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ORIGINAL INVESTIGATION

Rivastigmine but not vardenafil reverses cannabis-induced impairment of verbal memory in healthy humans

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Abstract
Rationale One of the most often reported cognitive deficits of acute cannabis administration is an impaired recall of previously learned information.
Objective The aim of the present study was to determine whether cannabis-induced memory impairment in humans is mediated via glutamatergic or cholinergic pathways.
Methods Fifteen occasional cannabis users participated in a double-blind, placebo-controlled, six-way cross-over study. On separate test days, subjects received combinations of pretreatment (placebo, vardenafil 20 mg or rivastigmine 3 mg) and treatment (placebo or 1,376 mg cannabis/kg body weight). Cognitive tests were administered immediately after inhalation of treatment was finished and included measures of memory (visual verbal learning task, prospective memory test, Sternberg memory test), perceptual-motor control (critical tracking task), attention (divided attention task) and motor impulsivity (stop signal task).
Results The results of this study demonstrate that subjects under the influence of cannabis were impaired in all memory tasks, in critical tracking, divided attention and the stop signal task. Pretreatment with rivastigmine attenuated the effect of cannabis on delayed recall and showed a trend towards significance on immediate recall. When cannabis was given in combination with vardenafil, there were no significant interaction effects in any of the tasks.
Conclusions The present data therefore suggest that acetylcholine plays an important role in cannabis-induced memory impairment, whereas similar results for glutamate have not been demonstrated in this study.

Keywords Memory · Cannabis · Glutamate · Acetylcholine

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Introduction

The acute effects of cannabis on cognitive functions have been assessed in numerous experimental studies (Chait and Perry 1994; Curran et al. 2002; Hall and Solowij 1998; Ramaekers et al. 2009a). These studies have generally shown that delta-9-tetrahydrocannabinol (THC, the principal psychoactive constituent of cannabis), in doses between 40 and 300 μg/kg, causes a dose-dependent reduction in performance in laboratory tasks measuring memory, divided and sustained attention, reaction time, tracking, and motor function (Curran et al. 2002; Ramaekers et al. 2009a; Ranganathan and D'Souza 2006). Impaired learning and recall are among the most often reported cognitive deficits observed during cannabis intoxication. In particular, immediate recall, delayed recall and recognition of items learned while being under the influence of THC are impaired, whereas recall of items learned before cannabis administration is generally not affected while recalled in an intoxicated state. This suggests that THC specifically impairs acquisition and consolidation of information into memory, but not its retrieval from memory (Ameri 1999; Miller and Branconnier 1983).

Probable sites for the amnestic effect of cannabis are the hippocampus and the prefrontal cortex (Bhattacharyya et al. 2009; Bossong et al. 2012), structures which are highly

involved in various forms of learning and memory. Large amounts of cannabinoid receptors (CB1) and anandamide, the endogenous cannabinoid, have been found in the hippocampus and the prefrontal cortex, suggesting a functional role of CB1 receptors in learning and memory (Davies et al. 2002; Egerton et al. 2006). Animal studies have also shown that CB1 receptors in these structures are particularly abundant on presynaptic cholinergic, GABA-ergic, and glutamatergic terminals (Davies et al. 2002; Lichtman et al. 2002).

The supposed physiological substrate of learning and memory is long-term potentiation (LTP), which involves changes in the connections between neurons. The NMDA glutamate receptor has long been known to be involved in LTP (Bliss and Collingridge 1993), and to be important for the acquisition and consolidation of information (Riedel et al. 2003). By binding at the presynaptic CB1 receptors, cannabinoids decrease the release of glutamate and disrupt LTP (Murray et al. 2007). However, to this date, the role of glutamate in cannabis-induced memory impairment in humans has not been determined. A classic approach to explore this relationship would be to reverse the THC-induced memory deficit by using glutamate agonists. However, such studies have never been conducted due to the neurotoxicity associated with these compounds. To circumvent this problem, glutamate concentrations could be indirectly increased with a non-toxic selective phosphodiesterase type 5 inhibitor (PDE5i). PDE5i's are presently marketed for erectile dysfunction, but there is substantial evidence that these drugs also promote memory acquisition and consolidation in animals (Devan et al. 2007; Reneerkens et al. 2012; Uthayathas et al. 2013). PDE5 breaks down the cyclic nucleotide cGMP (Bender and Beavo 2006), which presynaptically stimulates the release of glutamate (Neitz et al. 2011). Thus, selective PDE5i's increase central levels of cGMP (Prickaerts et al. 2002; Riazi et al. 2006), and consequently glutamate (Uthayathas et al. 2007, 2013), and may thereby reverse the effects of THC on glutamate.

Hippocampal acetycholine also plays a role in information acquisition and LTP (Hasselmo 2006). For instance, scopolamine, which blocks the muscarinic cholinergic receptors, has been shown to impair acquisition but not retrieval of information (Atri et al. 2004). In addition, cholinesterase inhibitors have been shown to reverse memory impairment in participants treated with scopolamine, and in Alzheimer's patients (Dawson and Iversen 1993; Ebmeier et al. 1992). Cannabinoids also act on the cholinergic system, and in fact, cannabis-induced memory impairments resemble those of cholinergic antagonists. By binding at the presynaptic CB1 receptors located at the cholinergic nerve terminals, cannabinoids inhibit acetylcholine release (Kathmann et al. 2001). However, until now, the hypothesis that cannabis-induced memory impairment is related to a decrement in acetylcholine release has been difficult to prove. Nava et al. (2000), for instance, showed that in rats, the decrease in extracellular hippocampal acetylcholine concentrations was delayed compared to the timing of the memory effect of THC (Nava et al. 2000). In addition, Lichtman and Martin (1996) were unable to show that a cholinesterase inhibitor (physostigmine) counteracted THC-induced memory impairment in rats (Lichtman and Martin 1996). However, eptastigmine, a more potent and long-lasting second-generation cholinesterase inhibitor, was able to reverse the memory deficit in rats (Braida and Sala 2000; Giacobini 2004; Lichtman et al. 2002). In humans, the hypothesis that cannabis-induced memory impairment is caused by a reduction in acetylcholine release and can be reversed by co-administration of a cholinesterase inhibitor has not yet been tested.

The aim of the present study was to determine whether cannabis-induced memory impairment in humans is mediated via glutamatergic or cholinergic pathways. The role of acetylcholine was investigated using the second-generation cholinesterase inhibitor rivastigmine. The clinically approved non-toxic selective PDE5i vardenafil was used to indirectly increase levels of glutamate. In addition to measures of memory impairment, some other cognitive tests were included, which have previously shown to be sensitive to the impairing effect of THC (Ramaekers et al. 2006, 2009a). These tests were included to demonstrate whether a possible interaction of rivastigmine or vardenafil with cannabis is specific to memory. As previous studies with rivastigmine and vardenafil have only been able to improve memory in memory-impaired subjects (Giacobini 2004; Pepeu and Giovannini 2010; Reneerkens et al. 2013b), we hypothesized that the memory-improving effects of rivastigmine and vardenafil would only be evident when our participants were under the influence of cannabis, but not when they were not intoxicated.

Methods

The study was approved by the standing Medical Ethics Committee of Maastricht University and was carried out in compliance with the current revision of the Declaration of Helsinki (amended in 2008, Seoul) and the International Conference on Harmonization guidelines for Good Clinical Practice. A permit for obtaining, storing, and administering cannabis was obtained from the Dutch drug enforcement administration. All subjects gave written informed consent and received financial compensation for their participation.

Subjects

A total of 21 occasional users of cannabis were recruited via advertisements placed around Maastricht University and in local coffee shops. Participants were screened using a health questionnaire and underwent a medical examination

(including an electrocardiogram (ECG), haematology and blood chemistry, urinalysis, and drug and pregnancy screening). The following inclusion criteria applied to participants: occasional use of cannabis (minimal 1 year experience, with a minimum and maximum use of 8 and 36 times/year), free from psychotropic medication; good physical health, as determined by medical examination and laboratory analysis; absence of any major medical, endocrine and neurological conditions; body mass index (weight/length^2) between 18 and 28 kg/m^2; and written informed consent. Exclusion criteria were as follows: history of drug abuse (excluding cannabis), as assessed by drug urine screens and questionnaires; excessive drinking (>20 alcoholic consumptions/week); pregnancy or lactation or failure to use contraceptives; hypertension (diastolic >100; systolic >170); and history of psychiatric disorders.

Design and treatments

The study was conducted according to a double-blind, placebo-controlled, six-way cross-over design. Pretreatment consisted of placebo, vardenafil 20 mg (VAR) or rivastigmine 3 mg (RIV), which was administered orally in identical looking capsules. Doses of vardenafil 20 mg and rivastigmine 3 mg represent regular therapeutic doses, and both drugs reach a T_{max} of around 1 h (Bischoff 2004; Gottwald and Rozanski 1999). Treatment consisted of placebo or 1,376 mg cannabis/kg body weight (150 μg THC/kg body weight). On separate test days, the following combinations were given: placebo+placebo, placebo+cannabis, VAR+placebo, VAR+cannabis, RIV+placebo, and RIV+cannabis.

Medicinal cannabis, type Bedrobinol, was provided by the Dutch Bureau for Medicinal Cannabis. Bedrobinol contained 11 % THC and <1 % cannabidiol. Cannabis or placebo was heated using a Volcano vaporizer (Storz-Bickel, Tuttlingen, Germany). The vapour was trapped in a valve balloon. Subjects put the mouthpiece of the balloon to their lips and inhaled deeply, held their breath for 10 s, and then exhaled. The volume of the balloon was inhaled in 7 to 10 subsequent breaths and emptied within 5 to 10 min.

Procedure

Prior to the first test day, subjects were trained extensively in all cognitive tests, and in using the vaporizer, in order to familiarize them with all tests and minimize practice effects. Subjects were not allowed to use alcohol or caffeine on the test day or the day prior to testing. Smoking was prohibited for 30 min prior and during test days. Subjects were asked to arrive at the site well rested. On each test day, subjects were instructed to have a standard breakfast before coming to the site, and no other food was allowed until the end of the test day. Subjects were instructed to continue their cannabis use as normal but were requested to abstain from cannabis from about 5 days prior to the test day, to make sure they were negative on the test day. Drug and alcohol tests were performed upon arrival, using an alcohol breath test and urine drug screen (assessing the presence of morphine, cocaine, cannabis, methamphetamine and amphetamine). For those with a negative alcohol and drug screen, pretreatment was administered, and 45 min later, the inhalation procedure started. The cognitive tests began within 10 min after finishing the inhalation procedure and were administered in two parts. Part one included visual verbal learning, critical tracking, prospective memory and stop signal tasks. Part two consisted of the Sternberg memory and divided attention task. In between the two parts, subjects had a break, in which they stayed at the site and could watch TV, read, or use the internet. Four blood samples were taken during the test day: immediately, 1, 1.5 and 2 h after cannabis/placebo inhalation was finished. See Table 1 for an overview of the test day. Test days always started at the same time in the morning and were separated by a minimum wash-out period of 7 days to avoid cross-condition contamination.

Performance tests

Visual verbal learning task (VVLT) The VVLT is a modified version of the Rey Auditory Verbal Learning Test (Rey 1964), validated by (Klaassen et al. 2002) and used in many studies since (e.g. Linssen et al. 2014). Thirty Dutch mono-syllabic meaningful nouns and adjectives were presented one by one on a computer screen. The words in the lists had been matched for abstraction. Six different lists were used for the different test days.

Subjects had to verbally recall as many words as possible (immediate recall) at the end of the list presentation. This procedure was repeated three times; immediate recall scores

Table 1 Overview of the test day

Time (h)	Activity
Arrival	Drug and alcohol screens
−45 min	Pretreatment
0	Cannabis administration
10 min–1 hr 5 min	Blood sample 1
	Test battery 1 (VVLT-immediate recall, CTT, PMT, SST, VVLT-delayed recall)
	Blood sample 2
1 hr 5 min–1 hr 30 min	Break
1 hr 30 min–2 hr 10 min	Blood sample 3
	Test battery 2 (SMT, DAT)
	Blood sample 4
3 hr 50 min	End test day

VVLT visual verbal learning task, *CTT* critical tracking task, *PMT* prospective memory task, *SST* stop signal task, *SMT* Sternberg memory task, *DAT* divided attention task

were summed to comprise the total immediate recall score. After a 30-min delay, subjects were asked to recall as many of the previously learnt words as possible (delayed recall). Hereafter, subjects were given a delayed recognition task containing 15 new words and 15 words from the previously shown list. The subjects' task was to indicate as fast as possible whether the presented word was a new one, or one from the original list.

Dependent variables were the total immediate recall score, the delayed recall score, the delayed recognition score and reaction time (RT) for recognition.

Prospective memory task (PMT) The PMT was developed to examine prospective memory performance (Ramaekers et al. 2009b). The subjects were shown the letter A or B in the middle of a computer screen. Subjects were required to respond to each letter as quickly as possible by pressing one of two response buttons (go trials). Letters were displayed for 3 s and the interval before displaying the following letter was 0.5 s. The test consisted of 240 trials, in which each of the two letters was presented equally often. A counter in the left corner of the screen continuously indicated the trial number. A prospective memory signal which consisted of a visual cue, i.e. a future trial number, appeared at random in the right corner of the screen for 1 s in 30 trials during the test. These signals indicated that subjects were required to withhold any response during this future trial number (no-go trial). Prospective memory trials were always the 5th, 10th or 15th trial (i.e. memory delays of 30, 60 and 90 s) following the onset of the prospective memory signal. The memory set did not exceed three prospective signals at a time. Dependent variables were RT on go trials and the proportion of inhibited responses on prospective memory trials (no-go trials).

Sternberg memory task (SMT) The SMT measures speed and efficiency of working memory (Sternberg 1966). The task consisted of three blocks; in each block, a memory set was presented which subjects had to remember. The number of items in the memory set increased during the task; in the first block, the memory set comprised one letter, in the second block, two letters, and in the third block, four letters. Subsequently, subjects were shown 90 letters one by one in the middle of the computer screen, and each letter was presented for maximally 2 s. In half of the trials, a letter from the memory set was presented, while the other half consisted of other letters. After each letter, the participant had to indicate as quickly as possible whether the presented letter belonged to the memory set or not, by using one of two response buttons. Different target letters were used in the different memory sets and on different test days. Dependent performance variables were RT and accuracy (correctly remembered letters) for each individual block.

Stop signal task (SST) The SST measures motor impulsivity, which is defined as the inability to inhibit an activated or precued response, leading to errors of commission. The current test is adapted from an earlier version used by Fillmore et al. (2002) and has been validated for showing stimulant and sedative drug effects (Ramaekers and Kuypers 2006). The task required subjects to make quick responses to visual go signals and to inhibit their response if a subsequent visual stop signal, i.e. '*', appeared in one of the four corners of the screen. The go signals were four letters (A, B, C, D) presented one at a time in the centre of the computer screen. Letters were displayed for 500 ms and a 1.5-s inter-stimulus interval was used before the next letter was displayed. The test consisted of 176 trials in which each of the four letter stimuli were presented equally often. Stop signals were presented 12 times at each of the four delays after the onset of a letter: 50, 150, 250 and 350 ms. Dependent variables were go reaction time, stop reaction time, response accuracy, omission (not responding on go trials) and commission errors (not inhibiting a no-go trial). Stop reaction time represents the estimated mean time required to inhibit a response. Stop reaction time is calculated by subtracting the stop signal delay from the RT on go trials associated with *n*th percentile of the RT distribution (Logan 1994).

Critical tracking task (CTT) The CTT measures the subject's ability to control a displayed error signal in a first-order compensatory tracking task (Jex et al. 1966). Error is displayed as a horizontal deviation of a cursor from the midpoint on a horizontal, linear scale. Compensatory joystick movements null the error by returning the cursor to the midpoint. The subject's compensatory response increases in frequency with an increasing phase lag. Control is lost at the point at which the compensatory response lags the cursor's last movement by 180°. The response frequency at this point is defined as the critical frequency, or lambda_c. The test included five trials of which the lowest and the highest scores were removed. The average of the remaining scores was taken as the final lambda-c score.

Divided attention task (DAT) The DAT measures the ability to divide attention between two tasks performed simultaneously (Moskowitz 1973). The primary task consists of the tracking task described above. The difficulty of the primary task is set at a constant level of 50 % of the subjects' maximum capacity, as determined in the training session. Tracking error is measured as the difference in millimetre between the average position of the cursor and the middle of the horizontal scale. As a secondary task, the subject monitors 24 single digits in the corners of the computer screen. These numbers change asynchronously and subjects have to react to the target number '2' by removing their foot as fast as possible from a pedal switch. In total, 75 targets and 375 non-targets were presented.

Mean absolute tracking error (in millimetres) and number of control losses were the performance measures of the primary task. Number of correct detections and average RT (in milliseconds) were the dependent variables of the secondary task.

Subjective high

Using 100-mm visual analogue scales (VAS), subjects were asked to indicate how 'high' they were feeling, with 0 indicating 'not high at all' and 100 indicating 'extremely high'. Subjects were asked to indicate this at baseline, immediately after cannabis, and at 1, 1.5 and 2 h after cannabis.

Pharmacokinetics

Blood samples (8 ml) were taken immediately and 1, 1.5 and 2 h hours after cannabis/placebo inhalation. Blood samples were centrifuged and serum was frozen at −20 °C until analyses for pharmacokinetic assessments. Cannabinoid concentrations (THC with its metabolites OH-THC and THC-COOH) were determined using a validated and accredited routine method for the analysis of cannabinoids in forensic blood samples. The procedure essentially consists of an automated solid-phase extraction and gas chromatography with mass spectrometric detection with a limit of quantification of 1 ng/ml which has also been successfully used in previous studies (Toennes et al. 2008).

Vardenafil and rivastigmine were determined in serum after liquid-liquid extraction and analysis using liquid chromatography-tandem mass spectrometry with very low limits of quantification, covering the range of therapeutic concentrations (0.2–200 ng/ml for vardenafil and 0.02–100 ng/ml for rivastigmine). Analyses were performed by the Institute of Legal Medicine, Goethe University of Frankfurt (Germany).

Statistics

The hypotheses that pretreatment with vardenafil or rivastigmine would prevent cannabis-induced memory impairment was tested in two separate general linear model (GLM) repeated measures ANOVA analyses. Effects of cannabis, vardenafil and their interaction were analysed by GLM 1 with vardenafil (two levels: present and absent) and cannabis (two levels: present and absent) as the main factors. Effects of cannabis, rivastigmine, and their interaction were analysed by means of GLM 2 with rivastigmine (two levels: present and absent) and cannabis (two levels: present and absent) as the main factors. The alpha criterion significance level was set at $p=0.05$. When a significant interaction effect was found, additional drug contrasts with sequential Bonferroni correction were performed. Pharmacokinetic results were analysed with a repeated measures ANOVA, with condition (two levels, var/riv+placebo and var/riv+cannabis, or three levels, pla+cannabis, var+cannabis, and riv+cannabis) and time (four levels: samples 1–4) as a factor. In case of a violation of the sphericity assumption, Greenhouse-Geisser estimates of sphericity was used to correct the degrees of freedom. All statistical tests were conducted using SPSS version 15.0.

Results

Six subjects quitted the study prematurely, due to matters unrelated to the study (four subjects were not able to combine participation with their work or study, and two participants did not comply with the study restrictions). Therefore, data from 15 subjects (nine males and six females) entered statistical analysis (see Table 2 for a summary of the participant's demographics and drug use characteristics).

An overview of mean scores of all tests is given in Table 3. Tables 4 and 5 provide overviews of p and F values of the statistical tests.

Side effects

Six subjects reported feeling nauseated or dizzy in the RIV+cannabis condition. On one occasion, this occurred shortly after administration of pretreatment, and on four other occasions, this occurred within 15 min after treatment. On one

Table 2 Subject demographics (mean, SD) and drug use characteristics ($N=15$)

Variable	Mean (SD)	
Age (years)	21.23 (1.76)	
Weight (kg)	67.33 (8.75)	
Cannabis use (times)/year	19.1 (17.76)	
Joints per occasion	1.43 (1.1)	
Years of cannabis use	4.1 (1.8)	
Alcohol drinks a week	11.6 (6.8)	
No. of regular tobacco users	5	
	Mean frequency of use (SD)	No. of subject having used
XTC	5.88 (9.8)	8
Amphetamine	4	1
Cocaine	11.67 (11.6)	3
Mushrooms	2.33 (1.4)	6
Salvia	1	1
Psychoactive truffles	3	1

Table 3 Mean scores (±SE) on subjective measures and cognitive tasks per drug treatment

	PLA+PLA	VAR+PLA	RIV+PLA	PLA+CAN	VAR+CAN	RIV+CAN
Subjective high baseline (mm)	3.2±1.4	4.8±3.4	2.3±1.1	1.8±0.9	2.0±1.4	1.9±0.9
Subjective high 0 min	9.2±4.1	8.2±3.5	11.6±3.7	73.2±4.1	75.3±5.2	69.1±6.5
Subjective high 60 min	8.1±4.1	6.5±2.7	12.1±5.6	65.9±5.6	67.2±4.3	69.0±3.7
Subjective high 90 min.	5.8±3.0	4.9±2.3	9.3±4.3	55.1±6.2	51.1±5.5	55.7±6.2
Subjective high 120 min	3.9±2.2	3.5±1.6	3.7±2.0	34.6±6.8	33.3±5.8	31.7±5.1
VVLT immediate recall (no)	44.93±3.25	45.47±3.54	43.87±3.15	32.67±2.97	36.20±3.23	37.60±3.01
VVLT delayed recall (no)	15.00±1.52	15.20±1.54	14.07±1.59	10.47±1.07	12.13±1.48	12.47±1.42
VVLT RT recognition (ms)	739.19±28.21	717.90±61.03	788.15±33.13	813.59±43.21	875.44±43.37	696.35±81.77
VVLT recognition (no)	26.47±0.78	25.67±2.22	25.73±1.00	25.50±0.40	23.67±1.30	20.40±2.53
PMT RT (ms)	729.81±52.35	732.98±56.40	744.88±43.81	797.62±76.70	820.92±54.82	785.25±62.63
PMT inhibited responses (no)	25.13±1.68	22.20±1.88	24.00±1.90	18.64±1.65	16.33±1.98	16.53±2.14
SMT RT block 1 (ms)	431.10±20.91	434.08±18.48	418.19±22.52	435.78±40.88	497.00±24.12	446.62±42.06
SMT RT block 2 (ms)	464.11±20.51	477.63±23.36	459.57±22.03	477.68±42.68	513.48±18.19	472.02±41.65
SMT RT block 3 (ms)	520.23±23.87	530.020±19.95	524.02±23.37	550.23±53.30	590.49±26.67	526.79±45.71
SMT accuracy block 1 (no)	87.33±0.50	86.80±0.60	81.73±5.79	76.33±7.57	86.47±0.87	80.87±5.80
SMT accuracy block 2 (no)	86.80±0.72	86.33±0.65	81.87±5.67	74.47±7.01	85.53±0.61	78.40±5.83
SMT accuracy block 4 (no)	86.47±0.79	86.33±0.77	80.53±5.56	73.67±6.74	84.87±1.20	78.20±5.80
SST stop RT (ms)	299.60±15.39	301.07±19.68	293.47±18.04	324.71±22.95	350.67±27.79	336.80±29.65
SST go RT (ms)	617.96±34.08	617.±38.85	635.52±44.70	653.49±47.61	707.43±49.08	655.96±53.36
SST commission errors (no)	14.27±3.35	15.20±2.76	12.87±2.75	16.13±3.75	16.13±2.69	17.40±3.87
SST omission errors (no)	1.20±0.50	1.53±0.84	0.73±0.37	1.43±0.50	8.07±4.57	2.53±0.74
SST response accuracy (no)	118.87±2.49	121.47±1.26	119.67±1.34	115.00±2.29	106.13±5.99	112.73±3.83
CTT lambda$_c$ (rad/s)	3.36±0.12	3.38±0.12	3.30±0.11	2.81±0.14	2.87±0.16	3.01±0.18
DAT correct detections (no)	44.27±1.19	40.13±3.13	44.80±0.70	40.67±1.46	41.93±1.29	38.00±3.32
DAT control losses (no)	14.60±5.36	11.20±2.92	8.87±2.15	23.67±4.84	27.07±8.73	20.80±5.77
DAT tracking error (mm)	19.62±0.85	19.22±1.57	18.40±1.00	22.26±0.52	20.99±0.65	19.60±1.50
DAT RT (ms)	2062.1±81	1864.2±155	1968.3±64	1979.6±54	2124.1±85	1907.1±156

PLA placebo, *VAR* vardenafil, *RIV* rivastigmine, *CAN* cannabis, *VVLT* visual verbal learning task, *PMT* prospective memory task, *CTT* critical tracking task, *SST* stop signal task, *SMT* Sternberg memory task, *DAT* divided attention task, *RT* reaction time

occasion, it was reported more than 1 h after treatment. As a result of these side effects, three subjects had to lie down for a couple of minutes before resuming with the tests, and one subject decided to stop any further testing that day (see missing data).

Missing data

One subject was unable to continue testing in the RIV+cannabis condition, resulting in missing data for DAT, SMT, VVLT delayed recall and recognition. Due to technical malfunctioning, computer responses were not registered for one person in the cannabis condition (resulting in missing data for SMT, VVLT recognition, PMT and SST). In addition, computer malfunctioning caused missing data for the DAT in the vardenafil condition for one person, VVLT recognition in the vardenafil condition for a second person, and VVLT recognition in the RIV+cannabis condition for a third person.

Subjective high

Both GLM's showed a significant effect of cannabis on the subjective high VAS measured immediately and at 1, 1.5 and 2 h after cannabis.

Visual verbal learning task

The interaction between rivastigmine and cannabis was significant for delayed recall and showed a tendency towards significance for immediate recall. There was no interaction between vardenafil and cannabis on any of the VVLT measures.

Cannabis significantly decreased immediate recall and increased recognition reaction time in both the vardenafil and rivastigmine comparisons. The delayed recall and recognition scores showed a significant impairing effect of cannabis in the vardenafil GLM comparison, while the effect on these

Table 4 Summary of the results of the GLM1 analyses, with factors vardenafil (present-absent) and cannabis (present-absent)

	VAR			CAN			VAR × CAN			
	p	*F*	η^2	*p*	*F*	η^2	*p*	*F*	η^2	df
Subjective high baseline	ns	0.21	.015	ns	1.44	.093	ns	0.16	.011	1.14
Subjective high 0 min	ns	0.07	.005	**<.001**	114.43	.891	ns	0.32	.023	1.14
Subjective high 60 min	ns	0.00	.000	**<.001**	116.82	.893	ns	0.21	.015	1.14
Subjective high 90 min	ns	0.40	.028	**<.001**	76.88	.846	ns	0.19	.013	1.14
Subjective high 120 min	ns	0.03	.002	**<.001**	34.26	.710	ns	0.01	.001	1.14
VVLT immediate recall	ns	1.66	.106	**.002**	13.76	.496	ns	0.87	.058	1.14
VVLT delayed recall	ns	1.38	.090	**.012**	8.29	.372	ns	2.11	.131	1.14
VVLT RT recognition	ns	4.80	.286	**.001**	18.40	.605	ns	0.46	.037	1.12
VVLT recognition	ns	0.15	.012	**.014**	8.33	.410	ns	2.44	.169	1.12
PMT RT	ns	0.09	.007	**.007**	10.03	.435	ns	0.26	.020	1.13
PMT inhibited responses	**.035**	5.55	.299	**.000**	27.17	.676	ns	0.12	.009	1.13
SMT RT block 1	ns	1.44	.100	**.003**	12.78	.496	ns	2.74	.174	1.13
SMT RT block 2	ns	0.04	.035	**.014**	8.15	.385	ns	0.25	.019	1.13
SMT RT block 3	ns	0.05	.004	**.001**	17.78	.578	ns	0.00	.000	1.13
SST omission errors	ns	0.16	.012	ns	0.66	.049	ns	0.00	.000	1.13
SST go RT	ns	0.39	.029	**.03**	5.96	.314	ns	0.58	.043	1.13
SST response accuracy	ns	3.99	.134	**.007**	10.17	.439	ns	3.99	.235	1.13
CTT $lambda_c$	ns	.263	.018	**<.001**	21.87	.610	ns	.09	.006	1.14
DAT correct detections	ns	.028	.002	**.008**	9.99	.435	ns	1.64	.112	1.13
DAT tracking error	ns	1.15	.081	*.059*	4.28	.248	ns	3.61	.217	1.13
DAT RT	ns	0.14	.010	ns	0.14	.018	**.026**	6.36	.328	1.13

VAR vardenafil, *CAN* cannabis, η^2 partial eta-squared values, *df* degrees of freedom, *VVLT* visual verbal learning task, *PMT* prospective memory task, *CTT* critical tracking task, *SST* stop signal task, *SMT* Sternberg memory task, *DAT* divided attention task, *RT* reaction time, *ns* non-significant. Significant effects are marked in bold; trends towards significance are marked in italics

variables showed a tendency towards significance in the other. Vardenafil did not affect any measures of the VVLT.

Contrast analysis showed that compared to cannabis, RIV+ cannabis significantly increased immediate recall score ($F_{1,14}$=8.77; p<.012) and delayed recall score ($F_{1,13}$=15.47, p<.01). Cannabis decreased delayed recall score compared to placebo ($F_{1,13}$=11.55, p<.012), and both cannabis and RIV+ cannabis decreased immediate recall compared to placebo ($F_{1,14}$=14.29, p<.01; $F_{1,14}$=7.62, p<.016). Rivastigmine did not differ significantly from placebo or RIV+cannabis. Mean (SE) immediate and delayed recall scores are presented in Fig. 1.

Prospective memory task

There were no significant interactions between vardenafil or rivastimine and THC.

The number of correctly inhibited memory trials was significantly decreased by cannabis in both the vardenafil and rivastigmine comparisons, and by vardenafil. Reaction time was significantly increased by cannabis in the vardenafil comparison.

Sternberg memory test

The Sternberg memory test was analysed with an extra factor workload (memory set; three levels). No significant interactions between vardenafil or rivastigmine and cannabis were found on any of the variables of the SMT.

Both GLM analyses showed that reaction time was significantly increased by cannabis ($F_{1,13}$=18.21, p=.001 and $F_{1,12}$=10.48, p<.01) and by workload ($F_{2,26}$=43.37, p<.001 and $F_{2,24}$=36.99, p<.001). No interaction was found between cannabis and workload.

Stop signal task

No interactions between cannabis and vardenafil or rivastimine were found.

The accuracy on go trials was negatively affected by cannabis in both the vardenafil and rivastigmine comparisons. Cannabis

Table 5 Summary of the results of the GLM2 analyses, with factors rivastigmine (present-absent) and cannabis (present-absent)

	RIV			CAN			RIV×CAN			
	p	*F*	η^2	*p*	*F*	η^2	*p*	*F*	η^2	df
Subjective high baseline	ns	0.42	.029	ns	0.92	.062	ns	0.86	.058	1.14
Subjective high 0 min	ns	0.07	.005	**<.001**	77.81	.848	ns	1.50	.097	1.14
Subjective high 60 min	ns	0.81	.055	**<.001**	113.33	.890	ns	0.02	.001	1.14
Subjective high 90 min	ns	0.39	.027	**<.001**	64.57	.822	ns	0,15	.011	1.14
Subjective high 120 min	ns	0.29	.022	**<.001**	35.81	.734	ns	0.29	.022	1.14
VVLT immediate recall	ns	2.87	.170	**.003**	13.28	.487	*.063*	*4.10*	.226	1.14
VVLT delayed recall	ns	2.35	.153	*.08*	3.60	.217	**.002**	14.61	.529	1.13
VVLT RT recognition	ns	1.78	.139	**.007**	11.14	.503	ns	1.65	.131	1.11
VVLT recognition	ns	1.79	.140	*.061*	4.37	.284	ns	1.27	.104	1.11
PMT RT	ns	0.02	.002	ns	3.06	.190	ns	0.62	.046	1.13
PMT inhibited responses	ns	3.46	.210	**<.001**	23.72	.646	ns	0.44	.033	1.13
SMT RT block 1	ns	0,07	.006	**.011**	9.14	.432	ns	1.62	.119	1.12
SMT RT block 2	ns	0.26	.022	**.035**	5.62	.319	ns	0.02	.002	1.12
SMT RT block 3	ns	0.65	.051	**.004**	12.68	.514	ns	0.52	.041	1.12
SST omission errors	ns	1.36	..095	**.03**	5.97	.315	ns	2.90	.183	1.13
SST go RT	ns	0.00	.000	ns	1.33	.093	ns	0.73	.053	1.13
SST response accuracy	ns	1.02	.073	**.012**	8.47	.394	ns	1.04	.074	1.13
CTT $Lambda_c$	ns	0.78	.053	**.001**	16.12	.535	ns	2.62	.158	1.14
DAT correct detections	ns	0.02	.001	**.002**	15.91	.550	ns	0.05	.004	1.13
DAT tracking error	**.004**	12.30	.486	**.006**	10.78	.453	ns	0.02	.002	1.13
DAT RT	ns	0.03	.002	ns	0.00	.000	ns	2.30	.150	1.13

RIV rivastigmine, *CAN* cannabis, η^2 partial eta-squared values, *df* degrees of freedom, *VVLT* visual verbal learning task, *PMT* prospective memory task, *CTT* critical tracking task, *SST* stop signal task, *SMT* Sternberg memory task, *DAT* divided attention task, *RT* reaction time, *ns* non-significant. Significant effects are marked in bold; trends towards significance are marked in italics

also increased reaction time in the vardenafil comparison and the number of omission errors in the rivastigmine comparison.

Critical tracking task and divided attention task

An interaction between vardenafil and cannabis was found on the reaction time of the DAT. However, contrast analyses showed no significant differences with cannabis or placebo. No interactions between cannabis and rivastigmine were found for the CTT or DAT scores.

A significant effect of cannabis on CTT score was found in both vardenafil and rivastigmine comparisons, with cannabis decreasing CTT scores. In the DAT, cannabis almost significantly increased tracking error in the vardenafil comparison, and this effect reached significance in the rivastigmine comparison. Also, rivastigmine significantly decreased tracking error. The number of correct detections was also significantly decreased by cannabis in both comparisons.

Pharmacokinetics

For two subjects, blood samples for all conditions are missing; three subjects are each missing two samples; and, for one subject, there is one sample missing. Repeated measures analyses demonstrated that there was no significant effect of condition or time on concentration of vardenafil or rivastigmine. For THC concentrations, a significant effect of time was demonstrated ($F_{1.01,12,09}$=19.52, p=.001), but there was no significant effect between cannabis conditions. Mean (standard error (SE)) concentrations of THC and its metabolites in the three cannabis conditions are shown in Table 6.

Discussion and conclusions

The current study investigated the role of cholinergic and glutamatergic mechanisms involved in cannabis-induced memory impairment. Subjects were first given vardenafil or rivastigmine, followed by a single dose of cannabis or placebo. Subsequently, performance was

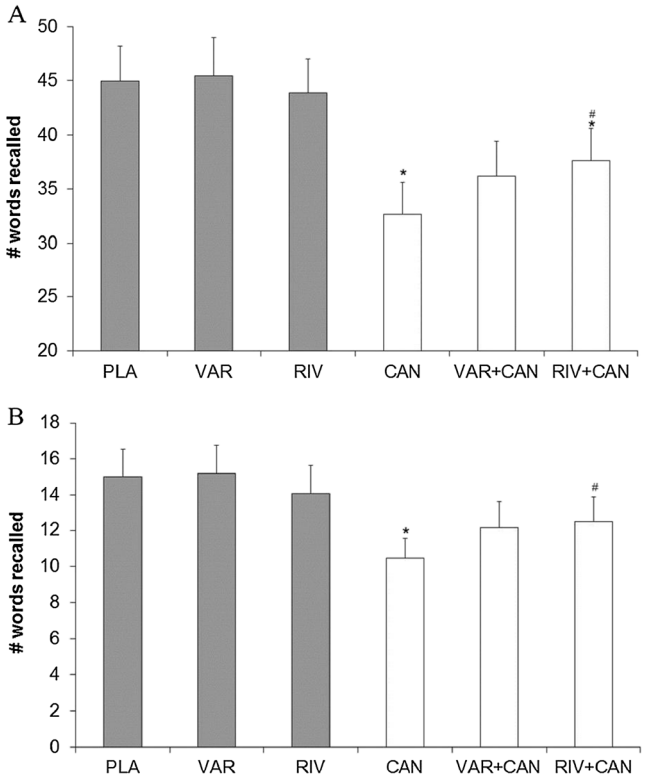

Fig. 1 Mean (SE) immediate (**a**) and delayed (**b**) recall score on the VVLT for each treatment condition. *Asterisk* indicates a significant difference from placebo (with sequential bonferroni correction); the *number sign* indicates a significant difference from THC (with sequential bonferroni correction); *VAR* vardenafil, *RIV* rivastigmine, *CAN* cannabis

measured on several memory tasks as well as on tasks tapping into other cognitive domains, in order to demonstrate specificity of the possible effects.

The results of this study demonstrate that subjects under the influence of cannabis were impaired in all memory tasks (Sternberg, VVLT and prospective memory). Specifically, cannabis increased reaction time in the Sternberg memory task and the VVLT recognition task, decreased immediate and delayed recall and recognition in the VVLT and the number of correct response inhibitions in the prospective memory task. In addition, cannabis also negatively affected performance in the critical tracking and divided attention tasks and in the stop signal task. These results are in accordance with previous studies demonstrating the impairing effects of cannabis on these tasks (Ramaekers et al. 2006, 2009a). In addition, the present results once more indicate that the impairing effects of cannabis on cognitive functions are found up until 2 h after administration of the drug; i.e. cannabis impaired performance on tests that were taken immediately after consuming cannabis, as well as tests that were taken 1.5 to 2 h later.

Pretreatment with rivastigmine showed a significant interaction with cannabis in the visual verbal memory task. When cannabis was given in combination with rivastigmine, delayed recall was less impaired. Separate drug-placebo contrasts confirmed that delayed recall after the rivastigmine+cannabis combination was significantly better compared to cannabis but was not significantly different from placebo. For immediate recall, the interaction effect between cannabis and rivastigmine showed a tendency towards an effect, with the combination of rivastigmine and cannabis apparently causing less impaired immediate recall than cannabis alone. When cannabis was given in combination with vardenafil, there were no significant interactions in any of the tasks.

In this study, we used the PDE5 inhibitor vardenafil, in an attempt to indirectly increase glutamate levels in the brain and to reverse the cannabis-induced memory impairment. Several studies with PDE5i's have previously demonstrated improved acquisition and consolidation in learning and memory tests, using predominantly rodents (Devan et al. 2007; Reneerkens et al. 2012). In humans, however, research on the cognitive effects of PDE5 inhibitors is scarce. One study investigating chronic dosing of the PDE5i udenafil showed improved executive function and performance on the mini-mental state examination in patients with erectile problems (Shim et al. 2011). However, studies using acute administration of vardenafil (Reneerkens et al. 2013b) or sildenafil (Schultheiss et al. 2001) have generally failed to show memory improving effects in healthy subjects. A possible explanation for this discrepancy between

Table 6 Mean (SD) concentrations of THC and its metabolites in serum (ng/ml) in the four samples taken during the day, for the three conditions in which THC was administered

Sample	Cannabis			VAR+cannabis			RIV+cannabis		
	THC	OH-THC	THC-COOH	THC	OH-THC	THC-COOH	THC	OH-THC	THC-COOH
1	30.7 (27.4)	3.3 (2.0)	17.2 (12.5)	22.8 (25.8)	4.9 (5.6)	19.5 (20.9)	30.5 (21.2)	3.4 (1.8)	14.1 (9.8)
2	6.3 (3.9)	2.2 (1.2)	17.3 (11.1)	4.2 (2.4)	1.9 (1.1)	17.9 (19.9)	4.5 (2.1)	1.9 (1.0)	15.4 (7.9)
3	4.3 (3.0)	1.9 (1.1)	17.6 (11.2)	3.3 (2.1)	1.8 (1.1)	18.0 (20.2)	3.2 (1.3)	1.5 (0.9)	16.2 (7.4)
4	2.5 (1.7)	1.3 (0.7)	14.7 (10.7)	2.1 (1.2)	1.4 (0.9)	16.4 (18.1)	1.9 (0.9)	1.3 (0.6)	13.8 (6.9)

VAR vardenafil, *RIV* rivastigmine, *OH-THC* 11-hydroxy-Δ^9-THC, *THC-COOH* 11-*nor*-9-carboxy-THC

animal and human effects of PDE5i's could be found in the translation in memory tests from animal to human studies. However, these tests are well established tests for studying human memory and should be sensitive enough to pick up any effects. Furthermore, the 20-mg dose of vardenafil used is comparable to effective doses in animals (Reneerkens et al. 2013a). In previous studies with healthy non-impaired humans, a ceiling effect might also have attenuated any improved cognition/memory performance. In the current study, we therefore hypothesized to demonstrate that cannabis-induced impairment of memory could be reversed by PDE5i's. Our results, however, show that acute treatment with vardenafil is also unable to improve cannabis-induced memory impairment in humans. This does not necessarily mean that glutamate is not involved in the cannabis-induced memory impairment, but rather that memory-improving effects of PDE5i's are difficult to demonstrate in humans.

Cholinesterase inhibitors, on the other hand, were previously found to be able to improve memory in impaired humans (Giacobini 2004; Pepeu and Giovannini 2010). In this study, memory-improving effects of rivastigmine alone were not demonstrated. This is most likely due to the fact that these were normal, young, healthy participants, who did not demonstrate memory impairments when not intoxicated. Cholinesterase inhibitors have also previously been demonstrated to reverse the cannabis-induced working memory impairment in rats (Braida and Sala 2000). Moreover, in the present study, we found some interaction of cannabis with rivastigmine, although this was only found for delayed recall of visually presented material. The other memory and cognitive tests did not show this interaction effect of cannabis and rivastigmine. In combination with previous results in rats, this is a strong indication that the acetylcholine system is, at least partly, involved in memory impairments seen after cannabis use. This is also in accordance with studies showing a reduced choline uptake in the hypothalamus and an inhibited synthesis of acetylcholine in rats after THC administration (Lindamood and Colasanti 1980). Some subjects experienced side effects when receiving the combination of rivastigmine and cannabis. These side effects may have caused subjects to perform below their maximal capacity. However, as all (except one) subjects were able to continue with the cognitive tests, we feel that these side effects had only minimal, if any, impact on the study results.

In conclusion, the present data suggest that acetylcholine plays an important role in cannabis-induced verbal memory impairment, whereas no experimental evidence was found for an involvement of glutamate.

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