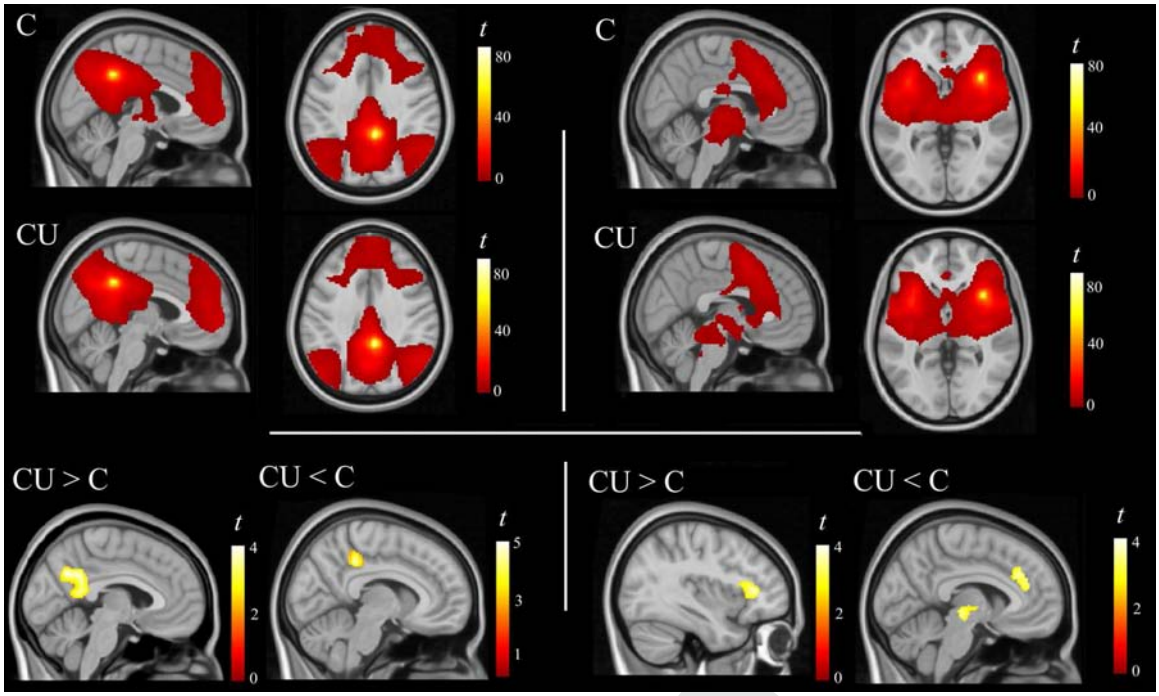
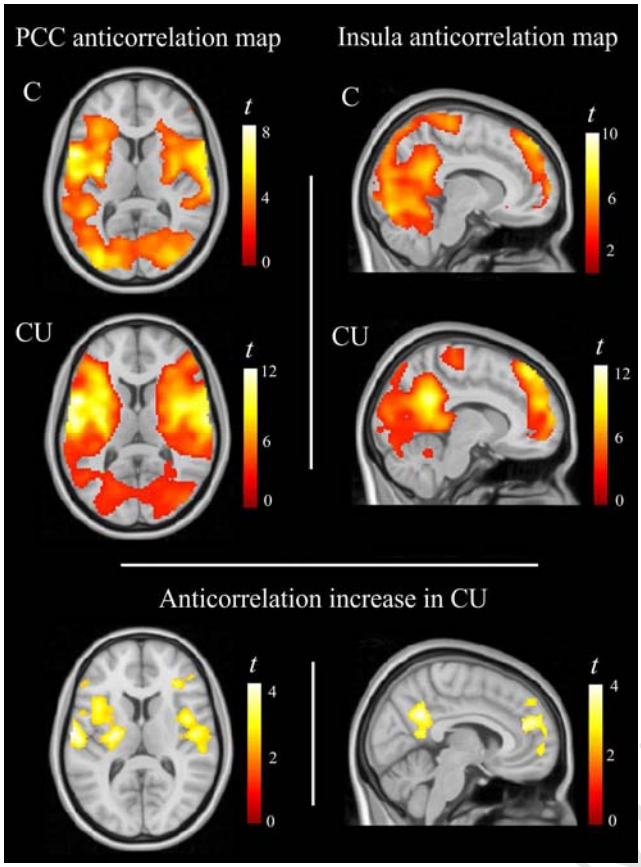
Table 1. Cannabis use and behavioral tests

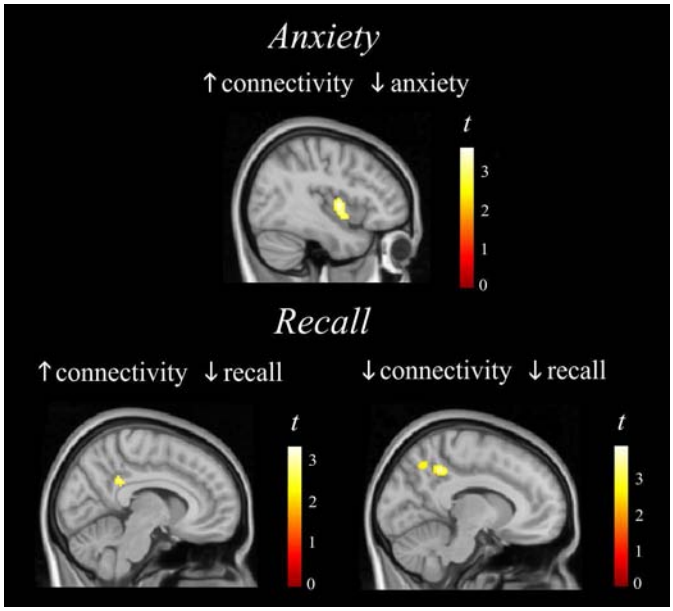
	Cannabis Mean (SD)
Age of use onset	14.9 (1.0)
Duration of use (years)	6.0 (2.5)
Total lifetime use (joints)	5268 (4265)
Average joints per year	899 (560)

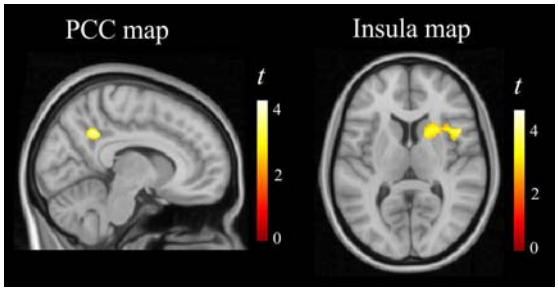
Anxiety and Memory Ratings	Cannabis Mean (SD)	Controls Mean (SD)	T	P	F*	P*
Trait Anxiety (STAI total score)	12.6 ± 4.3	9.0 ± 5.6	2.7	0.009		
State Anxiety (STAI total score)	12.3 ± 3.9	9.2 ± 4.4	2.8	0.008		
Verbal Memory Span (1st trial)	6.1 ± 1.6	7.5 ± 1.9	-2.9	0.006	5.4	0.023
Verbal Learning (over 5 trials)	20.6 ± 6.7	20.9 ± 6.0	-0.2	0.848	0.0	0.984
Recall (20-minute delayed)	11.2 ± 2.7	13.1 ± 1.8	-3.2	0.002	7.4	0.009
Forgetting Rate (5th trial – Recall)	1.9 ± 1.5	1.1 ± 1.5	2.1	0.042	3.5	0.069

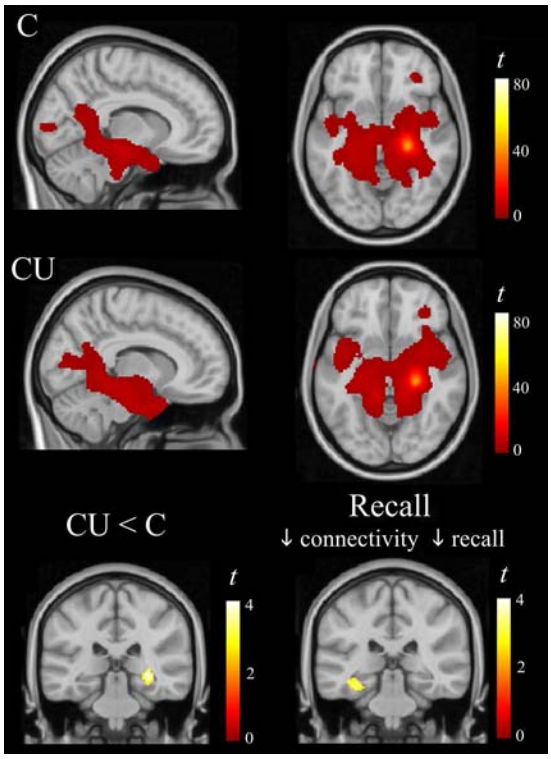
STAI, State–Trait Anxiety Inventory. Memory assessed with Rey Auditory-Verbal Learning Test (number of words). F* and P*, after controlling for years of education.

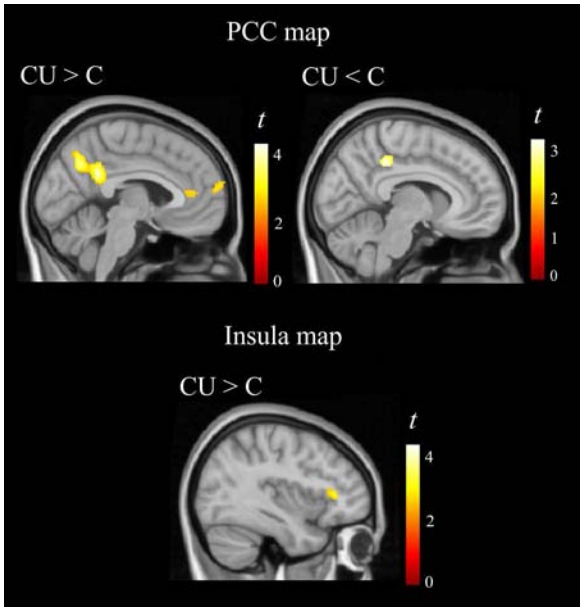












Authors' contribution:

RMS, RTF and JP were responsible for the study design. AF, AB, MF and MT conducted the clinical characterization and selection of participants. LBH, MLS, CSM, JD and JP designed the fMRI protocols, performed the neuroimaging, and analyzed fMRI results. JP wrote the initial manuscript draft. JAC, SB, BJH, and SN provided critical revision of the manuscript for important intellectual content. All the authors have supervised and approved the final version of the manuscript.

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Supporting Document:

Supplementary Table 1. Functional connectivity maps.

Supplementary Table 2. Functional connectivity maps (negative correlations).

Supplementary Table 3. Correlations between functional connectivity measurements and behavior ratings.

Supplementary Table 4. Correlations between functional connectivity measurements and amount of cannabis used.

Supplementary Table 5. Hippocampus functional connectivity results.

Supplementary Figure 1.Plots of the correlations between functional connectivity and behavior ratings.

Table S1. Functional connectivity maps.

	Control Subjects		Cannabis Users	
Default Network	*x y z*	*T*	*x y z*	*T*
PCC/Precuneus	6 -54 36	21.3	6 -52 34	28.4
R Angular gyrus	48 -60 38	9.9	52 -58 30	13.8
L Angular gyrus	-40 -62 34	7.3	-44 -70 36	9.6
Medial Frontal cortex	8 46 14	6.9	6 48 16	11.2
R lateral Frontal cortex	36 20 46	6.0	26 24 50	11.6
L lateral Frontal cortex	-22 24 46	7.3	-22 26 46	8.4
Anterior Cingulate cortex	-8 44 12	6.3	6 48 16	11.2
R lateral Temporal cortex	62 -34 -14	5.4	64 -26 -16	7.8
Thalamus	8 -12 10	4.2	-	-

Cannabis Users > Control Subjects

	Cluster size (voxels)	*x y z*	*T*
Ventral PCC (bilateral cluster)	1114	4 -54 32	4.1

Control Subjects > Cannabis Users

	Cluster size (voxels)	*x y z*	*T*
R dorsal PCC/Precuneus	453	10 -36 40	5.0
L dorsal PCC/Precuneus	173	-8 -32 42	4.2

	Control Subjects		Cannabis Users	
Insula Network	*x y z*	*T*	*x y z*	*T*
R Insula-Opercula complex	36 24 2	22.3	36 24 2	24.7
L Insula-Opercula complex	-36 12 0	23.3	-36 12 0	24.9
Anterior Cingulate cortex	-8 20 30	9.8	-10 22 30	6.5
Thalamus-Mesencephalon	4 -16 -2	7.8	4 -16 -2	4.1
R Amygdala	22 -4 -18	4.1	22 -4 -18	3.5
R Prefrontal cortex	38 36 2	8.9	38 36 2	12.8
L Prefrontal cortex	-32 40 26	6.5	-32 40 26	8.6
R Supramarginal gyrus	56 -40 32	6.1	60 -30 30	10.5
L Supramarginal gyrus	-58 -34 28	7.7	-60 -30 26	10.9

Cannabis Users > Control Subjects

	Cluster size (voxels)	*x y z*	*T*
L Insula	243	-36 32 8	3.8
L Inferior Parietal cortex	465	-56 -38 48	3.9
R Inferior Parietal cortex	466	54 -36 50	3.8

Control Subjects > Cannabis Users

	Cluster size (voxels)	*x y z*	*T*
Anterior Cingulate cortex	321	6 40 20	3.6
Brainstem	225	-2 -20 -4	3.8

x y z are coordinates given in Montreal Neurological Institute (MNI) space. PCC, posterior cingulate cortex; R, right; L, left. Statistics correspond to a corrected threshold $P_{FWE} < 0.05$ estimated using Monte Carlo simulations.

Table S2. Functional connectivity maps (negative correlations)

	Control Subjects		Cannabis Users	
PCC seed	*x y z*	*T*	*x y z*	*T*
R Insula-Operculum	50 6 0	6.9	48 4 -4	12.3
L Insula-Operculum	-40 4 -6	8.6	-54 8 6	12.6
R Sensorimotor cortex	54 -28 54	5.0	54 -32 60	8.2
L Sensorimotor cortex	-62 -18 40	6.3	-44 -40 56	7.2
Supplementary Motor Area	6 -4 58	6.6	8 -2 56	9.0
R Visual cortex	36 -78 -6	6.1	48 -56 -2	5.2
L Visual cortex	-34 -80 0	7.7	-50 -64 0	5.5
R Cerebellum	30 -56 -22	7.8	32 -54 -24	6.0
L Cerebellum	-32 -52 -24	7.2	-22 -64 -18	7.0
R Amygdala	-	-	20 -4 -24	4.8
L Amygdala	-	-	-16 -10 -24	4.8

Cannabis User anticorrelation > Control anticorrelation

	Cluster size (voxels)	*x y z*	*T*
R Insula-Operculum	1098	54 -20 4	4.1
L Insula-Operculum	349	-32 18 4	3.5
L Putamen	355	-22 -12 -10	4.1
Inferior Frontal gyrus	191	46 40 0	3.6
L inferior Parietal cortex	1263	-64 -12 12	4.3

Control anticorrelation > Cannabis User anticorrelation

	Cluster size (voxels)	*x y z*	*T*
Visual areas	665	-14 -82 -18	4.25

	Control Subjects		Cannabis Users	
Insula seed	*x y z*	*T*	*x y z*	*T*
PCC/Precuneus	-2 -58 22	7.0	0 -52 30	13.0
R Angular gyrus	32 -60 58	5.3	36 -70 48	7.9
L Angular gyrus	-32 -54 58	6.4	-32 -66 54	5.1
Medial Frontal cortex	6 60 30	5.0	6 52 26	9.4
L Visual cortex*	-24 -88 10	6.0	-20 -88 -12	4.1
R Visual cortex*	10 -84 8	5.4	10 -88 12	5.0
L Superior Parietal cortex	-32 -54 58	6.4	-34 -64 52	4.7
R Superior Parietal cortex	28 -64 60	5.3	34 -66 56	4.7

Cannabis User anticorrelation > Control anticorrelation

	Cluster size (voxels)	*x y z*	*T*
Ventral PCC/Precuneus	842	4 -54 30	4.0
R Angular Gyrus	559	48 -54 22	4.0
Medial Frontal cortex	619	8 50 24	3.6

Control anticorrelation > Cannabis User anticorrelation

	Cluster size (voxels)	*x y z*	*T*
L Superior Parietal cortex	808	-34 -52 62	4.1
R Superior Parietal cortex	374	26 -66 66	4.0
L Visual cortex*	597	-12 -54 -16	3.6

x y z are coordinates given in Montreal Neurological Institute (MNI) space. PCC, posterior cingulate cortex; R, right; L, left. Statistics correspond to a corrected threshold $P_{FWE} < 0.05$ estimated using Monte Carlo simulations. *extending to cerebellum.

Table S3. Correlations between functional connectivity measurements and behavior ratings (group by correlation pattern interactions)

	Cluster size (voxels)	*x y z*	*T*
Anxiety*			
Cannabis User neg. corr. > Control neg. corr.			
R Insula	116	38 -4 4	3.6
Verbal Recall**			
Cannabis User neg. corr. > Control neg. corr.			
Ventral PCC	65$^{\$}$	16 -48 30	3.2
Verbal Recall**			
Cannabis User pos. corr. > Control pos. corr.			
Dorsal PCC/Precuneus	760	-22 -52 46	4.1
Dorsal PCC/Precuneus	476	28 -44 38	4.0

*Insula network map. **Default network map. x y z are coordinates given in Montreal Neurological Institute (MNI) space. PCC, Posterior Cingulate Cortex. Statistics correspond to a corrected threshold $P_{FWE} < 0.05$, $^{\$}$except for ventral PCC subthreshold cluster extent.

Table S4. Correlations between functional connectivity measurements and amount of cannabis used (positive correlations in cannabis users)

	Cluster size (voxels)	*x y z*	*T*
Default Network			
Ventral PCC	70*	8 -50 36	4.1
Insula Network			
R Insula-Putamen	345	20 14 4	4.6
L Frontal cortex	126	-14 12 44	3.3

x y z are coordinates given in Montreal Neurological Institute (MNI) space. PCC, Posterior Cingulate Cortex. R. right; L, left. Statistics correspond to a corrected threshold $P_{FWE} < 0.05$, *except for ventral PCC subthreshold cluster extent.

Table S5. Hippocampus functional connectivity results

	Control Subjects		Cannabis Users	
Within-groups effects	*x y z*	*T*	*x y z*	*T*
R Hippocampus	28 -34 -10	15.1	28 -34 -12	13.0
L Hippocampus	-24 -22 -20	8.8	-24 -22 -20	8.3
R Medial temporal cortex	44 8 -22	5.0	40 16 -18	6.9
L Medial temporal cortex	-40 10 -24	5.4	-38 12 -26	7.1
Occipital/retrosplenial cortex	2 -62 20	3.9	2 -64 20	3.2
R Amygdala	28 0 -20	5.5	28 0 -20	5.1
L Amygdala	-26 -4 -18	4.9	-26 -4 -22	4.0
Brainstem	-4 -30 -20	3.7	-4 -30 -20	6.3

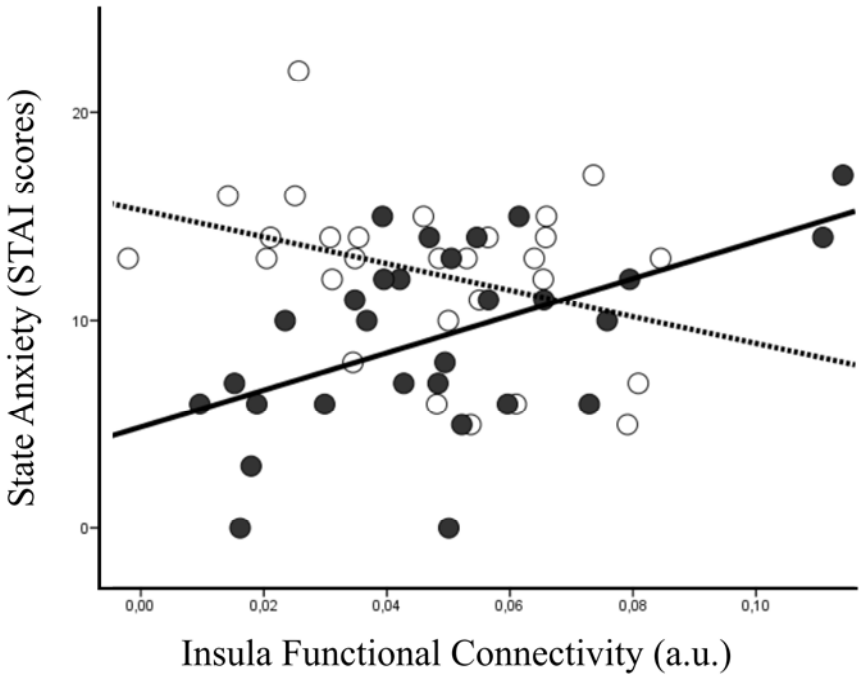
	Control Subjects > Cannabis Users		
Between-groups effects	*Cluster size (voxels)*	*x y z*	*T*
R Hippocampus	228	32 -34 -6	3.7

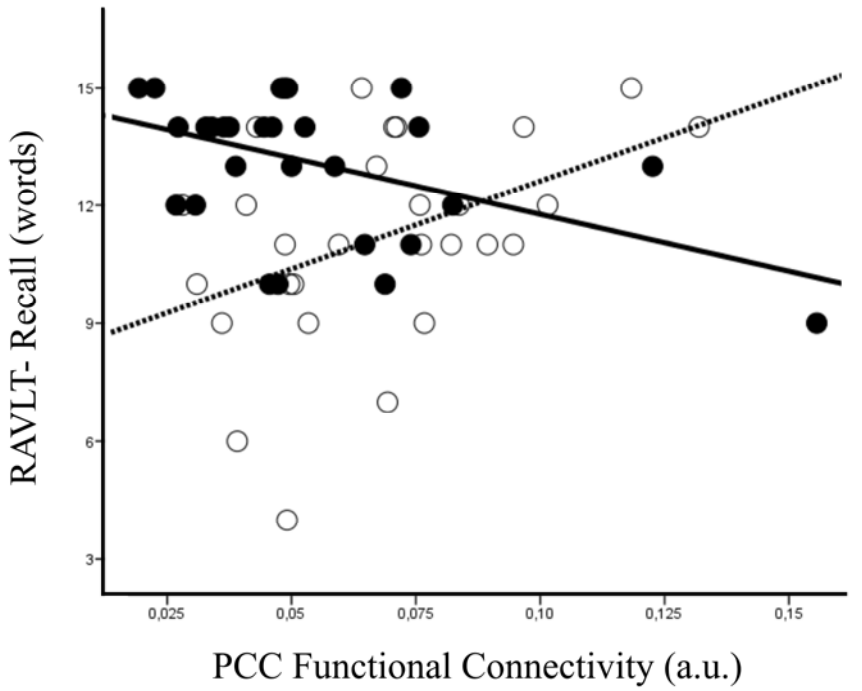
Verbal Recall

Cannabis User positive corr. > Control positive corr.

Correlations	*Cluster size (voxels)*	*x y z*	*T*
L Parahippocampal gyrus	218	-38 -36 -14	3.7

x y z are coordinates given in Montreal Neurological Institute (MNI) space. R, right; L, left. Statistics correspond to a corrected threshold $P_{FWE} < 0.05$ estimated using Monte Carlo simulations.





Supplementary Figure 1. Plots of the correlations between functional connectivity and behavioral measurements.Top plot; cannabis users (open circles, dashed line) showed stronger negative correlation between insula functional connectivity and state anxiety

compared with controls (filled circles, bold line) that showed a positive correlation (interaction t= 3.6, p= 0.0004 at x, y, z: 38 -4 4, displayed on top Figure 3). STAI, State–Trait Anxiety Inventory. Bottom plot, cannabis users (open circles, dashed line) showed stronger positive correlation between dorsal PCC/precuneus functional connectivity and verbal recall compared to controls (filled circles, bold line) that showed a negative correlation (interaction t= 3.7, p= 0.0003 at x, y, z: -8 -46 42, displayed on bottom-right Figure 3). RAVLT, Rey Auditory-Verbal Learning Test. A.u., arbritary units.

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