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Cognition and motor control as a function of Δ^9-THC concentration in serum and oral fluid: Limits of impairment

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Abstract

Cannabis use has been associated with increased risk of becoming involved in traffic accidents; however, the relation between THC concentration and driver impairment is relatively obscure. The present study was designed to define performance impairment as a function of THC in serum and oral fluid in order to provide a scientific framework to the development of per se limits for driving under the influence of cannabis. Twenty recreational users of cannabis participated in a double-blind, placebo-controlled, three-way cross-over study. Subjects were administered single doses of 0, 250 and 500 μg/kg THC by smoking. Performance tests measuring skills related to driving were conducted at regular intervals between 15 min and 6 h post smoking and included measures of perceptual-motor control (Critical tracking task), motor impulsivity (Stop signal task) and cognitive function (Tower of London). Blood and oral fluid were collected throughout testing. Results showed a strong and linear relation between THC in serum and oral fluid. Linear relations between magnitude of performance impairment and THC in oral fluid and serum, however, were low. A more promising way to define threshold levels of impairment was found by comparing the proportion of observations showing impairment or no impairment as a function of THC concentration. The proportion of observations showing impairment progressively increased as a function of serum THC in every task. Binomial tests showed an initial and significant shift toward impairment in the Critical tracking task for serum THC concentrations between 2 and 5 ng/ml. At concentrations between 5 and 10 ng/ml approximately 75–90% of the observations were indicative of significant impairment in every performance test. At THC concentrations >30 ng/ml the proportion of observations indicative of significant impairment increased to a full 100% in every performance tests. It is concluded that serum THC concentrations between 2 and 5 ng/ml establish the lower and upper range of a THC limit for impairment.

Keywords: Cannabis; Serum; Oral fluid; Impairment; Driving; THC threshold; Forensics

1. Introduction

The acute effects of Δ^9-tetrahydrocannabinol (THC) on isolated cognitive functions and psychomotor skills have been repeatedly assessed in experimental studies employing within subject, double-blind, placebo-controlled designs. These have generally shown that THC in doses between 40 and 300 μg/kg causes a dose-dependant reduction in performance on laboratory tasks measuring memory, divided and sustained attention, reaction time, tracking and motor function (Ameri, 1999; Curran et al., 2002; D'Souza et al., 2004; Hall and Solowij, 1998; Hampson and Deadwyler, 1999; Leweke et al., 1998; Lichtman et al., 2002; Ramaekers et al., 2004). Performance impairment after THC was usually highest during the first hour after smoking and declined to baseline over 3–4 h after THC use. From a public health perspective, a major concern about the acute effects of cannabis is the possibility of accidents if users drive or operate machinery while intoxicated (Hall, 2001). Cannabis induced impairment of driving has been demonstrated in on-the-road driving tests (Lamers and Ramaekers, 2001; Ramaekers et al., 2000; Robbe, 1994). The effects of cannabis on driving increased with dose and were larger and more persis-

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tent in driving skills requiring sustained attention. The degree of performance impairment observed after doses up to 300 μg/kg THC were equivalent to the impairing effect of an alcohol dose producing a blood alcohol concentration (BAC) ≥0.05 g/dl, the legal limit for driving under the influence in most European countries.

There is an increasing concern across EU and US member states regarding the connection between cannabis use and road traffic accidents. From a legal point of view there is a great challenge of measurability and accuracy of interpretation because the association between levels of THC and crash risk is not fully understood. In virtually all western countries the policy regarding driving under the influence of cannabis is in whole or in part based on the detection of any amount of THC, the pharmacologically most active ingredient of cannabis, or even its inactive metabolite THC–COOH in blood or urine of the driver. Law enforcement and policy makers often call for the adoption of per se laws with a zero limit for THC or any of its metabolites. Such 'zero-tolerance' laws are already in place in several US states and in other countries, such as Germany. Yet, there is little scientific evidence to show that detection of THC or THC–COOH in bodily fluids can be taken as proof of impairment in any circumstance. For example, THC or its metabolite can be detected in bodily fluids for days after smoking and may thus indicate past use rather than impairment. Moreover, impairment and crash risk after recent cannabis use has been shown to increase as a function of dose; little impairment is apparent at low doses whereas serious impairment develops at high doses (Drummer et al., 2004; Menetrey et al., 2005; Ramaekers et al., 2004). Thus, a body fluid sample in a collision-involved driver that is positive for THC merely indicates that the driver is a cannabis user. Plasma concentrations of THC have been shown to vary widely between 1 and 35 ng/ml in drivers suspected of driving under the influence (Augsburger et al., 2005) and between 1 and 100 ng/ml in fatally injured drivers (Drummer et al., 2004). How varying THC levels in plasma relate to driver behavior is presently unknown but can be simulated in experimental performance studies of the pharmacodynamics and pharmacokinetics of cannabis intoxication. Such studies will contribute to a science-based foundation for government policies and law enforcement practices on cannabis and driving in analogy to the system developed for alcohol.

The present study was designed to assess the effects of a medium (i.e. 250 μg/kg) and high dose (i.e. 500 μg/kg) of THC on skills related to driving throughout 6 h post smoking. Skills related to driving were assessed at regular intervals using laboratory tasks measuring perceptual-motor control (Tracking task), motor impulsivity (Stop signal task) and cognitive function (Tower of London). Likewise, blood and oral fluid were regularly collected in order to determine the pharmacokinetic profile of THC and its main metabolites throughout performance testing. The main goals were (1) to assess the association between cannabis induced performance impairment and THC concentrations in serum and oral fluid; (2) to assess the correlation between THC concentrations in serum and oral fluid; and (3) to determine threshold THC levels in serum at which performance impairment emerges.

2. Methods

2.1. Subjects

Twenty recreational cannabis users (14 males–6 females) aged 19–29 years participated in the study. Initial screening included a questionnaire on medical history. Subjects who were pre-selected were examined by the medical supervisor who also checked vital signs and collected blood and urine samples. Standard blood chemistry, haematology and drug screen tests were conducted on these samples. Inclusion criteria were experience with the use of cannabis (at least five times in the previous 12 month); free from psychotropic medication; good physical health as determined by medical examination and laboratory analysis; absence of any major medical, endocrine and neurological condition; normal weight; body mass index (weight/length^2) between 18 and 28 kg/m^2; and written informed consent. Exclusion criteria were history of drug abuse (including daily use of cannabis) or addiction; pregnancy or lactation; excessive drinking (>20 standard alcoholic consumptions a week); hypertension (diastolic >100; systolic >170); and history of psychiatric disorder.

The study was conducted according to the code of ethics on human experimentation as established in the declaration of Helsinki (1964) and amended in Edinburgh (2000). All subjects were fully informed of study procedures and aims. All subjects gave their written informed consent. A permit for obtaining, storing and administering cannabis was obtained from the Dutch drug enforcement administration.

2.2. Design, doses and administration

The study was conducted according to a three-way, double-blind, placebo-controlled, cross-over design. Subjects received THC placebo, 250 μg/kg THC and 500 μg/kg THC on three separate occasions. A minimum wash-out of 7 days transpired between treatments. Smoking started in the morning of test days (between 9.20 and 9.40 a.m.) and lasted for about 10 min. Subjects were instructed to smoke the cigarette according to a fixed procedure, i.e. inhale for 4 s, hold breath for 10 s and exhale/break for 15 s. This sequence was repeated until the cigarettes were smoked as completely as possible. Cannabis cigarettes were prepared beforehand for each individual from stock provided by the Dutch Bureau for Medicinal Cannabis. Cannabis cigarettes were prepared from batches containing 13% THC, a standard potency for cannabis sold at Dutch pharmacies for medical use. Weight adjusted doses of cannabis were prepared for each subject individually and mixed with tobacco to a standard sized cigarette. Placebo cigarettes equalled weight and size of active cannabis cigarettes, but consisted of tobacco only.

2.3. Procedures

Subjects were asked to refrain from any drugs during the study period. Subjects were not allowed to use alcohol on the day prior to an experimental session and were requested to arrive at experimental sessions well-rested. Drug and breath alcohol screens were performed prior to experimental sessions upon arrival of the subject. Drugs screens (Mahsan® diagnostika) assessed for the presence of morphine, cocaine, cannabis (cut-off level 50 ng/ml THC–COOH), methamphetamine and amphetamine in urine. THC or THC placebo cigarettes were only administered if a subject had passed the alcohol and drug screen on a given test day. In the case of a positive drug screen, subjects were sent home to return to the laboratory at a later time. Subjects were given a standardized breakfast prior to smoking. Performance test were conducted at fixed intervals during 6 h post smoking. The Critical tracking task was conducted at 15 min, 1 h 15 min, 3 h 15 min and 5 h 15 min post dosing; a Stop signal task was conducted at 30 min, 1 h 30 min, 3 h 30 min and 5 h 30 min post dosing; the Tower of London was conducted at 45 min, 1 h 45 min and 5 h 45 min post dosing. Subjects received a training session prior to onset of the experimental sessions in order to familiarize them with the tests and procedures. Training in Critical tracking and Stop signal task performance continued until the subject had performed each task with less than 5% variance from the average measured over three trials. Performance on the Tower of London task shows little practice effect. This task was administered once during training.

2.4. Cognitive and motor tasks measuring skills related to driving

Three performance tasks were employed for measuring skills related to driving.

The Critical tracking task (CTT) measures the subject's ability to control a displayed error signal in a first-order compensatory tracking task. 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 frequency at which the subject loses the control is the critical frequency or Lambda-c. The Critical tracking task measures the perceptual-motor delay lag (i.e. psychomotor control) during a closed loop operation (Jex et al., 1966) and is the closest laboratory analogue to on-the-road tracking performance as measured in real life driving (Ramaekers, 2003).

The Stop signal task (SST) measures motor impulsivity, which is defined as the inability to inhibit an activated or pre-cued response leading to errors of commission. The current test is adapted from an earlier version of Fillmore et al. (2002) and has been validated for showing stimulant en sedative drug effects (Ramaekers and Kuypers, 2006). The task requires subjects to make quick key responses to visual go signals, i.e. the letters ABCD presented one at a time in the middle of the screen, and to inhibit any response when a visual stop signal, i.e. "*" in one of the four corners of the screen, is presented at predefined delays. The main dependent variable is the stop reaction time on stop signal trials (i.e. stop reaction time) that represents the estimated mean time required to inhibit a response. Stop reaction time was calculated by subtracting the stop signal delay from the reaction time on go-trials associated with *n*-th percentile of the reaction time (RT) distribution. The *n*-th percentile corresponds to the percentage of commission errors (Logan, 1994).

The Tower of London (TOL) is a decision-making task that measures executive function and planning (Shallice, 1982). The task consists of computer-generated images of begin- and end-arrangements of three coloured balls on three sticks. The subject's task is to determine as quickly as possible, whether the end-arrangement can be accomplished by "moving" the balls in two to five steps from the beginning arrangement by pushing the corresponding number coded button (Veale et al., 1996). The total number of correct decisions is the main performance measure.

2.5. Pharmacokinetic assessments

Blood samples and oral fluid samples were taken between 0 and 6 h post drug. Blood samples were taken right after smoking (5 min) and every 15 min during the first hour after smoking. From then on blood samples were collected around the hour. Oral fluids were taken right before smoking and every 15 min during the first hour after smoking. From then on oral fluids were collected every hour.

Blood samples were taken using glass venotubes without an anticoagulant. Ten blood samples were collected per treatment condition. Blood samples (5 ml) were placed on ice immediately, centrifuged later and frozen at −20 °C until analyses for pharmacokinetic assessments. THC concentrations and its main metabolites (THC–COOH and OH–THC) were determined in the corresponding serum samples using solid phase extraction and gas chromatography with mass spectrometric detection with a limit of quantification of 0.5 ng/ml (Steinmeyer et al., 2002).

In the case of oral fluids, half of the subjects were sampled with an Orasure intercept® device for a quantitative analysis by GC–MS (Kauert et al., 2006) and half of the subjects were sampled by the UPlink/Dräger Test system®. The latter device is a rapid saliva test that was developed for use by the police during roadside drug testing. The UPlink/Dräger Test system® provides a qualitative (yes/no) indication of recent cannabis use but cannot be used for a quantitative analysis. The limits of detection for the GC–MS analyses and the Uplink/Dräger roadside testing device were 0.5 ng/g and 20 ng/ml, respectively.

2.6. Data analyses

Data sets were analyzed according to a three-step procedure. First, the presence of a significant overall effect of THC on each of the three performance tasks was established by means of repeated measures MANOVA with THC (three doses), time after smoking (three or four time points) and their interaction as main factors. Data collected during treatment with both doses of THC were then converted into difference scores from placebo for further analyses of the association between THC concentration and performance (i.e. difference score = performance during THC treatments − performance during placebo treatment). Second, linear regression analysis was conducted to establish linear relationships between changes (from placebo) in task performance during THC treatment and log-transformed THC concentrations in serum and oral fluid. The total number of data points included in these equations was defined by the number of subjects × maximal number test repetitions × the number of THC treatments. Third, individual THC concentrations in serum prior to performance assessments in each of the THC conditions were divided over six mutually exclusive categories (i.e. 0–1, 1–2, 2–5, 5–10, 10–30, >30 ng/ml in case of the Stop signal task and Critical tracking task and 0–1, 1–2, 2–5, 5–10, 10–20, >20 in case of the Tower of London task) covering the full range of THC concentrations. Corresponding change scores of task performance were then classified either as showing "impairment" or "no impairment" for all individual cases within each of these categories. Impairment was defined as a negative change score from placebo in case of the Tracking task and the Tower of London. Change scores greater than or equal to zero were defined as showing no impairment. In case of the Stop signal task, impairment was defined as a positive change from placebo, i.e. an increase in stop reaction time. Changes in stop reaction times less than or equal to zero were defined as showing no impairment. Binomial tests were then applied to measure whether the proportion of observations showing impairment or no impairment significantly differed from the hypothesized proportion. It was hypothesized that in case of no effect of cannabis on task performance the proportion of observations showing impairment or no impairment would be equal, i.e. 50%.

3. Results

Complete data sets ($N = 20$) were collected for the Critical tracking task and The Tower of London. In case of the Stop signal task data sets for nine subjects were incomplete due to technical malfunctions. All of these subjects were part of the subgroup whose oral fluid were sampled with Orasure intercept® for quantitative analysis. Consequently, no correlation between performance in the Stop signal task and THC in oral fluid was calculated due to the low number of complete data sets.

3.1. Pharmacokinetics and overall performance

Mean (S.D.) concentrations of THC, OH–THC, THC–COOH in serum and THC in oral fluid after both THC treatments are given in Table 1. These show that THC concentration in serum ($F_{1,19} = 14{,}93$; $p = .002$) and oral fluid ($F_{1,9} = 15{,}83$; $p = .003$) were dose-related. Mean serum THC concentrations after smoking the highest dose of THC were about 1.5–2 times as high as compared to smoking the lowest dose. Concentrations of THC in serum and oral fluid followed similar elimination curves over time.

A summary of the qualitative evaluation (yes/no) by the UPlink/Dräger Test system® of THC presence in oral fluid from a subset of 10 subjects is given in Table 2. These data show that the proportion of false negative evaluations range from 30 to 70% in samples collected during the first 6 h after smoking either the low or high dose of THC. The proportion of false negative evaluations further rose to 80–100% as the time post smoking progressed.

Homogeneity tests of variance indicated that variances in all three performance tasks were the same during THC and placebo treatments. Overall, mean performance in the Criti-

Table 1
Time course for mean (S.D.) concentrations of THC and its metabolites in serum (ng/ml; $N = 20$) and oral fluid (ng/g; $N = 10$) following smoking two doses of THC as assessed by gas chromatography–mass spectrometry (GC–MS)

Time relative to smoking (min)	Serum (GC–MS)						Oral fluid (GC–MS)	
	THC 500			THC 250			THC 500	THC 250
	THC	OH–THC	THC–COOH	THC	OH–THC	THCCOOH	THC	THC
−5	–	–	–	–	–	–	0	0
5	95.1 (63.2)	5.5 (6.0)	21.9 (15.6)	58.0 (47.7)	3.0 (2.7)	11.0 (10.6)	–	–
15	27.7 (13.8)	5.0 (4.6)	33.4 (24.1)	16.9 (11.1)	2.7 (2.5)	18.1 (15.9)	918 (702)	899 (630)
30	19.5 (9.8)	4.6 (4.2)	31.0 (23.9)	10.8 (7.6)	2.3 (2.3)	16.1 (14.7)	715 (443)	567 (388)
45	14.3 (8.1)	4.0 (4.0)	27.7 (23.4)	7.7 (5.0)	2.0 (1.8)	13.9 (12.5)	498 (317)	307 (279)
60	10.4 (5.9)	3.4 (3.1)	25.6 (21.6)	6.1 (3.7)	1.9 (1.8)	13.2 (12.8)	356 (414)	142 (92)
120	5.9 (2.7)	2.2 (1.6)	20.4 (17.4)	3.0 (1.4)	1.2 (0.9)	10.4 (9.6)	138 (87)	71 (65)
180	3.0 (1.7)	1.4 (0.9)	15.4 (12.4)	1.7 (0.8)	0.8 (0.6)	8.3 (7.5)	62 (46)	54 (67)
240	1.8 (0.9)	1.0 (0.7)	12.7 (11.0)	0.9 (0.5)	0.4 (0.4)	6.0 (4.7)	23 (13)	15 (10)
300	1.2 (0.8)	0.7 (0.5)	10.0 (8.6)	0.6 (0.4)	0.3 (0.3)	4.6 (3.4)	23 (12)	13 (8)
360	0.9(0.5)	0.5 (0.4)	8.4 (7.6)	0.5 (0.4)	0.3 (0.3)	4.9 (5.3)	21 (12)	13 (11)

Table 2
Qualitative evaluation by the UPlink/Dräger test system® of THC presence in oral fluid of 10 subjects at baseline and during 6 h post smoking two doses of cannabis (1, THC positive; 0, THC negative; 99, missing value)

Subject	THC dose	Base-line	15 min	30 min	45 min	60 min	120 min	180 min	240 min	300 min	360 min
1	THC 500	0	1	1	1	1	1	1	0	0	0
2	THC 500	0	1	1	1	1	0	1	0	0	0
3	THC 500	0	0	0	0	0	0	0	0	0	0
4	THC 500	0	1	1	0	0	1	0	1	0	0
5	THC 500	0	1	1	1	1	0	1	0	0	0
6	THC 500	0	0	0	0	0	0	1	0	0	0
7	THC 500	0	1	0	0	99	0	0	0	1	0
8	THC 500	0	1	0	0	0	0	0	0	0	1
9	THC 500	0	0	0	0	0	99	0	0	0	0
10	THC 500	0	1	1	99	1	1	99	0	99	0
False negatives (%)		0	30	50	60	50	60	50	90	80	90
1	THC 250	0	0	1	1	1	1	0	0	0	0
2	THC 250	0	1	1	1	1	0	1	0	0	1
3	THC 250	0	0	0	0	0	0	0	0	0	0
4	THC 250	0	0	0	0	0	1	0	0	0	0
5	THC 250	0	1	1	1	1	0	1	0	0	0
6	THC 250	0	1	0	0	0	0	1	0	1	0
7	THC 250	0	1	0	0	0	0	0	0	0	0
8	THC 250	0	1	1	0	0	0	0	0	0	0
9	THC 250	0	0	0	0	0	0	0	0	0	0
10	THC 250	0	0	0	0	0	0	99	0	0	0
False negatives (%)		0	50	60	70	70	80	60	100	90	90

Table 3
Mean (S.E.) performance change from placebo for two doses of THC smoking at three or four test repetitions after smoking

Variable	Drug	Repetition 1 (0–1 h)	Repetition 2 (1–2 h)	Repetition 3 (4–5 h)	Repetition 4 (5–6 h)
Critical tracking task ($N = 20$), Lambda-c (rad/s)	THC 500	−.60 (.21)	−.55 (.17)	−.27 (.13)	−.48 (.08)
	THC 250	−.21 (.13)	−.40 (16)	−.18 (13)	−.17 (.13)
Stop signal task ($N = 11$), stop reaction time (ms)	THC 500	60.6 (21.6)	64.6 (25.0)	12.9 (12.0)	7.0 (17.9)
	THC 250	17.5 (17.2)	10.9 (22.9)	−3.3 (20.11)	4.8 (11.4)
Tower of London ($N = 20$), correct decisions (#)	THC 500	−2.6 (0.59)	−3.2 (1.0)		−1.0 (.9)
	THC 250	−2.1 (1.1)	−2.2 (1.1)		.25 (.8)

For exact timing of performance testing relative to smoking see Section 2.3.

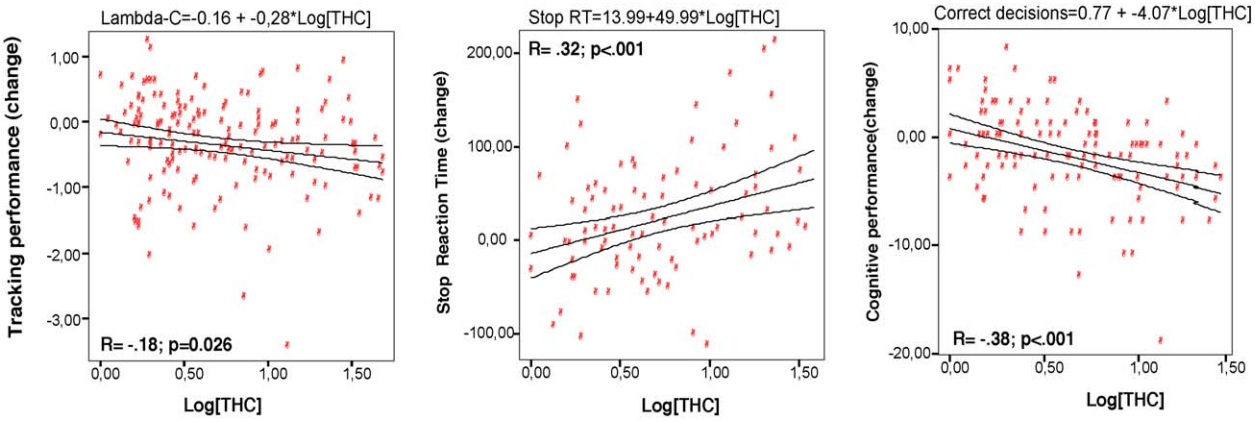

Fig. 1. Scatter plots showing linear (95% CI) relationships between serum log [THC] and changes in tracking performance (left), stop reaction time (middle) and cognitive performance (right) in 20 subjects.

cal tracking task ($F_{2,18} = 9.41$; $p = 0.002$), the Tower of London task ($F_{2,18} = 7.25$; $p = 0.005$) and the Stop signal task ($F_{2,9} = 5.15$; $p = .032$) were significantly affected by THC. Relative to placebo, THC reduced critical tracking performance and the number of correct decisions in Tower of London task and increased stop reaction time in the Stop signal task. Mean (S.E.) change scores from placebo for every performance parameter in each THC condition are given in Table 3. There was no significant interaction between THC and Time after smoking for any of the three parameters.

3.2. Associations between THC in serum/oral fluids and performance

Regression analysis showed weak but significant linear relations between THC in serum and changes (from placebo) in critical tracking ($p = 0.026$, $r = -0.13$), stop reaction time ($p < .001$, $r = 0.32$) and number of correct decisions ($p < .001$, $r = -0.38$). Scatter plots showing the linear relationships between serum THC and changes in performance measures are shown in Fig. 1. Similar linear equations were derived from the relationship between THC in oral fluid and performance in the critical tracking task ($p =$ ns, $r = -.18$) and the Tower of London task ($p = .006$, $r = -.35$). In addition, a strong linear relation was found between THC levels in oral fluid and serum ($N = 10$; $p < .001$, $r = .84$). A scatter plot showing the linear relationship between THC in oral fluid and serum is shown in Fig. 2. No correlations were found between changes in performance and THC–COOH. Also, the sum of THC and OH–THC did not provide higher correlations with performance change than THC alone.

3.3. THC threshold levels of impairment

Binomial tests showed a significant increase in the proportion of observations showing impairment in the critical tracking task for serum THC concentrations >2 ng/ml ($p < .05$). In case of the Stop signal task and the Tower of London task, significant increases in the proportion of observations showing impairment were found for serum THC concentration >5 ng/ml ($p < .05$).

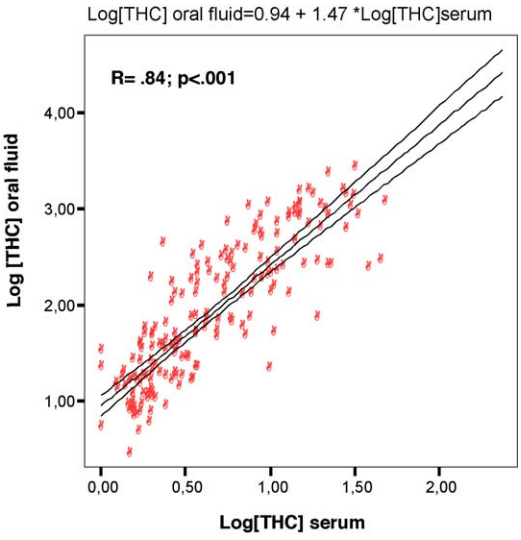

Fig. 2. Scatter plot showing the linear (95% CI) relationship between serum log [THC] and oral fluid log [THC] ($N = 10$).

Distributions of observations showing "impairment" and "no impairment" in each performance task as a function of serum THC are shown in Fig. 3.

4. Discussion

The main findings of this study were (1) that linear, but marginal relations exist between the magnitude of performance impairment and THC levels in serum and oral fluid; (2) that THC levels in serum and oral fluid are strongly correlated; and (3) that clear cut-off levels in serum THC can be determined above which performance impairment emerges.

The pharmacodynamic and pharmacokinetic effects of THC were as expected. THC significantly impaired cognitive and motor performance in the Critical tracking task, the Stop signal and the Tower of London task. THC induced performance impairment were severe and clinically relevant when compared to alcohol effects on the same tasks. During the first 2 h after smoking mean impairments observed during the Critical

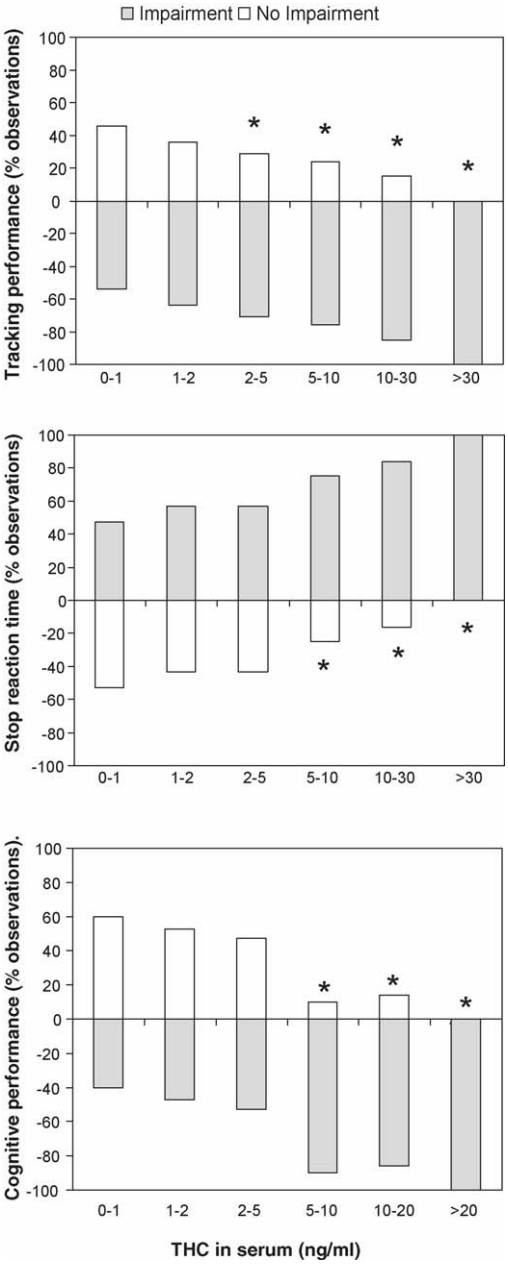

Fig. 3. Distributions of observations showing "impairment" and "no impairment" as a function of serum THC in each performance task ($^{*}p < .05$).

tracking task were generally equivalent to those observed for subjects performing the same task with blood alcohol concentration (BAC) >1.00 mg/ml (Ramaekers et al., 1996). Between 2 and 6 h after smoking, THC induced tracking impairment was comparable to BACs >0.5 mg/ml (Ramaekers et al., 1996). THC induced impairments observed in the Stop signal task and the Tower of London task were also noteworthy as previous studies have shown that performance on these tasks is not affected at BACs of 0.5 mg/ml (Lamers et al., 2003; Ramaekers and Kuypers, 2006). Comparative data for higher BAC levels are unfortunately not available, but it seems clear that it takes BACs >0.6 mg/ml to achieve equivalent impairments in the Stop signal task and the Tower of London task.

Both doses of cannabis produced maximal concentrations of THC during the initial absorption phase at 5 min post smoking. At this timepoint, mean THC concentrations were about 58 and 95 ng/ml after smoking the low and the high dose, with maximal peak concentrations of 160 and 240 ng/ml, respectively. After both doses, mean THC concentrations rapidly dropped to 1–2 ng/ml within 3–5 h after smoking. THC concentrations in oral fluid were much higher than those in serum, but their ratio appeared remarkably constant throughout the elimination phase. Regression analysis showed a strong linear relation between log-transformed THC in serum and oral fluid ($r = .84$) indicating that in general changes in serum THC co-varied very well with changes in oral fluid THC. Similar correlations between THC levels in both matrices have previously been reported after controlled THC administration (Huestis and Cone, 2004) and in regular drug users (Samyn and van Haeren, 2000). Together these results suggest that the presence of THC in oral fluid can be considered as a valid biomarker of recent cannabis exposure. It offers great opportunities for developing easy-to-use, non-invasive, roadside drug tests for qualitative assessments of THC in oral fluid in drivers. A range of on-site, oral fluid drug testing devices has already been developed in recent years but the reliability of these immunoassays has generally been sub-optimal (Verstraete, 2005). In the present study, the UPlink/Dräger drug tester produced a considerable high percentage of false negative evaluations, even shortly after smoking when THC levels where high. The device was taken off the market shortly after completion of this study and will be replaced by a new generation of oral fluid testers in the near future. The challenge being faced is to improve the analytic method of on-site immunoassays in order to achieve the same sensitivity and accuracy as with laboratory GC–MS techniques.

Regression analysis indicated linear relations between changes in performance impairment and log-transformed THC levels in both serum and oral fluid. However, the associated correlations were always rather low, in the range of 0.15–0.40. The lack of a strong association seems to indicate that serum THC cannot be taken as an accurate predictor of the magnitude of performance impairment. Similarly, the overall lack of a significant interaction between performance impairment in any of the performance tasks and time of testing suggested that THC induced impairment remained relatively stable over 5–6 h after smoking despite the prominent decline of THC in serum and oral fluid. The present data are in line with previous reports that have shown low or inconsistent correlations between performance measures and serum THC, particularly during the early distribution phase (Cone and Huestis, 1993; Kelly et al., 1993; Reeve et al., 1983; Robbe, 1994). The implication is that magnitude of performance impairment is not a suitable parameter for defining threshold levels of THC in serum.

A more promising way to define threshold levels of impairment was found by comparing the proportion of observations showing impairment or no impairment as a function of THC concentration. That approach is not affected by the large variability in performance impairment. It classifies positive or negative performance changes from placebo as showing either "impairment" or "no impairment", irrespective of its magnitude. Binomial

tests were used to test the statistical significance of deviations in the proportion of observations showing impairment or non-impairment from the theoretically expected distribution within six successive serum THC concentration ranges. It was hypothesized that in case of no effect of cannabis on task performance the proportion of observations showing impairment or no impairment would always equal, i.e. 50%. The approach worked out surprisingly well on all accounts. The proportion of observations showing impairment progressively increased as a function of serum THC in every performance task. Binomial tests showed a significant increase in the proportion of observations showing impairment in the critical tracking task for serum THC concentrations >2 ng/ml. Between 2 and 5 ng/ml the proportion of "impaired" observations was about 71% and gradually increased to a full 100% at THC concentrations >30 ng/ml. In the Stop signal task and the Tower of London task, significant increases in the proportion of observations showing impairment were found for serum THC concentration >5 ng/ml. Between 5 and 10 ng/ml the proportion of "impaired" observations were 75 and 90%, respectively, and increased to a full 100% at THC concentrations >30 and 20 ng/ml, respectively.

Several THC threshold limits can be defined on the basis of the present results. First, there is the lower limit above which performance impairment emerges in some but not all tasks related to driving, i.e. 2 ng/ml. The proportion of observations showing tracking impairment significantly increased at this concentration, whereas performance in the Stop signal task and the Tower of London task was still unaffected. It shows that tracking performance is more sensitive to the effects of THC concentrations between 2 and 5 ng/ml in comparison to tasks measuring motor impulsivity or cognitive function. Previous studies have also indicated that detrimental effects of low doses of THC are more prominent in highly automated behaviours, such as road tracking control, as compared to more cognitive driving tasks requiring conscious control (Ramaekers et al., 2004). The lower limit, however, also implies that there is no performance impairment at serum THC concentrations below 2 ng/ml. In terms of driving under the influence of cannabis this may be of particular importance in relation to residual THC concentrations of 0–2 ng/ml that can be found in frequent THC users (Giroud et al., 2001) or even in non-users who have been passively exposed to cannabis. Traces of THC in serum or urine are not likely to be found after passive exposure to smoke of a single cannabis cigarette (Niedbala et al., 2004), but have been demonstrated after passive inhalation under extreme exposure conditions, i.e. 16 cannabis cigarettes for 5 days (Cone et al., 1987). Second, there is the upper limit above which performance impairment was evident for all observations. This was the case at THC concentrations >30 ng/ml. The upper limit clearly is the most liberal definition of a THC threshold that would predict absolute impairment in all performance domains in each and every individual. Finally there is the relative impairment limit at which performance impairment emerged in a significantly large proportion of observations across all performance domains, i.e. at THC concentrations between 5 and 10 ng/ml. Within this range, frequency distributions of observations showing impairment/no impairment showed a marked shift towards impairment in every performance test, i.e. approximately 75–90% of the observations were indicative of impairment.

In theory, it would also be possible to calculate limits of impairment in oral fluid from the linear regression equation between THC in oral fluid and serum. It should be noted, however, that the oral fluid/serum ratio in the present study differs markedly from that reported in another study (Huestis and Cone, 2004). In the present study THC levels in oral fluid were generally 10–30-folds higher as compared to corresponding THC levels in serum. In the study by Huestis and Cone (2004), THC concentrations in serum and oral fluid were very similar with oral fluid/serum ratios ranging between 0.5 and 2. It is presently unknown why these ratios differ so markedly in both studies but it may be related to between-subject variations in THC contamination of the oral cavity while smoking cannabis or differences in methods of collecting oral fluid. Huestis and Cone (2004) collected oral fluid under stimulated (citric acid type, sour candy) conditions, whereas in the present study oral fluid was collected under non-stimulated conditions. Collectors that stimulate oral fluid usually reduce drug concentration compared to a non-stimulated manner (Drummer, 2005). It thus seems wise at present to primarily employ oral fluid testing for obtaining a first indication of recent cannabis use until methods for collection and analyses of oral fluid have been standardized. In case of a THC positive result, additional analyses should be conducted in serum in order to establish a quantitative evaluation of THC levels.

The present study will be criticized for the face validity of the performance tasks and their ability to reflect driver impairment or crash risk. Though it is evident that the present laboratory tasks did not measure actual driving, it should be noted that the laboratory tasks do possess sufficient content validity. The test battery is representative of mental and behavioural functions that are relevant to driving. Previous work has also demonstrated that some laboratory tests, though not all, can be predictive of real life driving performance. For example, drug induced changes in Critical tracking task performance have been shown to significantly correlate ($r = -.45$) to drug induced changes in road tracking performance as measured in an on-the-road driving test (Ramaekers, 2003). Whether drug induced driver impairment as shown in experimental laboratory or driving studies is also predictive of crash risk, however, may be more difficult to determine. It appears that construct validity of experimental studies, i.e. their sensitivity to pharmacological drug effects, is generally higher than that of epidemiological studies designed to establish crash risk. For example, it is no problem in experimental studies to demonstrate driver impairment for BACs as low as 0.2–0.5 ng/ml (Ramaekers et al., 1992; Vermeeren et al., 2002; Verster et al., 2002). Yet, epidemiological surveys have repeatedly demonstrated that crash risk only starts to increase at BACs >0.5 mg/ml (Borkenstein, 1978; Drummer et al., 2004; Movig et al., 2004). Likewise, experimental studies have repeatedly shown driver impairment at low concentrations of THC (Ramaekers et al., 2000, 2004) whereas epidemiological studies have provided heterogeneous reports on the association between crash risk and low THC concentrations (Drummer et al., 2004; Laumon et al., 2005; Longo et al., 2000). Yet despite these

differences in construct validity, both experimental and epidemiological studies can provide mutually supporting results. In the present study, slight and selective impairment of tracking performance was already notable at THC ranges between 2 and 5 ng/ml, but impairments became truly prominent across all performance domains at serum THC concentrations between 5 and 10 ng/ml. These ranges seem to correspond well to recent epidemiological data that have shown a concentration-dependent increase in crash risk in drivers positive for THC (Drummer et al., 2004; Laumon et al., 2005). Significant odds ratios (OR) of crash risk for THC concentrations ranging between 1 and 2 ng/ml and 2 and 5 ng/ml in whole blood were 1.45 and 2.13, respectively (Laumon et al., 2005). At THC concentrations >5 ng/ml in whole blood the ORs ranged from 2.1 to 6.6 (Drummer et al., 2004; Laumon et al., 2005). When converted to THC concentrations in serum as employed in the present study these ranges would be equivalent to 2–4, 4–10 and >10 ng/ml, respectively.

The present data thus supports epidemiological data and shows that THC serum concentrations between 2 and 5 ng/ml establish the lower and upper range of a per se limit for defining general performance impairment above which drivers are at risk. It should be stressed, however, that the predictive validity of such a per se limit is confined to the driving population at large, and not necessarily applicable to each and every driver as an individual. Individual drivers can widely differ in their sensitivity for THC induced impairment as evinced by the weak correlations between THC in serum and magnitude of performance impairment in the present study. Even at a 5 ng/ml limit only 70–90% of the observations were indicative of impairment, meaning that in 10–30% of the observations there was no impairment at all. The purpose of a per se limit is to indicate the average THC concentration above which drivers are at risk and should be interpreted as such.

In summary, the present study showed a strong and linear relation between THC in serum and oral fluid. Linear relations between the magnitude of performance impairment and THC in oral fluid and serum, however, were low. Classification of performance into observations showing either impairment or no impairment provided a much better insight into the relation between serum THC and performance impairment. An initial shift toward impairment was evident in the critical tracking task for serum THC concentrations between 2 and 5 ng/ml. At concentrations between 5 and 10 ng/ml approximately 75–90% of the observations were indicative of impairment in all three performance tasks. At THC concentrations >30 ng/ml the proportion of "impaired" observations increased to a full 100% in all three performance tasks. It is concluded that serum THC concentrations between 2 and 5 ng/ml establish the lower and upper range of a legal THC limit.

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