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ORIGINAL INVESTIGATION

Acute subjective effects after smoking joints containing up to 69 mg Δ9-tetrahydrocannabinol in recreational users: a randomized, crossover clinical trial

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Abstract
Rationale An increase in the potency of the cannabis cigarettes has been observed over the past three decades.
Objectives In this study, we aimed to establish the impact of Δ9-tetrahydrocannabinol (THC) on the rating of subjective effects (intensity and duration of the effects), up to 23 % THC potency (69 mg THC) among recreational users.
Methods Recreational users ($N = 24$) smoked cannabis cigarettes with four doses of THC (placebo 29, 49 and 69 mg of THC) on four separate test days in a randomized, double-blind, placebo-controlled, crossover study. The participants filled in three different questionnaires measuring subjective effects during the exposure up to 8 h post-smoking. The 'high' feeling, heart rate, blood pressure and THC serum concentrations were also regularly recorded during these 8 h.
Results THC significantly increased the high feeling, dizziness, dry-mouthed feeling, palpitations, impaired memory and concentration, and 'down', 'sedated' and 'anxious' feelings. In addition, THC significantly decreased alertness, contentment and calmness. A cubic relationship was observed between 'feeling the drug' and 'wanting more'. The THC-induced decrease in 'feeling stimulated' and increase in anxiety lasted up to 8 h post-smoking. Sedation at 8 h post-smoking was increased by a factor of 5.7 with the highest THC dose, compared to the placebo.
Conclusions This study shows a strong effect of cannabis containing high percentages of THC on the rating of subjective effects. Regular users and forensic toxicologists should be aware that the THC-induced increase in 'feeling sedated' continues longer with a 69 mg THC dose than with a 29 mg THC dose.

Keywords Cannabis · THC · Subjective effects · Acute · High doses · Mood rating

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Introduction

The prevalence of lifetime and regular cannabis use has increased in most developed countries (EMCDDA 2012 and World Drug Report 2006). The estimated cumulative (lifetime) incidence of cannabis use has been estimated to be between 16 and 20 % in Western and Central Europe versus ~40% in the USA and New Zealand (Degenhardt et al. 2008). Cannabis is mainly used for recreational purposes and most often smoked. It is prepared from the plant Cannabis sativa and Δ9-tetrahydrocannabinol (THC) is its main psychoactive ingredient. European cannabis users preferably smoke cannabis cigarettes ('joints') made of a mixture of herbal cannabis and tobacco (including nicotine), whereas North America users prefer pure cannabis (Korf et al. 2004; EMCDDA

2004). Users commonly believe cannabis is an innocuous drug and look for effects such as euphoria, feeling of well-being and detachment, relaxation and altered perception of time.

Experimental human studies have previously been conducted to investigate the subjective effects observed after using cannabis cigarettes (Miller et al. 1983; Nemeth-Coslett et al. 1986; Heishman et al. 1989; Wachtel et al. 2002; Curran et al. 2002; Ramaekers et al. 2006; Metrik et al. 2011). These studies indicate that cannabis administration produces dose-related effects on several physiological and subjective variables (Nemeth-Coslett et al. 1986). Further, the psychoactive effects of cannabis in healthy volunteers are primarily due to THC (Wachtel et al. 2002), and the high feeling is a reliable index of intoxication with THC (Miller et al. 1983). Adverse effects have usually been studied by self-administered questionnaires on cannabis use (Johns 2001). A review of the psychiatric effects of cannabis mentions that panic attacks or anxiety were reported in 22 % of cannabis users from New Zealand aged 18–25 (Johns 2001; Thomas 1996). Many of these adverse effects were reported to be dose-related and generally short-lived (Johns 2001).

In these previous studies, however, the percentages of THC in cannabis cigarettes were lower than those of some cannabis cigarettes currently used. An increase in the potency of the cannabis over the past three decades has been observed (Cascini et al. 2012; Mehmedic et al. 2010; Trimbos Institute 2012), as well as a wider use of more potent forms of cannabis (Hall and Swift 2000). Nowadays, highly potent cannabis can be sold as 'sinsemilla', i.e. a seedless cultivated cannabis and/or 'netherweed', e.g. cannabis originating from Dutch grown hemp. The average levels of THC in netherweed cannabis sold in the Netherlands rose from 11.3 % in 2000/2001 to 20.4 % in 2003/2004 (Trimbos Institute 2012). The average THC content of netherweed in 2011 was 16.5 %. Previous studies on subjective effects of cannabis administered cigarettes of 2.8 % THC potency (total THC dose between 9 and 28 mg in Miller et al. 1983; Heishman et al. 1989; Chait et al. 1988; Wachtel et al. 2002; Metrik et al. 2011). The highest THC doses studied so far were up to 35–40 mg (Ramaekers et al. 2006 with administration of European cannabis cigarettes with 13% THC potency; Nemeth-Coslett et al. 1986 with administration of 1 g NIDA cannabis cigarettes with 4.99 % THC potency). It is unknown what the subjective effects among recreational users are after smoking European cannabis cigarettes with 16.5–20 % THC potency.

From a public health perspective, the major concern about the acute effects of cannabis is the possibility of accidents if users drive or operate machinery while intoxicated (Hall and Degenhardt 2009). In previous studies on this subject, psychomotor and subjective effects were usually examined up to 1–2 h after inhalation (Heishman et al. 1989; Chait et al. 1988; Metrik et al. 2011). Studies were also conducted with a longer observation of the subjects, up to 5–6 h post-smoking (Wachtel et al. 2002; Ramaekers et al. 2006). In the latter study, THC-induced effects lasted up to 5 h 15 min post-smoking (Ramaekers et al. 2006), but no study has investigated the subjective effects after smoking cannabis with a follow-up longer than this.

Dose-related differences in puffing and inhalation parameters, and large inter-subject variability in puff and inhalation volume, have been observed (Heishman et al. 1989). Individuals' stimulus expectancies significantly influence positive and negative affects (Metrik et al. 2011). Therefore, a study investigating the subjective effects of smoking cannabis should preferably have a crossover design in order to allow within-individual comparisons.

In the present study, we aimed to investigate the subjective effects among recreational users after smoking joints containing up to 23 % THC (69 mg THC). The study has been conducted with European joints, a follow-up of 8 h and a crossover design allowing within-individual comparisons.

Materials and methods

Design and participants

Twenty four recreational cannabis users (all males) aged 18–45 years participated in this study (Hunault et al. 2008, 2009). They were recruited through advertisements in local newspapers and were pre-selected on the basis of their average cannabis use. Inclusion criteria included experience with the use of cannabis (between 2 and 9 cannabis cigarettes/month) to exclude both novice users and users with considerable tolerance to effects of cannabis (Perez-Reyes et al. 1991); free from psychiatric disease; good health as determined by medical examination, an electrocardiogram and laboratory analyses (standard blood chemistry, haematology and drug screen tests); normal weight and body mass index; written informed consent. Exclusion criteria were: use of medications; excessive consumption of alcohol (more than 14 drinks/week) or history of drug abuse; any respiratory disease, liver disease or cardiovascular disease. The screening of the participants (including medical examination, medical history and substance use history) were performed in a standardised way. The study was carried out in accordance with the Declaration of Helsinki, approved by the Medical–Ethical Committee of the University Medical Center Utrecht, and all participants gave written informed consent.

Design and cannabis intake

A double-blind, crossover design was used to compare the effects of four different cannabis cigarettes smoked on four

different test days. Each 1 g cannabis cigarette contained 700 mg tobacco mixed with 300 mg cannabis. The Central Pharmacy Department of the University Medical Center Utrecht prepared the cannabis cigarettes in a standardised way, using three different medicinal cannabis batches containing 0.003, 9.75 and 23.12 % THC. The placebo batch was supplied by the National Institute on Drug Abuse (NIDA, USA) and the other batches were obtained from the Office for Medicinal Cannabis (Dutch Ministry of Health). The 'placebo' cannabis cigarettes contained 100% of the placebo batch; the low-dose cannabis cigarettes 100% of the 9.75 % batch; the middle-dose cannabis cigarettes 50% of the 9.75 % batch and 50% of the 23.12 % batch, and the high-dose cannabis cigarettes 100% of the 23.12 % batch. The corresponding dose levels per cigarette were 0 mg (placebo), 29.3 mg (low dose), 49.1 mg (middle dose) and 69.4 mg (high dose). Besides THC, cannabis may also contain cannabidiol (CBD) which is not psychoactive but can interfere with THC metabolism. The CBD concentration was less or equal to 0.36 % (0.32, 0.34 and 0.36 % in the low-, middle- and high-dose THC preparations, respectively).

The specific THC doses (29.3, 49.1 and 69.4 mg) were based upon a publication of the Trimbos Institute that reported systematic measurements of THC in cannabis cigarettes obtained from coffee shops in the Netherlands. For netherweed, an 'average' potency of 20.3 % and high concentrations up to 30% were reported in 2004 (Niesink et al. 2004). In the Netherlands, the majority of cannabis users smokes cannabis mixed with tobacco (Korf et al. 2004), which is the reason why a cannabis–tobacco mixture was chosen in this study.

The order of administration of cigarettes was balanced and participants were randomly allocated to treatment order. Participants smoked one of the four cannabis cigarettes on each testing day, with a 'washout' period of at least 1 week between treatments. Smoking started in the morning of test days. Subjects were instructed to smoke the cigarettes according to a computer-paced procedure (3 s for getting ready, 2 s for inhalation, 3 s for breath-holding and 32 s for normal breathing and relaxation). The whole cigarette was smoked in about 22 min.

Procedure

Participants were asked to abstain from any drugs (except alcohol) 15 days before and during the study period. Participants arrived the evening prior to each test day and stayed overnight in our unit. Urine drug screens were performed prior to experimental sessions upon arrival of the subjects. Drugs screen tests (DrugControl®) were used to assess the presence of amphetamines, barbiturates, benzodiazepines, cocaine metabolites, methaqualone, opiates, 3,4-methylenedioxy-*N*-methylamphetamine (MDMA or ecstasy), 3,4-methylenedioxyamphetamines (MDA) and THC in urine (cutoff level 50 ng/ml THC-COOH). Participants were immediately excluded if drugs other than cannabinoids were detected in their urines. Further, participants with a baseline THC serum concentration higher than the limit of quantification (LOQ) were excluded from the analyses since this indicates they had smoked a cannabis cigarette aside from the study. On each testing day, participants performed a series of tests and filled in questionnaires at fixed time points.

Visual analogue scales (VAS)

Figure 1 shows at which time points the different questionnaires were filled in. 'High' was taken every 5 min for the first half hour, then at 43 min, 50 min, 1.4 h, 2 h, 3 h, 3.5 h, 4 h, 4.5 h, 5 h, 6 h, 7 h and 8 h after the onset of smoking. Participants were asked to estimate their 'high' feeling on a 100-mm-long visual scale (anchored by *0* not at all and *100* tremendously high).

VAS_A was taken at baseline, 35 min, 65 min, 108 min, 4 h, 6 h and 8 h after onset of smoking. This nine-item VAS (Kirk et al. 1998; Curran et al. 2002; Wachtel et al. 2002) was used as a measure of acute bodily symptoms that are commonly associated with cannabis use (dizziness, dry mouth, feeling palpitations, stimulation, impaired memory, impaired concentration, down feeling, sedation and anxiety). Each scale is anchored with 'no symptom present' on the left side of the scale to 'severe symptom' on the right side of the scale.

VAS_B was taken at baseline, 108 min and 4 h after onset of smoking. This 16-item VAS (Bond and Lader 1974) was used to measure three mood factors: alertness, contentment and calmness.

VAS_C was taken only at 4 h post-smoking. This four-item VAS (Kirk et al. 1998; Curran et al. 2002) was used to measure participants' feeling about the cannabis cigarette they had smoked. The four items were: (1) the overall effects of the cannabis cigarette ('feel the drug', anchored as 'not at all' on the left side of the scale to 'a lot' on the right side); (2) how much they liked the effects of the cannabis cigarette ('like drug', anchored 'dislike a lot' to 'like a lot'); (3) how much they felt 'high' ('high/stoned', anchored 'not at all' to 'a lot'); and (4) whether they wanted more of the drug ('want more', anchored 'not at all' to 'very much').

Physiological variables

Heart rate and blood pressure were monitored with a Passport 2® monitor model (Datascope, USA). Both were measured every 5 min for the first half hour, then at 43 min, 50 min, 85 min 116 min, 3 h, 3 h 30 min, 4 h, 4 h 30 min, 5 h, 6 h, 7 h and 8 h after the onset of smoking. During the smoking of the cannabis cigarette, an upper limit for the heart rate was set at 170 bpm, and a lower limit for the mean arterial blood

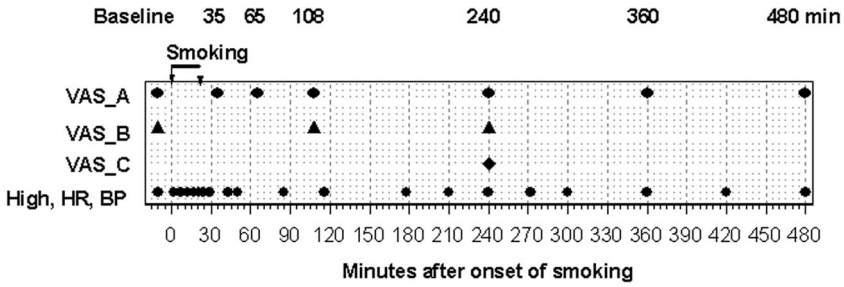

Fig. 1 Schematic diagram of the sequence of events during the experimental sessions

pressure was set at 55 mmHg to limit the health risks for participants. The subjects were instructed to stop smoking temporarily if they reached these levels.

Serum Δ9-THC levels

Venous blood samples were taken between 0 and 8 h post-drug from a cannule in the participant's forearm. Blood samples were taken at baseline and right after the onset of smoking (5 min) and every 5 min during the first half hour after smoking. From then on, blood samples (7.5 ml/time) were collected at 42 min, 55 min, 1 h 30 min, 2 h, 3 h, 5 h and 8 h. Samples were stored into Vacutainer® serum separator tubes (BD, USA), allowed to clot 0.5–2 h, then centrifuged and stored at −20 °C pending analysis. Serum concentrations of THC, 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) were determined using solid phase extraction (Kintz and Cirimele 1997; Gustafson et al. 2003) and liquid chromatography electrospray tandem mass spectrometry detection (Maralikova and Weinmann 2004). THC is metabolised into the active metabolite 11-OH-THC and inactive metabolite THC-COOH within a few minutes after the onset of cannabis smoking (Huestis et al. 1992). The limits of quantification (LOQ) were 0.5, 0.5 and 1.0 μg/l for THC, 11-OH-THC and THC-COOH, respectively. To reduce variation, all samples from each participant were analysed in the same batch.

Statistical analyses

The effect of THC dose on the rating of subjective effects was tested by mixed models analyses ('PROC MIXED' in SAS version 9.2) including dose (four levels), time (three or seven levels), the interaction terms dose × time and 'use of tobacco' (binary covariate) as fixed factors. Since observations were clustered within individuals, a random intercept per patient was used; residual correlation was modeled using an autoregressive (1) structure. Data were carefully inspected to determine which contrasts should be performed to compare the subjective effects between THC treatment conditions and placebo, and each of the THC dose condition to each other. In addition, analyses were performed to study differences in subjective effects using a contrast at the last time point measurement (240 or 480 min). Effects were considered significant when values were lower than or equal to 0.001 (instead of 0.05 usually, so as to compensate for multiple comparisons).

Results

Sample characteristics and smoking duration

A description of the participants is given in Table 1. Four, six, four and three participants for the placebo, 29.3, 49.1 and 69.4 mg THC doses, respectively, had a baseline THC serum concentration higher than the LOQ and were therefore excluded from the analyses. One participant with 8 out of 14 blood samples missing was also excluded from the analyses. The analyses finally include 20, 18, 20 and 20 participants for the placebo, 29.3, 49.1 and 69.4 mg THC cigarettes, respectively.

Table 1 Participants' demographics and drug use history

	N values
Total number of participants	24
Included in the placebo analyses	20
Included in the low THC dose analyses	18
Included in the middle THC dose analyses	20
Included in the high THC dose analyses	20
Race (Caucasian)	23
Previous use of netherweed	16
Cocaine exposure (occasional)	4
Ecstasy exposure (occasional)	1
	Mean (SD)
Age (years)	24.1 (4.0)
BMI (kilograms per square meter)	22.1 (1.7)
Past year cannabis use (number of joints, monthly)	7.7 (3.7)
Duration of cannabis use (years)	7.7 (4.2)
Past year number of tobacco cigarettes smoked daily[a]	8.4 (5.8)
Past year alcohol consumption (grams of ethanol per day)	19.4 (15)

[a] Among tobacco smokers (n = 18). Five participants did not smoke pure tobacco

The average duration of smoking was 19 min (SD 3.4), 21.6 min (SD 5), 23.1 min (SD 4.6) and 24.4 min (SD 4.4) for the 29.3, 49.1 and 69.4 mg THC doses, respectively. Some people were obliged to stop smoking temporarily because they reached the maximum heart rate limit or the minimum blood pressure limit: one person stopped with the low dose, four with the middle dose and three with the high dose. During these stops, the cannabis cigarette usually stopped burning and had to be lit up again when the participant restarted to smoke. Participants who transitorily stopped smoking did so after at least 4 min had elapsed (median time 14 min after onset of smoking, range 4–28 min).

First 2 h after the onset of smoking

The time required to reach the maximal highrating was slightly delayed (range 11–16 min) compared to the time required to reach the peak THC serum concentration (Fig. 2). High rating declined after the peak with a rapid phase within the first 3.5 h post-dose (Fig. 2). An overall significant effect of THC on high rating was observed (Table 2).

Scores on the VAS_A scale (dizziness, dry mouth, palpitations, impaired memory and concentration, down, sedated and anxious feelings) reached maximum within the two first hours post-dose (see Fig. 3 showing some of the variables). THC dose effect was significant (Table 2). In Table 2, the empty cells mean that no contrast was used to compare the THC conditions to placebo, nor between the different THC doses, when the interaction term THC dose × time was significant.

Relative to placebo, VAS_B scores decreased in the THC conditions at 108 min (Fig. 4). THC dose effect was significant (Table 2). The scores were lower after smoking cigarettes containing cannabis than after smoking a placebo cigarette. At 108 min after the onset of smoking, participants were much less alert, content and calm after having smoked the highest dose than after having smoked the placebo cigarette (e.g. on Fig. 4b, the contentment score decreases from 87 with the placebo to 62, using the high dose).

4 h post-dose

Significant differences in VAS_C scores were observed between THC doses at 4 h post-dose (Table 3). Scores of 'feel a drug effect' rose with increasing THC doses (Fig. 5a), with significant differences between THC treatment conditions relative to placebo, and between the high dose relative to the low dose (Table 3). Scores of 'like the drug' and 'want more of this drug' were significantly increased between THC treatment conditions and placebo (Table 3, Fig. 5b and c). A cubic relationship was observed between the rating of 'wanting more' and the rating of 'feeling more', as illustrated in Fig. 6 ($y = 0.0002x^3 - 0.0506x^2 + 3.0498x + 11.735$). Only three participants continued rating the item 'wanting more of the drug' higher while rating 'feeling the effect of the drug' higher.

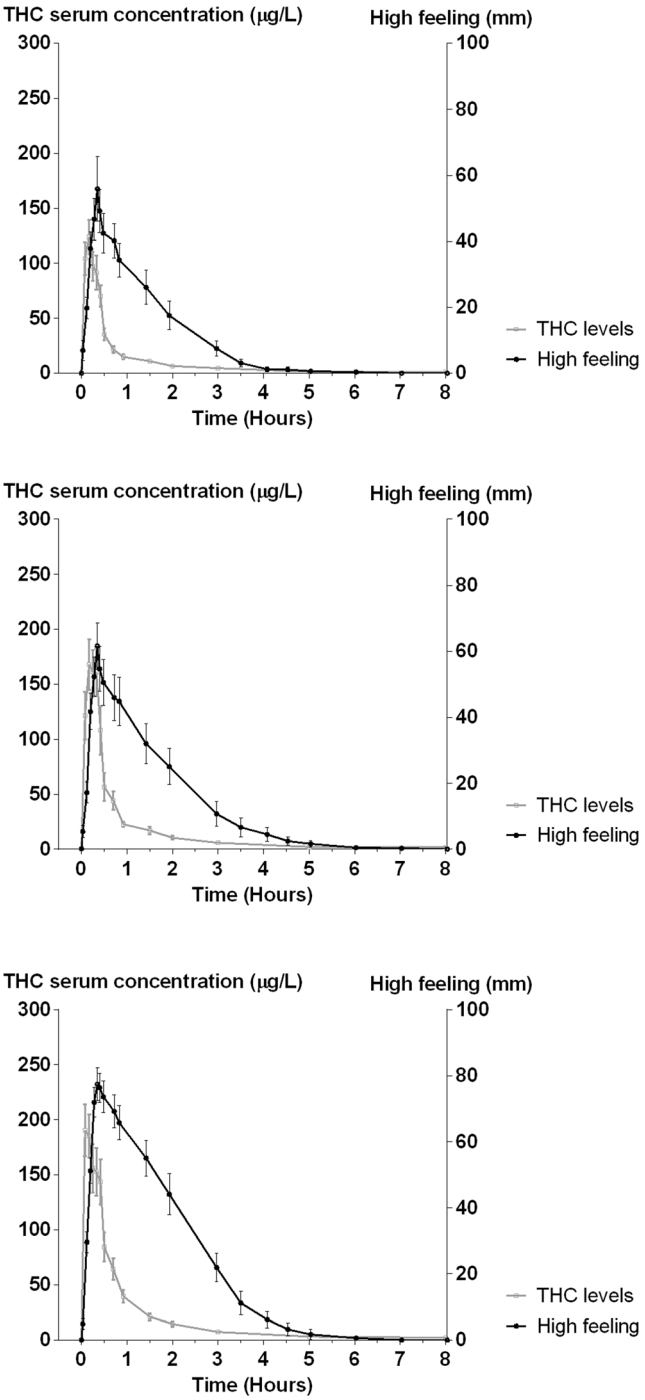

Fig. 2 THC concentration and rating of 'high' over time for the 29.3, 49.1 and 69.4 mg THC doses, respectively. Data represent the mean (SE) responses of 18, 20 and 20 participants, respectively

8 h post-dose

At 8 h post-smoking, 'high' and VAS_A measurements were available. THC-induced decrease in stimulation and increase

Table 2 Mixed effects models to test the effect of Δ9-tetrahydrocannabinol (THC) dose on the rating of subjective effects

	Overall *p* values		Contrasts					
	THC dose	Dose × time	L vs. P	M vs. P	H vs. P	M vs. L	H vs. L	H vs. M
'High'	<0.0001	<0.0001	–	–	–	–	–	–
VAS_A								
Dizziness	<0.0001	<0.0001	–	–	–	–	–	–
Dry mouth	<0.0001	<0.0001	–	–	–	–	–	–
Palpitations	<0.0001	<0.0001	–	–	–	–	–	–
Stimulated	<0.0001	0.19	0.002	<0.0001	<0.0001	0.017	0.014	0.92
Impaired Memory	<0.0001	<0.0001	–	–	–	–	–	–
Impaired Concentration	<0.0001	<0.0001	–	–	–	–	–	–
Down	<0.0001	0.0006	–	–	–	–	–	–
Sedated	<0.0001	<0.0001	–	–	–	–	–	–
Anxious	0.0003	0.12	0.28	0.035	<0.0001	0.27	0.0015	0.0195
VAS_B								
Alertness	<0.0001	<0.0001	–	–	–	–	–	–
Contentment	<0.0001	<0.0001	–	–	–	–	–	–
Calmness	0.0027	0.0003	–	–	–	–	–	–

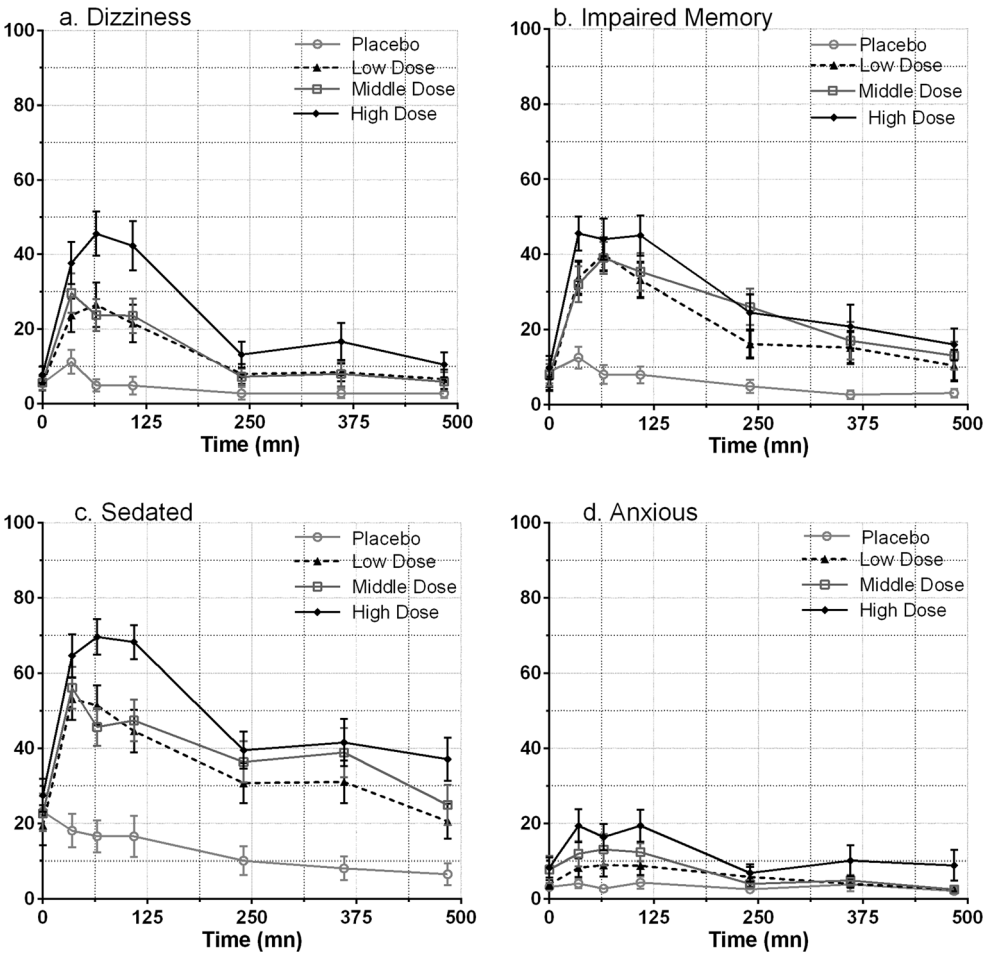

Fig. 3 Self-reported mean VAS_A scores of **a** dizziness, **b** impaired memory, **c** sedation and **d** anxiety over time for the 0, 29.3, 49.1 and 69.4 mg THC doses, respectively. Data represent the mean (SE) responses of 20, 18, 20 and 20 participants, respectively

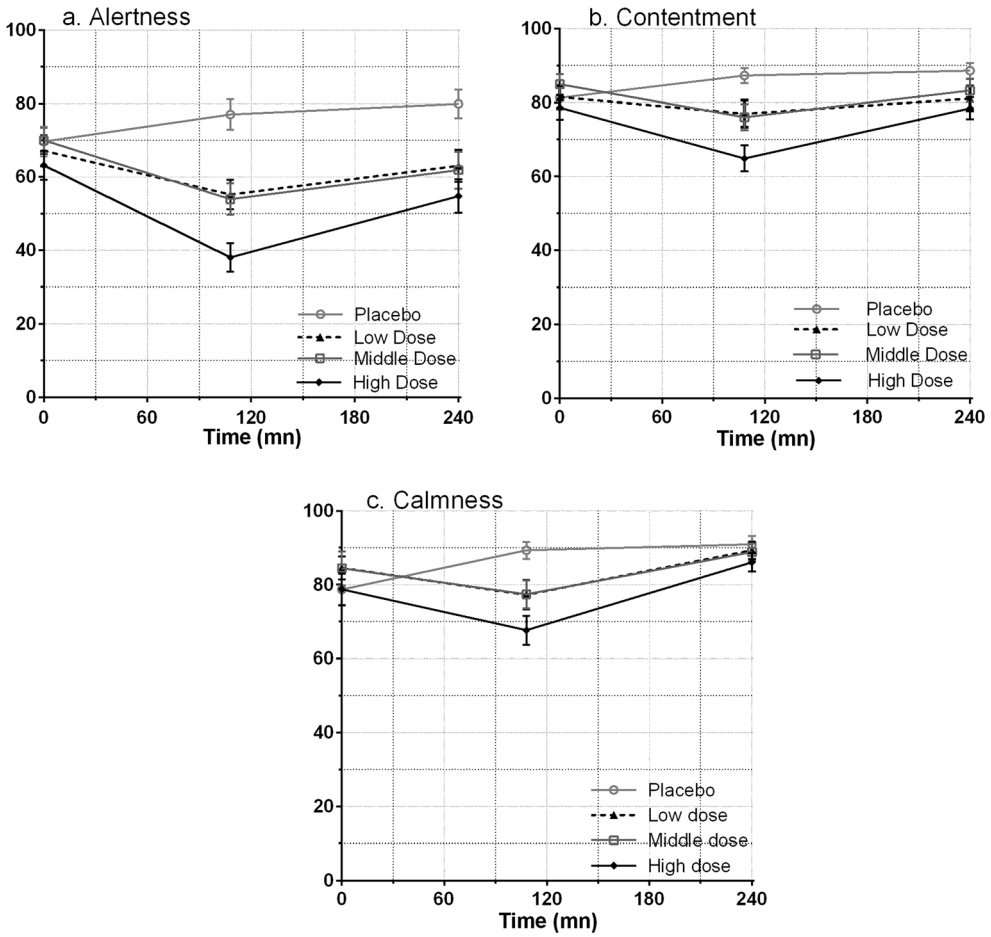

Fig. 4 Self-reported mean VAS_B scores of feeling **a** alert, **b** contented and **c** relaxed over time for the 0, 29.3, 49.1 and 69.4 mg THC doses, respectively. Data represent the mean (SE) responses of 20, 18, 20 and 20 participants, respectively

in anxiety lasted up to 8 h post-smoking as indicated by the absence of THC × time interaction (Table 2). The average rating of 'feeling sedated' was 37.1 mm with the high dose vs. 6.5 mm with the placebo (increase by a factor of 5.7; Fig. 3c).

Discussion

This study provides new and unique data about the extent and duration of acute subjective effects after smoking cigarettes containing tobacco and cannabis at THC doses up to 69 mg. Significant subjective effects were observed. Effects were most pronounced in the first 2 h post-smoking but a significant increase in sedation was still measurable 8 h after onset of smoking.

The observed subjective effects were most pronounced with the 69 mg THC dose. The maximum rating of high was reached within a few minutes in all THC treatment conditions but was 1.4 times higher with the high THC dose (69 mg) than with the low THC dose (29 mg). Besides, the rating of dizziness was doubled with the highest dose compared to middle and low doses (29 and 49 mg THC) up to 2 h post-smoking. Furthermore, 8 h after the onset of smoking, sedation was increased by a factor of 5.7 with the highest THC dose (69 mg) compared to placebo. The observed subjective effects were felt as unpleasant with the middle and high THC doses, relative to the low dose, as illustrated by the ratings of 'like the drug' and 'want more of the drug' which were highest with the low THC dose (29 mg). The cubic relationship observed between the items 'feel the effect of the drug and 'want more of the drug' reflects that the participants wanted more of the drug up to a certain point, but when they felt the effect of the drug was too much, they rated the item 'want more of the drug' lower.

The lack of significant interaction between dose and time after smoking for the items 'stimulated' (decreased) and 'anxious' (increased) suggests that these subjective effects induced by THC continued up to 8 h after the onset of smoking. This implicated that the observed subjective effects lasted longer in the present study than in previous studies (Nemeth-Coslett et al. 1986; Chait et al. 1988; Wachtel et al. 2002; Ramaekers et al. 2006; Metrik et al. 2011). The effects of cannabis are known to affect skills related to driving a vehicle or flying an aeroplane (Ashton 2001; Asbridge et al. 2012). The duration of this impairment in performance on driving simulator tasks and on open and closed driving courses has been estimated to last up to approximately 6 h (Ramaekers et al. 2006), but our

Table 3 Mixed effects models to test the effect of Δ9-tetrahydrocannabinol (THC) dose on the rating of subjective effects at the last time measurement

	Last time measurement (minutes)	Dose	L vs. P	M vs. P	H vs. P	M vs. L	H vs. L	H vs. M
High	480	1.0	–	–	–	–	–	–
VAS_A								
Dizziness	480	0.47	–	–	–	–	–	–
Dry mouth	480	0.0105	0.19	0.29	0.001	0.79	0.056	0.026
Palpitations	480	0.50	–	–	–	–	–	–
Stimulated	480	0.0104	0.18	0.048	0.001	0.55	0.057	0.18
Impaired memory	480	0.10	–	–	–	–	–	–
Impaired concentration	480	0.004	0.32	0.014	0.0008	0.15	0.021	0.36
Down	480	0.07	–	–	–	–	–	–
Sedated	480	<0.0001	0.007	0.001	<0.0001	0.63	0.0099	0.031
Anxious	480	0.14	–	–	–	–	–	–
VAS_B								
Alert	240	<0.0001	0.0004	<0.0001	<0.0001	0.22	0.20	0.96
Happy	240	0.055	–	–	–	–	–	–
Calm	240	0.72	–	–	–	–	–	–
VAS_C[a]								
Feeling a drug effect	240	<0.0001	<0.0001	<0.0001	<0.0001	0.42	0.0005	0.002
Liking the effects of the drug	240	0.0004	0.0001	0.0005	0.0005	0.51	0.48	0.98
Wanting more of this drug	240	<0.0001	<0.0001	<0.0001	0.0016	0.082	0.004	0.24

[a] VAS_C was taken only at 240-min post-smoking

results suggest that this impairment could, in fact, be longer. One possible explanation is the persistence of substantial blood THC concentrations up to several days after drug discontinuation. This phenomenon has been observed among heavy chronic cannabis users but can also occur among recreational users (Karschner et al. 2009).

The three active cigarettes used in the present study reflect plausible THC contents nowadays. THC doses higher than 35–40 mg THC have been administered in few studies before now. In one study (Nemeth-Coslett et al. 1986) investigating the effects of cannabis on subjective ratings and tobacco smoking, eight volunteers smoked 4.99% 1 g THC cigarette (total THC dose of 40 mg). However, the volunteers had to smoke tobacco ad lib 15 min following cannabis smoking so the follow-up time to observe the effects of cannabis was very short. A more recent study (Ramaekers et al. 2006) investigated the effects of smoking joints containing a THC-rich breed of cannabis, with a 5 h 15 min follow-up. The THC content was 13%, so that the total amount of THC consumed was 35 mg assuming an average body weight of 70 kg, whereas joints sold in coffee shops in the Netherlands often contain a higher THC dose. Around 1970, when cannabis use spread rapidly in the Netherlands, the average cannabis cigarette contained approximately 10 mg of THC. In 2004, systematic measurements of THC in netherweed revealed that the mean concentrations of THC had increased up to 20.3% in December 2003–January 2004 (NiesinK et al. 2004). In 2011, the average THC content of netherweed was 16.5 % and in 2012 the average THC percentage of the cannabis samples sold as 'most potent' was 16.9 %. Dutch joints with netherweed are made of 700 mg tobacco mixed with 300 mg cannabis, therefore they contained on average about 60.9 mg THC (300 × 20.3/100) in 2004 and 49.5 mg in 2011 (300 × 16.5/100). Because the effects of THC are dose-dependent, current cannabis users may experience more harmful effects than their predecessors.

Not all of the administered THC dose was actually delivered to the participant because of the percentage being lost in sidestream smoke and combustion. A study using a cigarette-smoking machine in a constant draft mode (i.e. the cigarettes were artificially inhaled in a single puff) estimated the total amount lost to pyrolysis to be less than 30% of the original THC content, with cigarettes containing pure cannabis (Perez-Reyes 1990). Further, the percentage lost in sidestream smoke has been estimated to be between 40 and 50 % of the original THC content of the cigarettes (Perez-Reyes 1990). In our study, the loss in sidestream smoke might be considerable given the controlled smoking protocol followed, with 32-s rest while the joint continued to burn, and an overall 22-min smoking time. The previous studies conducted to study the cannabis smoking dynamics have been performed using cigarettes containing pure cannabis. The dynamics of cigarettes containing cannabis mixed to tobacco is probably different. However, the loss related to sidestream would not impact our

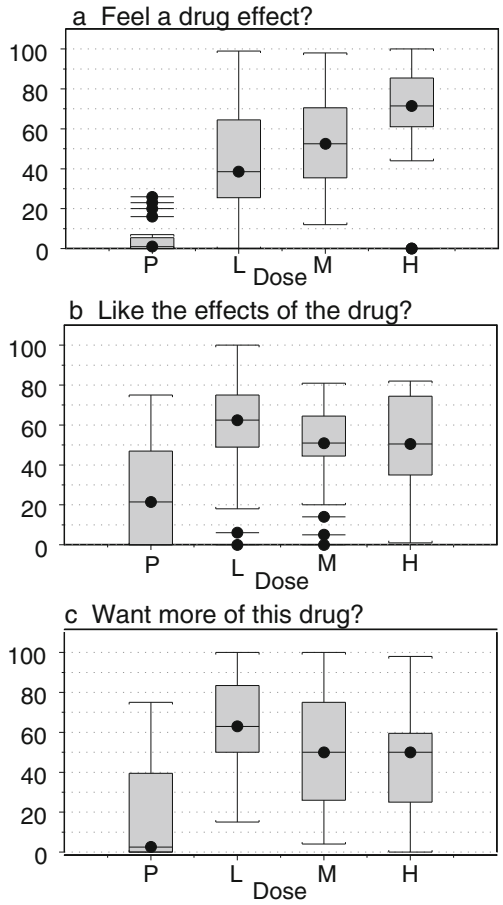

Fig. 5 Subjective effects of THC on VAS_C scores of **a** feeling a drug effect, **b** liking the effects, **c** wanting more of the drug at 4 h post-smoking for the 0, 29.3, 49.1 and 69.4 mg THC doses, respectively. Data represent the mean responses of 20, 18, 20 and 20 participants, respectively. The *boxplots* contain the following statistical measures: minimum, 1st quartile (*bottom of the box*), median (*dark line in the middle of the box*), 3rd quartile (*top of the box*) and maximum and outliers (*dots outside the box*, defined as values that do not fall in the inner fences)

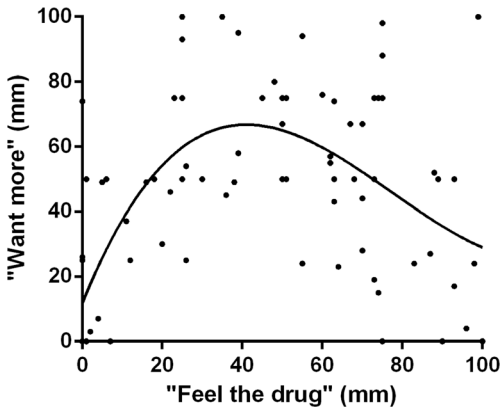

Fig. 6 Scatter plot with 'Feeling the drug' and 'Wanting more of the drug'. The plot contains 78 data points (20, 18, 20 and 20 subjects for the placebo, low, middle and high THC doses, respectively). The *line* represents the fitted cubic relationship between the two variables

findings since the THC doses administered in our study resulted in significant mean serum THC peaks (123.8 μg/l (SD 65.5), 168.0 μg/l (SD 100.3) and 190.4 μg/l (SD 106.8) for the 29.3-, 49.1- and 69.4-mg THC doses, respectively; Hunault et al. 2008).

Δ9-THC is considered to be largely responsible for psychological and physical effects (Ashton 2001). THC binds to cannabinoid receptors and interferes with important endogenous cannabinoid neurotransmitter systems. THC has been reported to increase the release of dopamine in the nucleus accumbens and prefrontal cortex (Tanda et al. 1997; Ameri 1999). The subjective effects may be linked to the density of cannabinoid/anandamide receptors in limbic, cerebellar and related areas of the brain. The first metabolite of THC, 11-OH-THC is also psychoactive and could also contribute to the psychological and physical effects observed. However, 11-OH-THC is rapidly metabolised to THC-COOH (psycho-inactive) so the effects were probably mainly due to THC. The effect of THC may be affected by another cannabinoid present in the cannabis plant, cannabidiol (CBD), which is not psychoactive. According to a recent study in rats, CBD can potentiate the psychoactive and physiological effects of THC, most likely by delaying the metabolism and elimination of THC through an action on the CYP450 enzymes that metabolise both drugs (Klein et al. 2011). Very low percentages of cannabidiol were present in the joints in our study, so effects cannot be related to this.

Our study has some limitations as only VAS_A and HIGH were measured up to 8 h post-smoking and not all VAS were measured at the same time points. In particular, the first measurement of the VAS_B was at 108 min post-smoking. The peak effects on alertness, contentment and calmness may have been missed and therefore limit the interpretation of dose-related effects for these items.

Secondly, participants were placed in an artificial experimental situation with a standardised smoking pace and were obliged to smoke the whole joint and to perform different tasks. In real life, participants may not go so far and would possibly stop smoking earlier. Regular, experienced users are presumably able to titrate their own dose and 1–3 'hits' of high potency sinsemilla or netherweed would be enough to produce the desired effects (a single intake of smoke from a joint is called a hit; NHTSA 2004).

A further issue that could be seen as a limitation is the inclusion of tobacco in the cannabis cigarettes as opposed to pure cannabis as in previous studies from the USA. In this study, we wanted to assess the effects of cannabis cigarettes with high THC content similar to the cigarettes currently available on the Dutch market. In Europe, cannabis is usually smoked in a mixture with tobacco contrary to the USA where cannabis is commonly smoked in pure form (EMCDDA 2004). Recreational European users commonly mix cannabis with tobacco in order to increase the burning efficiency of the cigarette and to reduce the overall costs of the cigarette (van

der Kooy et al. 2009). Interaction between nicotine and THC has been reported in mice which were exposed to 5−10 mg/kg of THC over 5 consecutive days (Valjent et al. 2002 Jan). The authors of this study observed that nicotine increased the latency of response in a tail immersion test and a hot plate test. Another study using a small-scale smoking machine determined that mixing cannabis with tobacco increases the vaporisation efficiency of THC (van der Kooy et al. 2009). The amount of THC inhaled rose from 32.70 ± 2.29 mg/g for a 100 % cannabis cigarette to 58.90 ± 2.30 mg/g for a 25 % cannabis cigarette. In our study, nicotine may have strengthened the behavioral and physiological effects of THC.

In summary, a significant THC effect was observed on the rating of subjective effects. Regular users and forensic toxicologists should be aware that the THC-induced decrease in stimulation is larger and continues longer with a THC dose equal to 69 mg.

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Conflict of interest None

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