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Deficits in learning and memory: Parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users

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The consumption of cannabis has been linked to impairments in human learning and memory, as well as aspects of executive functioning. Cannabis-related impairments in learning and memory in chronic cannabis users, it has been argued, are caused by the effects of cannabis on hippocampal functioning. The current study involved two experiments. Experiment 1 compared 35 current users of cannabis and 38 well-matched controls on a face–name task, previously shown to activate the hippocampal region. Based on the results of experiment 1, experiment 2 used fMRI and a modified version of the face–name task, to examine cortical and (para)hippocampal activity during learning and recall in 14 current users of cannabis and 14 controls. Results of experiment 1 showed that cannabis users were significantly worse with respect to learning, short and long-term memory performance. Experiment 2 showed that despite non-significant differences in learning and memory performance, cannabis users had significantly lower levels of BOLD activity in the right superior temporal gyrus, right superior frontal gyrus, right middle frontal gyrus and left superior frontal gyrus compared to controls during learning. Results also showed that cannabis users had significantly higher BOLD activity in the right parahippocampal gyrus during learning. Hypoactivity in frontal and temporal cortices, and relative hyperactivity in the parahippocampus identify functional deficits and compensatory processes in cannabis users.

Introduction

The consumption of cannabis has been linked to impairments in human learning and memory, as well as aspects of executive functioning (Grant et al., 2003; Pope et al., 2001; Pope, 2002; Rogers and Robbins, 2001; Solowij et al., 2002; Pope and Yurgelun-Todd, 1996; Bolla et al., 2002). The effects of cannabis in animals are mediated by cannabinoid CB_1 receptors, which are expressed at especially high densities in the dentate gyrus (DG) and cornu amonis (CA) 3 regions of the hippocampus (Herkenham et al., 1991; Tsou et al., 1998). The hippocampus is a brain structure strongly implicated in both declarative and episodic memory (Sperling et al., 2001; Crane and Milner, 2002; Zeineh et al., 2003). Cannabis-related impairments in learning and memory in chronic cannabis users, it has been argued, are caused by the effects of cannabis on the hippocampus via their influence on CB_1 receptors (Herkenham et al., 1991; Tsou et al., 1998). Despite some of the neuropsychological literature indicating learning and memory deficits in chronic cannabis users (Grant et al., 2003; Pope et al., 2001; Pope, 2002; Rogers and Robbins, 2001; Solowij et al., 2002), the neurobiology underlying these deficits has yet to be fully clarified.

Evidence now suggests that cannabis impairs learning and memory in rodents (Heyser et al., 1993; Terranova et al., 1996; Nava et al., 2001) and non-human primates (Evans and Wenger, 1992), which may be related to the neurotoxicity potential of cannabis for hippocampal neurons. The effects of cannabis are mediated through the CB_1 receptor in the brain (Matsuda et al., 1990; Herkenham et al., 1991; Tsou et al., 1998), with evidence that chronic cannabinoid administration in rats causes distinct hippocampal morphological changes (Scallet, 1991; Landfield et al., 1988). While there is still inconclusive evidence that cannabis is neurotoxic in humans, Matochik et al. (2005) showed that frequent cannabis users had lower grey matter tissue densities in the hippocampus bilaterally, providing some evidence for the effects of cannabis on human hippocampal integrity.

Animal studies have also shown that cannabis exerts some of its impairing effects via the hippocampus during learning (Carta et al., 1998; Collins et al., 1995; Gessa et al., 1997, 1998; Nava et al., 2001), consistent with the high density of hippocampal endocannabinoid receptors (Herkenham et al., 1991; Tsou et al., 1998). Impairments to learning and memory have been shown in chronic cannabis users (Grant et al., 2003; Pope et al., 2001; Pope, 2002; Rogers and Robbins, 2001; Solowij et al., 2002), with imaging studies specifically demonstrating decreased memory-related blood flow in the prefrontal cortex and lower activation in the parahippocampus during verbal memory and associative learning tasks

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(Block et al., 2002; Jager et al., 2007). Studies in animals also suggest that some of the memory-impairing effects of cannabinoids occur in the prefrontal cortex (Diana et al., 1998; Jentsch et al., 1997; 1998; Verrico et al., 2003), with human imaging studies in cannabis users also demonstrating prefrontal hypoactivity (Amen and Waugh, 1998; Yurgelun-Todd et al., 1999; Lundqvist et al., 2001; Eldreth et al., 2004; Bolla et al., 2005; Pillay et al., 2004; Gruber and Yurgelun-Todd, 2005; Chang et al., 2006). Given the effects of cannabis on hippocampal and cortical functioning in animals, together with some of the neuropsychological and imaging evidence, there remains a need to use paradigms which engage cortical and hippocampal-dependent learning and memory in humans, thereby elucidating the long-term effects of cannabis use on different neuronal networks of the brain.

The hippocampal region plays a crucial role in forming new associations or episodic memories, including memories for faces (Sperling et al., 2001; Crane and Milner, 2002; Zeineh et al., 2003). Using a face–name task, Zeineh and colleagues showed that learning associations between faces and names most prominently activates the anterior CA 2 and 3 fields and the DG of the hippocampus. Based on the extant literature concerning behavioural and functional activity differences in cannabis users related to learning and memory (Grant et al., 2003; Pope et al., 2001; Pope, 2002; Rogers and Robbins, 2001; Solowij et al., 2002, Block et al., 2002; Jager et al., 2007), the current study reports two separate experiments. In experiment 1, high functioning regular users of cannabis and demographically matched controls were compared behaviourally, using a face–name task previously shown to engage the hippocampal formation (Zeineh et al., 2003). Arising from the observation of performance differences in experiment 1, experiment 2 compared brain activity, under fMRI conditions, between cannabis users and drug-naïve controls using a modified version of the face–name task. Given the effect of chronic cannabis use on this structure, and the potentially taxing effects of face–memory learning on hippocampal functioning, we hypothesised the following. In experiment 1, cannabis users would show inferior learning and memory performance for face–name associations, with performance related to life-time cannabis use; and in experiment 2, using a modified version of the face–name task, cannabis users would show dysfunctional hippocampal and prefrontal-dependent activity during learning, with altered activity related to life-time cannabis consumption.

Experiment 1

Material and methods

Subjects'

35 current users of cannabis and 38 controls were recruited from the general public and academic institutions around Dublin city. All participants underwent a comprehensive telephone screening, during which detailed information concerning past and present psychiatric, neurological and substance use was taken. Information pertaining to any form of treatment (counselling, psychological, psychiatric), past or present, was carefully detailed, with any potential participant describing any major life-time psychiatric event or head injury (e.g., head trauma resulting in a loss of consciousness, seizure or stroke) considered ineligible for the study. Cannabis and control participants also completed inventories for mood (BDI II) and drug use (questionnaire taken from the Addiction Severity index Lite-CF) (see Questionnaires section) prior to testing, to screen for depression and past or concurrent abuse of other substances. Therefore, cannabis and control participants were additionally considered ineligible if they reported concurrent or past dependence on other drugs (e.g., alcohol, amphetamines, benzodiazepines, cocaine, MDMA, hallucinogens and opiates) at the practice session prior to testing. No cannabis or control participants reported the current or past use of any other psychoactive substances (e.g., nicotine, neutraceuticals). All information concerning drug use in each participant was indexed in years (lifetime) and recent (last 30 days) and fully recorded prior to testing.

Cannabis participants were required to have regularly consumed cannabis (5–7 days/week) for the previous 2 years in order to be eligible as cannabis users for experiment 1. All cannabis users provided a positive urine sample for Δ^9-tetrahydrocannabinol (Δ^9THC) prior to behavioural testing, with additional screening for methadone, benzodiazepines, cocaine, amphetamine, opiates, barbiturates and tricyclic antidepressants (Cozart® RapiScan, UK) taking place. While the identification and quantification of cannabis metabolites in urine may have proved advantageous as a potential predictor of cognition and brain functioning, past studies have reliably shown that estimates of recent use, life-time use and age of onset of use, are reliable predictors of behavioural impairments and BOLD activity in cannabis users (Block and Ghoneim, 1993; Bolla et al., 2002, 2005; Pope and Yurgelun-Todd, 1996; Solowij et al., 2002; Solowij, 1995; Pope et al., 2003; Chang et al., 2006). Control participants were also tested for Δ^9THC and the above adulterants. Nine control participants reported past infrequent use of cannabis (<10 times lifetime use). None of the controls used cannabis in the 30 days preceding study participation (see Table 1). The sample of cannabis users reported a mean lifetime consumption of 5.7 years (range=1.5–17), a mean 23 days (range=7–30) of use in the 30 days preceding study participation and had been abstinent from cannabis, on average, for 15 h (range=2–45) prior to testing. All research participants provided informed consent and were financially compensated.

Table 1
Experiment 1

	Control (n=38)	Cannabis (n=35)
Age	22.0±0.4	22.3±0.5
Years of education	16.0±0.3	16.0±0.3
Verbal intelligence score (NART)	121.8±0.6	120.1±0.8
Beck depression inventory II score	5.2±0.7	4.7±0.6
Males/females	29/9	32/3
Years of alcohol use	5.2±0.4	6.9±0.5*
Alcohol use in the last month (no. days)	6.9±0.9	9.2±1.0
Alcohol use age of onset (years)	16.7±0.3	15.7±0.4
Amphetamine use (years)	1.0±1.0	2.3±0.5
Amphetamine use in the last month (no. days)	0.0±0.0	0.0±0.0
Cocaine use (years)	2.0±0.7	1.3±0.3
Cocaine use in the last month (no. days)	0.0±0.0	0.0±0.0
MDMA use (years)	0.0±0.0	0.9±0.2
MDMA use in the last month (no. days)	0.0±0.0	0.2±0.1
Hallucinogenic use (years)	0.1±0.8	0.2±1.0
Hallucinogenic use in the last month (no. days)	0.0±0.0	0.0±0.0
Cannabis use (years)	0.2±0.1	5.7±0.6***
Cannabis use in the last month (no. days)	0.0±0.0	23.1±1.0***
Cannabis age of onset (years)	18.7±1.0	16.5±0.4**
Years of nicotine use	0.0±0.0	0.0±0.0
Nicotine use in the last month (no. days)	0.0±0.0	0.0±0.0

Mean and SEM for control and cannabis groups on demographic and drug use history (*p<0.05; **p<0.01, ***p<0.001 versus control group).

Learning and memory face–name pairs task

The face–name learning task was adapted from a paradigm in Zeineh et al. (2003), and modified to provide a serial-learning format similar to that of the *Rey Auditory Verbal Learning Test* (RAVLT) (Rey, 1941, 1964). The task structure included learning, distraction and recall phases, with the learning phase requiring participants to study eight serially presented pairs of faces and names (each presented for 3.5 s). A distracter task was inserted between each learning and recall phase to prevent rote rehearsal of the face–name associations. The distracter task required participants to press a button (the "1" key on the key pad) each time a central visual display (an empty circle) contained a black star (see Fig. 1). Eight distracter trials, separated by intervals of 2 to 5 s were presented prior to the beginning of the cued recall phase. During each recall trial participants were presented (in random order) with one of the eight 'learning phase' faces (for 3.5 s), and required to verbally respond with the correct name association. The learning, distraction, and recall procedure was repeated five times for the original set of faces, following which the procedure was conducted with a new set of unfamiliar faces, which acted as a "diversion memory set." Immediately following the recall phase of the diversion set, participants were once again presented with the original set of faces (in random order) and asked to correctly identify their names with a verbal response. This constituted the "short delay" component of the face–name cued recall task. Approximately 25 min later, participants were again presented with the original set of faces (in random order), and asked to correctly identify the name (with a verbal response) associated with each face. This constituted the "long delay" component of the face–name cued recall task. Finally, participants completed the recognition component of the task, during which they were presented with 24 faces (each presented for 3.5 s in a randomised order), eight of which were part of the original set and 16 of which were from either the diversion set or previously unseen. Here, participants were required to press a button (i.e., the "1" key on the key pad) only when they were presented with a face from the original set of faces.

A series of dependent measures were derived from this task: learning curve (trials 1–5), learning performance (sum of trials 1–5), short and long delay recall, percentage recall consistency (100/ sum of trials 1–4) × (sum of conjoint recalls of faces between trials 1, 2; 2, 3; 3, 4; 4, 5), which is a measure of both working memory and organization of memory (based on Delis et al., 1987) and the percentage recognition score, which constituted the number of original faces correctly identified. The task was programmed and run using *E-Prime version 1.1* (Psychology Software Tools, Pittsburgh, USA).

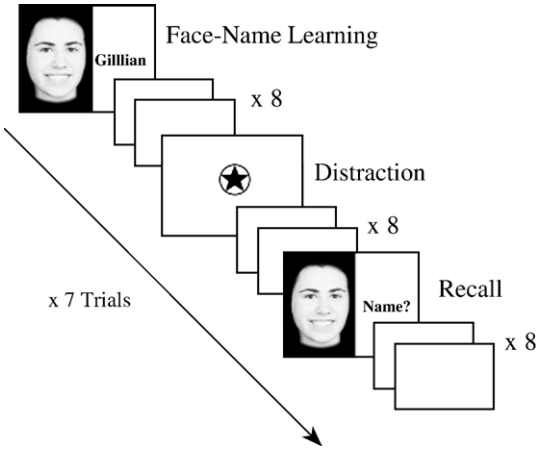

Fig. 1. Experiment 1. Face–name cued recall task in which participants were required to learn and recall face–name pairs over a total of 7 trials.

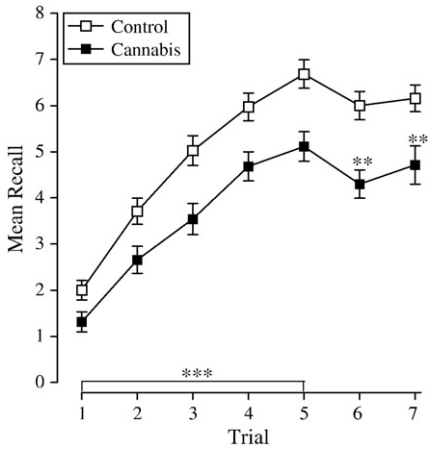

Fig. 2. Experiment 1. Mean performance on the first five trials, trial 6 (short delay) and trial 7 (long delay) of the face–name cued recall task in controls and cannabis users (means and standard error means). Learning curves (trials 1–5) were analyzed using a repeated measures design. Performances on trials 6 and 7 were analyzed using independent *t*-tests (**$p<0.01$; ***$p<0.001$).

Questionnaires

The National Adult Reading Test (NART) (Nelson and O'Connell, 1978) and the Beck Depression Inventory-II (Beck et al., 1996) were administered to all participants during the testing session. Information concerning recent and lifetime alcohol and drug use (see Table 1) was obtained from all participants using a questionnaire taken from the Addiction Severity Index *Lite*-CF (McLellan et al., 1992).

Results

Demographics and drug use

Demographic data for the two groups are shown in Table 1. Overall, the groups did not differ significantly on any variable except lifetime alcohol use ($p<0.05$). Despite this group difference, there were no associations between reported alcohol use and face–name memory performance. 45% of the cannabis group reported past MDMA use, with 4 cannabis users reporting minor usage of MDMA in the last month, despite urinalyses indicating an absence of MDMA in these participants. A comparison of cannabis users with no history of MDMA use ($n=19$) and those with a history of use ($n=16$), on all components of the face–name cued recall task revealed no significant differences, nor were there any significant correlations between MDMA use and behavioural performance in those subjects with a history of MDMA use. Exploration of MDMA use in the cannabis group also showed that both lifetime and recent use were significantly positively skewed. These data were, therefore, appropriately log transformed and used, together with lifetime alcohol use, as co-variates in all analyses of behavioural data.

Memory performance

Memory performance for the two groups is shown in Fig. 2. A two (group) by five (trial) repeated measures analysis of variance (ANOVA) found a significant main effect of trial ($F=106.7$, $df=4,68$, $p<0.001$), reflecting an increase in memory performance over the first five trials. There was also a significant group effect ($F=12.7$, $df=1,71$, $p<0.01$) with mean scores indicating cannabis users had lower overall levels of

recall performance when compared to controls. These group differences still remained when co-varying for lifetime alcohol (F=8.3, df=1,70, p<0.01), lifetime MDMA (F=4.4, df=1,70, p<0.05) and recent MDMA use (F=13.0, df=1,70, p<0.01). Analyses revealed no trial by group interaction (F=1.3, df=4,68, p=0.3), suggesting there was no difference between groups in the gradient of the learning curves. Independent t-tests showed that the two groups significantly differed on the short (p<0.01) and long (p<0.01) delay recall components of the task. Univariate analyses showed that inferior short delay performance remained when controlling for lifetime alcohol ($F_{1,70}$=6.0, p<0.05), lifetime MDMA ($F_{1,70}$=5.0, p<0.05) and recent MDMA use ($F_{1,70}$=8.7, p<0.01), as did long delay performance when controlling for lifetime alcohol ($F_{1,70}$=5.2, p<0.05), lifetime MDMA ($F_{1,70}$=3.7, p<0.05) and recent MDMA use ($F_{1,70}$=7.8, p<0.01).

Figs. 3 and 4 show the significant differences between the two groups on learning performance (p<0.001) and percentage recall consistency (p<0.01). There was no significant group difference on percentage recognition (p=0.2). The inferior learning performance of cannabis users remained when controlling for lifetime alcohol ($F_{1,70}$= 8.9, p<0.01), lifetime MDMA ($F_{1,70}$=7.7, p<0.01) and recent MDMA use ($F_{1,70}$=13.5, p<0.001) as did recall consistency when controlling for lifetime alcohol ($F_{1,70}$=6.6, p<0.05), lifetime MDMA ($F_{1,70}$=6.1, p<0.05) and recent MDMA use ($F_{1,70}$=10.7, p<0.01).

Drug use correlations

There were no correlations between cannabis abstinence or self-reported use of cannabis (e.g., years of use, days of use in last month and age of onset of use) and behavioural performance on the face–name cued recall task in the cannabis-using group.

Finally, correlations between supraspan (recall performance on the first trial), and recall consistency were observed in the cannabis-using group (r=.56, p<0.001), in the control group (r=.54, p<0.001) and when the samples were combined (r=.67, p<0.001).

Discussion

The results indicate that high functioning chronic cannabis users and non-drug-using controls differed significantly on a task previously shown to actively engage hippocampal functioning. Despite the reported life-time alcohol and MDMA use (which were not found to relate to task performance), the control and cannabis groups were well matched for education, verbal IQ, and mood, thereby avoiding some of the common confounds of previous studies (a cohort effect), which may contribute to group differences. The group differences in this study appear to be consistent with recent findings indicating deficits in learning and memory (Grant et al., 2003; Pope et al., 2001; Pope, 2002; Rogers and Robbins, 2001; Solowij et al., 2002), supporting the notion that cannabis may impair learning, short and long-term memory processing.

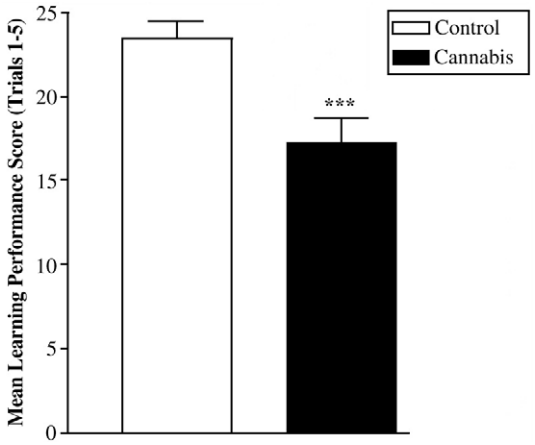

Fig. 3. Experiment 1. Mean learning performance in the control and cannabis groups on the face–name cued recall task (***p<0.001).

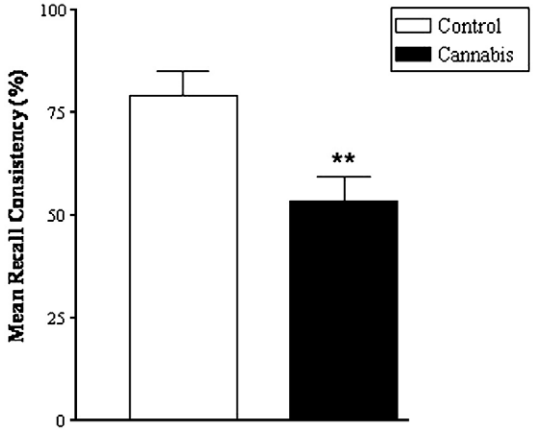

Fig. 4. Experiment 1. Mean percentage recall consistency in the control and cannabis groups on the face–name cued recall task (**p<0.01).

The present results do not appear to be influenced by cannabis intoxication at the time of testing the cannabis-using group. While there is evidence indicating that significant cognitive impairment accompanies cannabis use up to 24 h after smoking (Robbe and O'Hanlon, 1993), we found no significant associations between hours of abstinence and task performance in our sample of cannabis users. Unlike previous studies (Block and Ghoneim, 1993; Bolla et al., 2002; Pope and Yurgelun-Todd, 1996; Solowij et al., 2002), we found no relationship between the frequency (days) of use in the preceding month and memory performance, nor did we observe any association between life-time cannabis use or age of cannabis use onset and performance, like previous studies (Block and Ghoneim, 1993; Solowij, 1995; Pope et al., 2003).

The face–name cued recall task has previously been shown to selectively engage the hippocampal formation (Zeineh et al., 2003). The behavioural effects of cannabis on learning and memory appear to be mediated by CB_1 receptors, which, as noted, are expressed at especially high densities in the hippocampus proper and dentate gyrus regions (Herkenham et al., 1991; Tsou et al., 1998). Our high functioning cannabis-using group also showed inferior recall consistency on the face–name task, which may have been due to working memory dysfunction (Alexander et al., 2003) or a reduced organization of memory during learning (Waters and Waters, 1976; Sternberg and Tulving, 1977). In support of deficits in working memory, correlation analyses showed a significant positive relationship between supraspan (recall performance on the first trial), an indirect measure of working memory capacity, and recall consistency in this study. In addition, the learning curves for the two groups were mostly parallel (supported by the lack of a *trial*×*group* interaction), suggesting that there was no deficit in learning over-and-above that observed on the first trial, which may support prefrontal/working memory impairment. Studies suggest the memory-impairing effects of cannabinoids are the result of prefrontal dysfunction (Diana et al., 1998; Jentsch et al., 1997, 1998; Verrico et al., 2003), with imaging studies confirming

prefrontal hypoactivity in cannabis users (Amen and Waugh, 1998; Yurgelun-Todd et al., 1999; Lundqvist et al., 2001; Block et al., 2002; Eldreth et al., 2004; Bolla et al., 2005; Gruber and Yurgelun-Todd, 2005; Chang et al., 2006; Jager et al., 2007).

The hippocampal region plays an important role in forming new associations or episodic memories, including memories for faces (Sperling et al., 2001; Crane and Milner, 2002; Zeineh et al., 2003), with observations that learned associations between faces and names most prominently activate the anterior CA 2 and 3 fields and the DG of the hippocampus (Zeineh et al., 2003). The results here suggest that learning and cued recall for face–name associations are significantly compromised in high functioning regular cannabis users, possibly due to cannabis use and its effects on hippocampal and/or prefrontal neuronal functioning. Based on the extant literature concerning both behavioural and functional activity differences in cannabis users related to learning and memory (Grant et al., 2003; Pope et al., 2001; Pope, 2002; Rodgers et al., 2001; Solowij et al., 2002, Block et al., 2002; Jager et al., 2007), and the results of experiment 1, experiment 2 used functional magnetic resonance imaging (fMRI) to further explore the effects of cannabis on both hippocampal and prefrontal functioning within the context of learning and memory processing for faces.

Experiment 2

Material and methods

Subjects'

14 cannabis users and 14 control subjects meeting the same criteria as in experiment 1 were recruited from the general public and academic institutions around Dublin. None of these individuals participated in experiment 1. Twelve controls reported past infrequent use of cannabis (no more than 10 times). None of the controls used cannabis in the 30 days preceding study participation (see Table 2). Cannabis users in experiment 2, as in experiment 1, were required to have consumed cannabis 5–7 days/week for the past 2 years. All participants in the cannabis group were additionally required to have smoked a minimum of 500 joints in their life-time. This additional criterion was introduced to maximize the examination of possible "dose-response" relationships between this index of cannabis consumption and BOLD activity, as addressed in previous studies (Bolla et al., 2005; Chang et al., 2006). Information concerning alcohol, nicotine and cannabis use in each participant was indexed in years (life-time) and recent (last 30 days). Other drug use information in each participant was indexed by the total number of separate occasions (life-time), total number of recent separate occasions (last 30 days) and the length of time (days or months) since a substance was used. Cannabis and control participants did not report the concurrent or past misuse of any other psychoactive substances (e.g., nicotine, neutraceuticals), which may have altered BOLD activity. Cannabis users tested positive for Δ^9THC. The cannabis group reported, on average, 7.2 years (range=2–16) of life-time cannabis use, consumption of 7925 joints (range=988–33,653), an average of 19 days of use in the last 30 days (range=1–30) and a mean abstinence of 80.8 h (range=3–686). All participants were right-handed as confirmed by the Edinburgh Handedness Inventory (Oldfield, 1971) during the telephone screening process. Control and cannabis participants completing the study were neurologically normal (as confirmed by a registered radiographer who examined each structural MRI). All research participants provided informed consent and were financially compensated.

Table 2
Experiment 2

	Control (n=14)	Cannabis (n=14)
Age	24.1±1.3	24.4±1.4
Years of education	16.6±0.4	16.0±0.5
Verbal intelligence score (NART)	122.6±0.7	122.9±0.9
Beck Depression Inventory II Score	3.6±0.8	6.1±1.3
Females/males	2/12	2/12
Years of alcohol use	7.7±1.3	9.0±1.2
Alcohol use in the last month (no. days)	2.2±0.2	2.9±0.3
Alcohol use age onset (years)	16.1±0.6	15.4±0.5
Last alcohol use (h)	118.3±33.5	197.6±153.4
Years of nicotine use	0.0±0.0	0.0±0.0
Nicotine use in the last month (no. days)	0.0±0.0	0.0±0.0
Amphetamine use (no. times)	2.9±2.9	2.6±1.0
Amphetamine use in the last month (no. times)	0.0±0.0	0.0±0.0
Last amphetamine use (months)	36.0±36.0	42.7±13.7
Cocaine use (no. times)	2.2±2.1	6.0±2.9
Cocaine use in the last month (no. times)	0.0±0.0	0.0±0.0
Last cocaine use (months)	40.5±31.5	13.6±6.7
MDMA use (no. times)	3.3±2.8	9.4±3.7
MDMA use in the last month (no. times)	0.0±0.0	0.0±0.0
Last MDMA use (months)	84.0±45.4	32.0±10.6
Hallucinogenic use (no. times)	20.0±0.0	10.4±3.4
Hallucinogenic use in the last month (no. times)	0.0±0.0	0.0±0.0
Last hallucinogenic use (months)	12.0±0.0	15.0±3.8
Cannabis use (years)	0.0±0.0	7.2±1.1
Lifetime joints (number)	5.5±1.5	7925.9±2253.7
Days of use in last month (number)	0.0±0.0	19.1±2.7
Joints in last month (number)	0.0±0.0	82.8±19.4
Cannabis age of onset (years)	17.8±0.3	17.0±0.9
Cannabis abstinence (h)		80.8±49.8
Cannabis withdrawal score (out of 32)		10.5±1.8
Cannabis craving scores		
Compulsivity (out of 21)		6.7±1.2
Emotionality (out of 21)		8.4±1
Expectancy (out of 21)		11.6±1.1
Purposefulness (out of 21)		11.6±1.2

Mean and SEM for control and cannabis groups on demographic and drug use history.

Learning and memory for faces task

The task used for the imaging procedure was adapted from that used by Zeineh et al. (2003) and modified[1] from experiment 1, into a face–number associative learning paradigm. The modified task structure involved button-press responses during recall so as to avoid possible head motion associated with verbal responses. It consisted of two runs of three blocks, with each block containing rest, learning, distraction and recall phases. The beginning of each block involved participants resting for 30 s. During the learning phase, participants were required to learn eight serially presented face–number pairs (presented for 3 s each). The numbers paired with each face were randomly selected from a set including 11, 12, 13, 14, 21, 22, 23 and 24. During recall, these numbers were selected using two handheld keypads (left keypad contained the 1

[1] An initial pilot study comparing the face–name and face–number versions of the task found identical learning and recall performance.

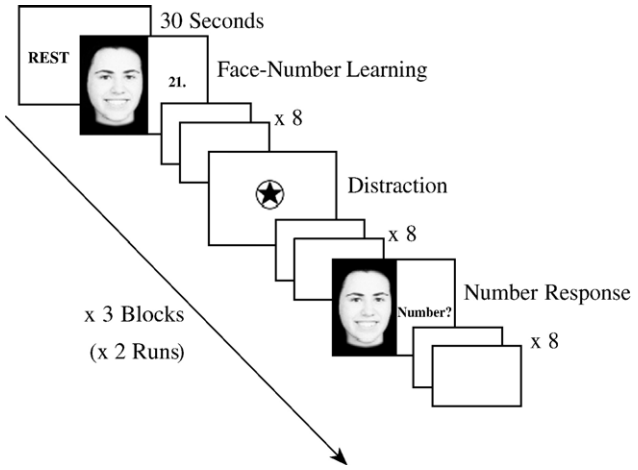

Fig. 5. Experiment 2. Face memory task administered during fMRI data collection. Participants were required to learn and recall face–number pairs over a series of 6 blocks.

and 2 keys, right keypad contained the 3 and 4 keys). Following the presentation of each face–number pair, a fixation crosshair was presented for a variable period of 1 to 7 s. The face–number association remained consistent throughout each learning phase, with each learning phase lasting 60 s. Eight distracter trials were presented between each learning and recall phase, lasting a total of 22 s. The distracter task required participants to press a key (the 3 key on the right hand-held keypad) each time the central visual display, an empty circle, contained a black star (see Fig. 5). The eight distracter trials were separated by delay intervals of 3 to 5 s. During each recall trial, participants were presented (in random order) with one of the eight 'learning phase' faces (for 3 s), and required to respond with the correct double digit number for each face. A fixation crosshair of variable duration (1 to 7 s) followed the presentation of each face. Each recall phase was 60 s in duration, with a different presentation order administered during each of the six recall blocks. Each run of the face–memory task lasted 570 s. Dependent measures for the behavioural task were the learning curve (percentage correct recall on each of the six recall trials) and percentage learning performance (Mean percentage of trials 1–6). The task was programmed using *E-Prime version 1.1* (Psychology Software Tools, Pittsburgh, USA).

Questionnaires

In addition to the National Adult Reading Test (NART) (Nelson and O'Connell, 1978), Beck Depression Inventory-II (Beck et al., 1996) and a drug and alcohol use questionnaire (McLellan et al., 1992) used in experiment 1, cannabis users also provided information concerning withdrawal and cannabis craving prior to scanning. The Marijuana Craving Questionnaire (Heishman et al., 2001) is made up of 12 statements, which the participant has to rate according to a seven-point Likert-type scale from "strongly disagree" to "strongly agree". Responses to the questionnaire are then divided into four specific constructs made up of *compulsivity*, *emotionality*, *expectancy* and *purposefulness* related to cannabis use. Information regarding withdrawal, modified from a cocaine withdrawal checklist (Brower et al., 1988) was obtained using a thirty two-item checklist, where participants were required to rate, on a scale of 0 (none) to 3 (severe), symptoms they had experienced in the previous 24 h.

fMRI acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror that reflected the visual display, which was projected onto a panel placed behind the participants' head outside the magnet. The mirror was mounted on the head coil in the participants' line of vision.

Each scanning sequence began with a reference scan to resolve sensitivity variations. A parallel Sensitivity Encoding (SENSE) approach (Pruessmann et al., 1999) with a reduction factor of 2 was utilised for all T1-weighted image acquisitions. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV 230 mm, thickness 0.9 mm, voxel size $0.9\times0.9\times0.9$) were then acquired (total duration 325 s), to allow subsequent activation localization and spatial normalization.

Functional data were collected using a T2* weighted echo-planar imaging sequence that acquired 32 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE = 35 ms, TR = 2000 ms, FOV 224 mm, 64×64 mm matrix size in Fourier space). The functional scans had a total duration of 570 s per run.

Data processing and analyses

All analyses were conducted using AFNI software (http://afni.nimh.nih.gov). Following image reconstruction, the two 3-D time series (runs 1 and 2) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was also removed using edge detection techniques.

A block analysis was performed to estimate the activation for the learning and recall periods separately. These ON-OFF block regressors were convolved with a standard haemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Multiple regression analyses were then used to determine the average level of block activation as a percentage change relative to the distraction period (baseline). The baseline activation was derived from averaging the distraction periods in each block over both runs of the task.

The percentage change map (block activation) voxels were re-sampled at 1 mm^3 resolution, then warped into standard Talairach space and spatially blurred with a 3-mm isotropic rms Gaussian kernel. Group activation maps for each condition of the task (learning and recall) were determined with one-sample *t*-tests against the null hypothesis of zero activation change (i.e., no change relative to the distraction-period baseline). Significant voxels passed a voxelwise statistical threshold ($t = 3.4$, $p < 0.005$) and were required to be part of a larger 278 μl cluster of contiguous significant voxels. Thresholding was determined through Monte Carlo simulations and resulted in a 5% probability of a cluster surviving due to chance.

To compare activations between the control and cannabis groups, thresholded group *t*-test maps for each condition in both groups were combined to form OR maps. For example, the selection OR map includes the significant voxels from either group. This process was performed independently for the learning and recall periods. The mean activation for clusters in the OR map was calculated for the purposes of a whole brain analysis, and these data were used for group independent t-tests.

We also performed a small-volume correction region of interest (ROI) analysis, given *a priori* interest in hippocampal involvement in this task. A second volume threshold was applied for voxels that fell within an anatomically defined medial temporal lobe region that included the hippocampus and parahippocampal gyrus. Significant voxels passed the same voxelwise statistical threshold

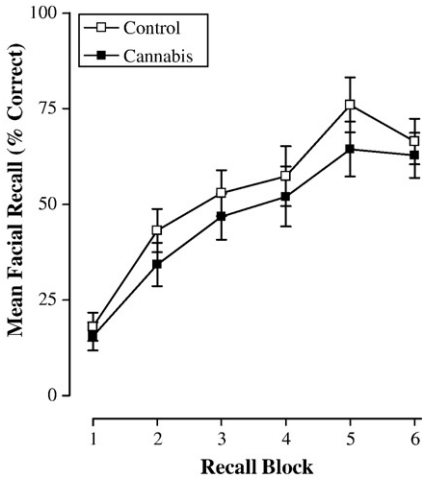

Fig. 6. Experiment 2. Mean percentage performance on trials 1–6 of the fMRI face memory task in controls and cannabis users (means and standard error means).

(t=3.4, p<0.005) and were required to be part of a 114-μl cluster of contiguous significant voxels.

All between-groups analyses of mean activation clusters were conducted using independent t-tests in SPSS (SPSS Inc).

Results

Demographics and drug use

Table 2 shows the group demographic and drug use history for both samples. The groups did not significantly differ on age, years of education, pre-morbid intelligence or alcohol and other drug use.

Memory performance

Fig. 6 shows the learning curves for the two groups over the 6 blocks of recall trials. A two (group) by six (trial) repeated measures analysis revealed an effect of trial (F=32.9, df=5,22, p<0.001), but no effect of group (F=0.78, df=1,26, p=0.39) and no trial by group interaction (F=0.4, df=5,22, p=0.85).

fMRI

Whole brain analyses. Table 3 lists the areas of significant activity during the learning and recall phases of the face–memory task. Fig. 7 also demonstrates the general patterns of activation in both the cannabis and controls groups during the learning phase of the face–memory task. Five regions were found to have passed the voxel and cluster-size threshold for the learning phase and included the right superior temporal gyrus (RSTG, BA 39), right superior frontal gyrus (RSFG, BA 6 and BA 9), left superior frontal gyrus (LSFG, BA 8), and right middle frontal gyrus (RMFG, BA 8). The cannabis group showed significantly lower levels of BOLD activity in the RSTG, BA 39 (p<0.01), RSFG, BA 6 (p<0.001), RSFG, BA 9 (p<0.01), RMFG, BA 8 (p<0.001) and LSFG, BA 8 (p<0.05) (see Figs. 8b and c). Areas significantly active across the control and cannabis groups for the recall phase were the right middle temporal gyrus (RMTG, BA 39) and left superior temporal gyrus (LSTG, BA 39). There were no group differences in BOLD activity during recall.

Region of interest (ROI) analyses. Table 3 also demonstrates the results of a small volume correction region of interest (ROI) analysis during the learning phase, which included the hippocampal and parahippocampal regions. Five areas were found to be significantly active, consisting of the right parahippocampal gyrus (RPHG, BA 27); left PHG (LPHG, BA 27); LPHG, BA 19; LPHG, BA 30 and the

Table 3
Experiment 2

	BA	HS	Vol (μl)	Centre of Mass			P	Direction of significance
				x	y	z		
Learning condition (whole brain analysis)								
Structure								
Superior temporal gyrus	39	R	2063	50.5	57.8	21.3	**	Ctrl>THC
Superior frontal gyrus	6	R	1561	14.4	−26.7	55.1	***	Ctrl>THC
Superior frontal gyrus	9	R	1226	16.3	−45.2	38.6	**	Ctrl>THC
Middle frontal gyrus	8	R	590	33.9	−25.6	44.8	***	Ctrl>THC
Superior frontal gyrus	8	L	479	−12.0	−39.4	48.1	*	Ctrl>THC
Recall condition (Whole brain analysis)								
Structure								
Middle temporal gyrus	39	R	1920	47.1	63.0	22.7	ns	
Superior temporal gyrus	39	L	581	−51.0	58.3	27.0	ns	
Learning condition (ROI analysis)								
Structure								
Parahippocampal gyrus	27	R	832	20.8	30.7	−6.4	ns	
Parahippocampal gyrus	27	L	741	−22.9	29.8	−6.8	ns	
Parahippocampal gyrus	19	L	351	−21.3	51.6	−5.0	ns	
Parahippocampal gyrus	30	L	201	−11.3	44.5	2.3	ns	
Parahippocampal gyrus	–	R	181	25.7	7.8	−23.3	*	THC>Ctrl
Parahippocampal gyrus	–	R	138	19.8	54.2	−4.5	ns	

Regions of activation during the learning and recall phases of the face memory task.
Shown are the regions for whole brain and small volume correction ROI analyses. Positive values for x, y and z Talairach coordinates denote, respectively, locations that are right, anterior and superior relative to the anterior commissure. Table abbreviations indicate: BA—Brodmann area; HS—hemisphere; Vol—activity cluster volume in microliters, Ctrl—control group, THC—cannabis group. Only positive activations are reported.

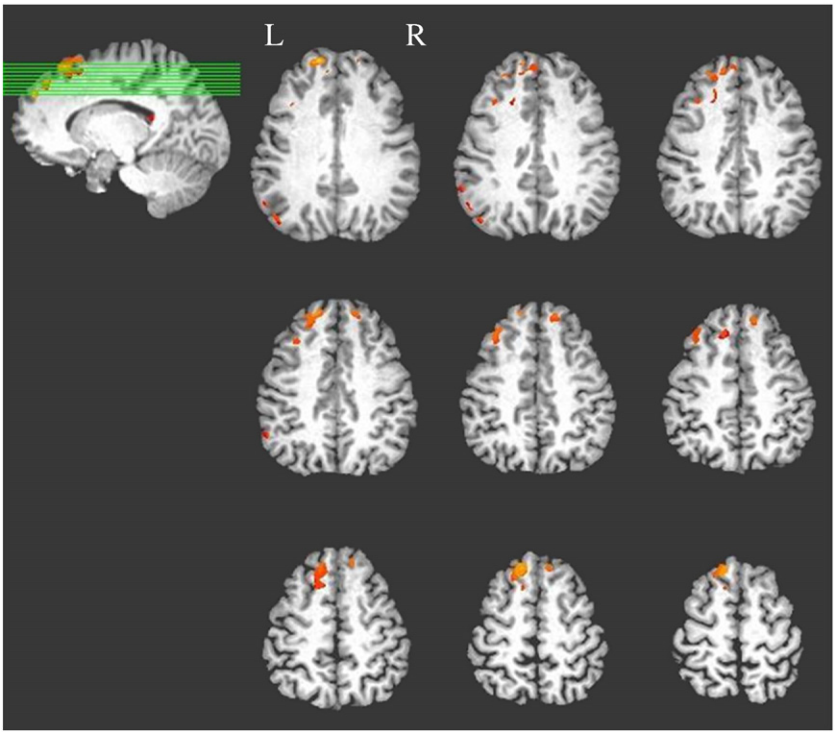

Fig. 7. Experiment 2. Activation *t*-test maps ($p=0.005$) showing horizontal sections during the learning phase of the face–memory task across the whole brain (left is left and right is right).

RPHG. A between-groups comparison indicated that the cannabis group had significantly greater activity in the right parahippocampal gyrus ($x=25$, $y=7$, $z=-23$) during the learning phase (see Fig. 8a), when compared to control participants. There were no significant clusters of activation during recall.

Performance and BOLD correlations

There were no correlations between BOLD activity (whole brain and ROI results) and task performance scores in either the control or cannabis groups.

Drug-use correlations

There were no correlations between cannabis abstinence or self-reported use of cannabis (e.g., years of use, lifetime joints, days of use in last month, joints in last month and age of use onset) and behavioural performance on the face–memory task in the cannabis-using group. Nor were there any associations between these measures and BOLD activations in the cannabis group.

Drug craving and withdrawal correlations

Fig. 9 shows two significant correlations. Cannabis withdrawal scores were positively associated with activity in LPHG, BA 19 during learning ($r=0.53$, $p=0.05$) and negatively associated with BOLD activity in the LSTG, BA 39 during recall ($r=-0.59$, $p<0.05$). Finally, we found no significant correlations between reported craving (compulsivity, emotionality, expectancy and purposefulness) and behavioural performance or BOLD activity.

Discussion

Experiment 2 investigated the effects of chronic cannabis use on learning and recall-related brain activity. In a sample of high functioning cannabis users, demographically well matched to a comparison control group, we found evidence of hypoactivity in the right superior temporal gyrus, right superior frontal gyrus, right middle frontal gyrus and left superior frontal gyrus during associative learning. These were observed in the absence of group differences in recall-related activity or recall performance. The lack of a performance effect may be due to the smaller sample size and lower statistical power of experiment 2: The absence of performance effects can be advantageous, however, enabling us to discount performance-related effects (e.g., error-related activity, frustration) from confounding the group comparison (Murphy and Garavan, 2004). Cortical hypoactivation in cannabis users has previously been argued to reflect the sub-acute effects of cannabis at the time of testing (Block et al., 2002). The present differences in BOLD activity do not appear to be influenced by cannabis intoxication at the time of testing the cannabis-using group. We also failed to observe any significant association between self-reported measures of use, such as the frequency (days) of use or number of joints in the month prior to testing, and either task performance or BOLD activity.

Animal studies suggest some memory-impairing effects of cannabinoids are the result of a dysfunction in the prefrontal cortex (Diana et al., 1998; Jentsch et al., 1997, 1998; Verrico et al., 2003), with human imaging studies also demonstrating prefrontal hypoactivity in cannabis users (Amen and Waugh, 1998; Yurgelun-Todd et al., 1999; Lundqvist et al., 2001; Block et al., 2002; Eldreth et al., 2004; Bolla et al., 2005; Pillay et al., 2004; Gruber and Yurgelun-Todd, 2005; Chang et al., 2006; Jager et al., 2007). Prefrontal areas are critical to working memory (WM) function (D'Esposito et al., 1995; 1999; Petrides, 2000; Blumenfeld and Ranganath, 2006), with the suggestion that the organization of learning is highly contingent upon WM processes (Tulving and Pearlstone, 1966; Alexander et al., 2003). Patients with LSFG lesions exhibit WM

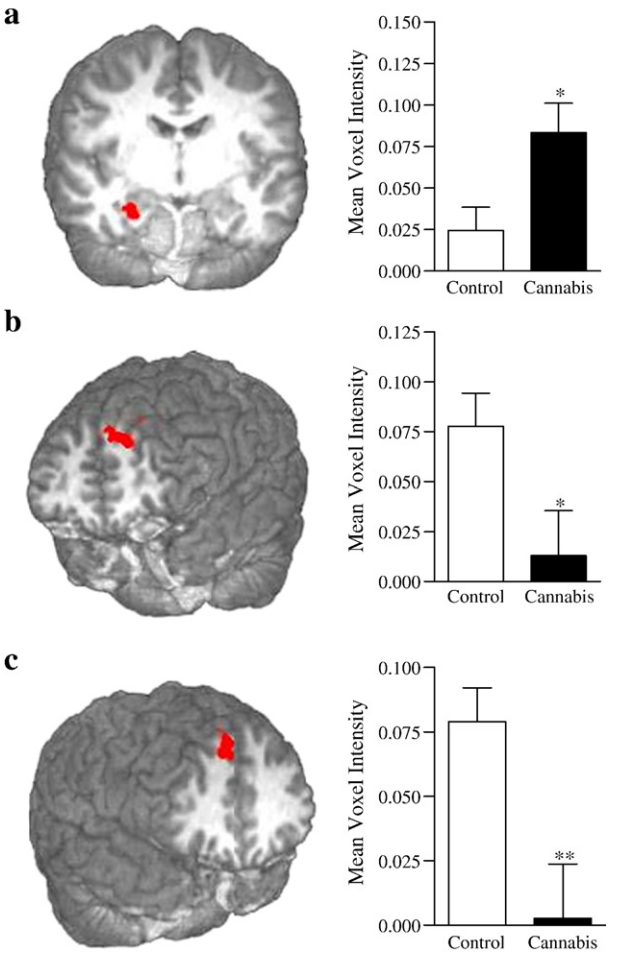

Fig. 8. Experiment 2. Mean brain activity (voxel intensity) in three brain regions where the cannabis and control groups significantly differed in BOLD activity during the learning phase of the face–memory task. (a) right parahippocampal gyrus ROI; (b) left superior frontal gyrus and Brodmann area 8; (c) right superior frontal gyrus and Brodmann area 9 (*$p<0.05$; **$p<0.01$).

deficits when compared with control groups (du Boisgueheneuc et al., 2006), and there is evidence of prefrontal hypoactivity in cannabis users during verbal and associative learning (Block et al., 2002; Jager et al., 2007). Recently, Hermann et al. (2007) showed that cannabis users have diminished N-acetylaspartate/total creatine (NAA/tCr) in the dorsolateral prefrontal cortex (DLPFC), suggestive of reduced cortical neuronal and axonal integrity. Our results showing reduced bilateral SFG activity during facial associative learning may, therefore, indicate a dysfunction related to WM processing, associated with reduced neuronal functioning in this area. These differences, however, may well have preceded cannabis use.

Cannabis users also demonstrated increased right parahippocampal gyrus activity during the learning phase, when compared to control participants. The face–memory associative learning task has previously been shown to selectively involve the hippocampal formation (Zeineh et al., 2003). The parahippocampal gyrus includes the entorhinal cortex, an area known to have extensive connections with the hippocampus and DG, and is thought to be involved in the translation of temporary hippocampal information storage during learning (Rolls, 2000). The behavioural effects of cannabis appear to be mediated by CB_1 receptors, which are expressed at especially high densities in the hippocampus proper and DG regions (Herkenham et al., 1991; Tsou et al., 1998). Our findings of parahippocampal hyperactivity are contrary to a recent study conducted by Jager and colleagues (2007), which showed that cannabis users demonstrated parahippocampal hypoactivity during an associative learning task. This inconsistency may reflect the differing recall demands of the two tasks; in the present study participants were required to recall the digits associated with a face, whereas Jager et al. required recognition of previous picture pairs.

Previous studies have found that greater activity in the parahippocampal region during learning predicts subsequent recall, suggesting a strong association between parahippocampal neuronal activity and memory encoding (Fernandez et al., 1998; Brewer et al., 1998; Wagner et al., 1998). Jansma et al. (2004) have suggested that hyperactivity may represent a greater 'neurophysiological' effort in order to maintain normal behavioural performance, which in this case, may account for the absence of significant recall deficits in the cannabis user group due to relative parahippocampal hyperactivity during learning. Previous research studies using other cognitive paradigms have also reported increased hippocampal and parahippocampal activity in cannabis users (Eldreth et al., 2004; Bolla et al., 2005). The frontal and temporal cortical hypoactivity and parahippocampal hyperactivity observed in the present study may, therefore,

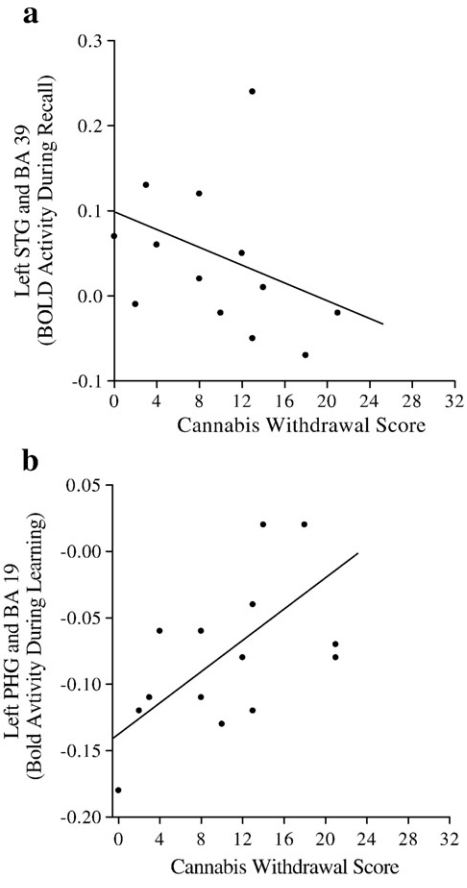

Fig. 9. Experiment 2. Correlations between (a) withdrawal score and LSTG, BA 39 BOLD activity during recall ($r=-0.59$, $p<0.05$) and (b) LPHG, BA 19 BOLD activity during learning ($r=0.53$, $p=0.05$).

suggest a neural compensatory mechanism, whereby the latter is compensating for the cannabis-related lack of prefrontal-mediated involvement in memory formation. Interestingly, different patterns of activity in other drug-using groups, such as cocaine users, have also been observed during fMRI paradigns, which are assumed to reflect a reliance on sub-optimal circuits (Hester and Garavan, 2004). This conclusion may be consistent with the behavioural results of experiment 1, during which a prefrontal deficit was identified as the likely source of poorer memory performance, and where the reliance upon compensatory neural circuits would prove behaviourally sub-optimal in a much larger sample of cannabis users.

Adolescent and adult users seeking treatment for cannabis dependence (Crowley et al., 1998; Budney, Novy and Hughes, 1999; Budney et al., 2003; Dawes et al., 2006) and frequent users not seeking treatment (Wiesbeck et al., 1996) have both been shown to experience withdrawal symptoms. In the present study, we assessed cannabis withdrawal prior to scanning using a questionnaire designed to elucidate potential physical, affective and behavioural withdrawal symptoms (Brower et al., 1988). The cannabis-using group in our study had a mean score of 10.5 (range=0–21) of reported withdrawal, which suggests that they were experiencing a low-moderate level of withdrawal during the testing session. Rating scores were also found to be positively correlated with LPHG activity during learning and negatively correlated with LSTG activity during recall. These results might suggest that the cannabis-using group was experiencing a level of withdrawal that affected memory-related brain activity, although there were no associations observed between withdrawal scores and cortical areas where group differences were present.

Experiment 1 of the current study demonstrated learning and memory deficits in a group of high functioning cannabis users on a task previously shown to engage activity within the hippocampal region. These behavioural findings taken from a sample of moderate cannabis users concur with the extant literature, which has shown deficits related to learning and memory, which may result from prefrontal and/or hippocampal impairment. Using a modified version of this task, sensitive to memory impairments, experiment 2 has demonstrated learning-related functional brain alterations in a cohort of equally high functioning cannabis-users with heavier use than those of experiment 1. Hypoactivity in frontal and temporal cortices, and relative hyperactivity in the parahippocampus during learning, may suggest discordant compensatory and adaptive functioning to overcome diminished activity in normal neural networks. These results may help reconcile learning and memory impairments in cannabis users, challenging the strongly held view that such deficits are (para) hippocampal in origin, with evidence to suggest that deficits in associative learning are also related to prefrontal dysfunction.

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