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Cannabis smoking impairs driving performance on simulator and real driving: A randomized, double blind, placebo-controlled, crossover trial

Running title: Driving performance under THC smoking conditions

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ABSTRACT

Driving experiments in real conditions are considered as a "gold standard" to evaluate the effects of drugs on driving performance. Several constraints are difficult to manage in these conditions, so driving simulation appears as the best alternative. A preliminary comparison is crucial before being able to use driving simulation as a valid evaluation method. The aim of this study was to design a driving simulation method for assessing drugs effects on driving. We used cannabis (THC) as a positive control and assessed whether THC affects driving performance in simulation conditions and whether these effects are consistent with performance in real driving conditions. A double-blind, placebo-controlled, two successive 2-way cross-over design was performed using cigarettes containing 20 mg of THC. Healthy occasional users of THC, aged 25-35 years, who had a consistent driving experience were included. The first two sessions were realized in simulation conditions and the last two sessions were in real driving conditions. Driving performance was estimated through inappropriate line crossings (ILC) and the standard deviation of the vehicle's lateral position.

Participants felt significantly drowsier and more tired after THC, whatever the driving condition. Driving stability was significantly impaired after THC, both in simulated and real driving conditions. We also found that ILC were significantly more numerous in driving simulation conditions, as compared to real driving. In conclusion, the driving simulator was proven to be more sensitive for demonstrating THC-induced effects on driving performances. Driving simulation appears to be a good qualitative predictor of driving safety after drug intake.

Keywords: Driving simulation, Drug effect, Driving performance, Cannabis, Lateral control

INTRODUCTION

Road accidents and casualties are a major public health issue. Indeed, about 1.25 million people die each year as a result of road traffic crashes, an average of over 3400 fatal events every day. Road accidents represent 2.2% of the overall death reports in the world in 2012, ranking 9th in the principal death causes on a global scale. Moreover, they are the leading cause of death among young people, aged 15–29 years. Beyond fatal casualties, between 20 and 50 million people are injured every year in road accidents. In financial terms, the cost of road accidents (and related traumas) represents about 3% of the gross domestic product of industrialized countries and rises to 5% in some low- and middle-income countries [1]. Beyond infrastructure-related, vehicle- and environmental-related risks, most accidents are a consequence of human factors and behavior. Among them are tiredness, sleepiness [2], excessive speed, health state and the consumption of alcohol, legal and illegal psychoactive substances [3,4].

Alcohol and cannabis (THC) are amongst the psychoactive substances most widely used by drivers. Roadside surveys conducted in 13 countries across Europe, in which blood or oral fluid samples from 50 000 drivers were analyzed, revealed that alcohol was present in 3.48% of the cases, illicit drugs in 1.9%, medicines in 1.36%, combinations of drugs or medicines in 0.39% and alcohol

combined with drugs or medicines in 0.37% [5]. In USA, the weekend nighttime prevalence of THC use by drivers has increased by 48% between 2007 and 2014, while prevalence of alcohol use in the same studied period was significantly lower. According to authors, "*changes in State policy on marijuana use, including medical and recreational use, may have contributed to an increase in marijuana use by drivers*" in USA [6]. Recently, after a systematic review of the literature from January 1998 to February 2015, statistically significant associations between drug use and road traffic crashes involvement were found for THC in 23 out of 36 studies [7]. Another meta-analysis of studies examining acute THC consumption and motor vehicle collisions, with adequate control groups, found a near doubling of risk of a driver being involved in a motor vehicle collision resulting in serious injury and death [8]. All these results highlight that driving under the influence of THC is still a growing health problem. However, epidemiological approaches cannot establish causal relationships, due to the possibility of confounding factors.

As a consequence, experimental studies are conducted to investigate the impairing effects of THC, using basic cognition and psychomotor functions such as attention, vigilance and reaction time [9]. In this approach, traditional (possibly computerized) neuropsychological and psychomotor test batteries are currently used and developed. For instance, Stav et al. assessed visual sensitivity, motor performance, useful field of view and memory (MMSE) in older drivers, but did not find clear predictability of these tests on driving performance [10]. Other studies used a similar test battery and found better correlations [11,12]. Nevertheless all these studies failed to predict driving risk for multiple reasons, the environment of testing being very neutral (i.e. research labs), the proprioceptive and cognitive feedback being completely removed from the experiment. Finally and most importantly, driving is a very complex task requiring multiple cognitive and sensorimotor functions being simultaneously activated which might explain the poor results of the in-lab protocols using single or limited number of tasks.

More realistic tests have been performed using real driving. Real driving is often considered as a "gold standard". These ecological protocols have been notably tested in healthy subjects submitted to sleep deprivation [13-15], using in particular trajectory control as a good indicator of driving performance in different circumstances. A series of on-road studies conducted in the Netherlands evaluated the effects on smoked THC on actual driving performance, demonstrating dose-dependent THC impairment in road tracking [16-19].

However, actual driving experiments are difficult to organize and can also be susceptible to interruption before completion, if the driving instructor or the subject feels it is unsafe to continue, due for example to adverse effects caused by the drug under investigation [20]. It would be valuable to understand whether and in what measure driving simulation behavior can be a good predictor of actual driving, to avoid these real driving constraints. As a matter of fact, driving simulation is attractive, involving easily controllable situations, presenting the great advantage of involving the subjects into virtually dangerous, but actually safe, situations.

Influence of THC on driving performance in simulated conditions was examined in several studies [21,22]. However, the validation of simulators is the key component of any study that wants to investigate the impairing effects of any drugs on driving conditions. Given the state-of-the-art, it seems that a comparison between data collected in simulation conditions and data collected in actual driving conditions is mandatory, before concluding that driving simulation is actually a good predictor of actual driving behavior (see for instance Davenne et al. [23]).

To date there are few studies directly comparing driving simulator performance with real driving [13,24-27], notably after sleep deprivation, alcohol or benzodiazepine intake. Most of these studies highlight that a relative validity of simulators is satisfactory for many variables, but that generalization to real driving conditions can be questioned (in absolute terms). Moreover, to our knowledge such a comparison has only been performed after an oral intake of THC, i.e. dronabinol [28] and never under THC smoking conditions.

In our study, an innovative and original approach was applied to avoid the most of confounding factors, using several controls as the transposition of the same scenario during simulated and real driving sessions, the monitoring of tiredness with actimetry measure to verify normal sleep before sessions and the use of a timed smoking computerized procedure that minimize exposure variability. The aim of this study was to setup and validate a driving simulator method for assessing drugs effects on driving. To achieve it, we used THC as a positive control, and assess whether THC affects driving performance in the simulator and whether these effects are consistent with performance in real driving.

MATERIAL AND METHODS

Participants

Twenty Caucasian moderate tobacco smokers (<8 cigarettes per day) and occasional users of THC aged 25-35 years, who had been in possession of a driver's license for at least 3 years and had a driving experience (at least 3.000 kms per year) participated in the study. Occasional smokers were defined as those who reported smoking THC less than once a month. Only males were included due to evidence for gender differences in the effects of THC [29]. Before the study, subjects were medically screened, interviewed about past and current drug uses. During the clinical assessment, subjects completed several diagnostic questionnaires including the Spielberger State-Trait Anxiety Inventory [30], the Raine Schizotypal Personality Questionnaire [31], the Eysenck Personality Inventory [32], the Sensation Seeking scale of the Zuckerman Personality Questionnaire [33] and the Horne & Ostberg questionnaire for the chronotype [34]. After that, subjects underwent a psychiatric interview, a physical examination including an electrocardiogram and urinary drug screen for opiates, benzodiazepine, cannabis, amphetamine, cocaine. Subjects were excluded if they meet criteria of psychiatric disorder. Other criteria for exclusion included history of drug or alcohol related problems, body mass index outside the range 19-26 kg/m^2, smoking more than 8 tobacco cigarettes per day, and relevant abnormalities on electrocardiogram.

Finally, they were required to have normal or corrected to normal vision, as verified during baseline testing and to have no signs of simulator sickness after a 30-minute practice drive in the driving simulator. They were instructed to abstain from alcohol for 24h before and their compliance was verified by testing breath alcohol levels on the morning of each session.

As a safety precaution, participants agreed that they would not drive to and from the laboratory. Subjects were instructed not to drive for 12h after each session. Participants were also instructed to maintain a regular sleep-wake schedule (i.e., to sleep from 11 p.m. to 7 a.m. for the 3 days before each of the 4 test days). Actimeters (Actiwatch®, CamNtech, Cambridge UK) were used to check compliance with the protocol, by quantifying nocturnal sleep episodes. Individuals were only included if they had a mean sleep efficiency (ratio of time asleep to time in bed) of at least 85% during the 3 days of recording [15].

The study was approved by the local Ethics Committee and the French Drug Agency. The study, registered by ClinicalTrials.gov web site (Identifier: NCT01118364) was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization guideline for Good Clinical Practice. All subjects gave written informed consent before participation. All subjects received 700 € for their participation, as a minimal compensation for burden related to the procedures used for the trial as compared to other pharmacological studies. This amount was agreed upon by the local Ethics Committee. A permit for obtaining, storing and administering THC was obtained from the Department of Narcotics of the French Drug Agency.

Design, dose and administration

The study design was a randomized, placebo-controlled, double blind, cross-over design (two successive 2-way cross-over), consisting a total of four sessions being separated by at least four weeks (figure 1). The driving simulation sessions took place in two centers (Marseille and Toulouse) and on-road driving in another and single center for the session on the A62 freeway (Bordeaux).

Randomizing the order of all sessions was very complex in terms of organization because the sessions was performed in the three different cities, which were more than 500 kilometers away (all subjects from Toulouse and Marseille went to Bordeaux for the real driving session) and also in terms of safety (a subject may begin the study by a on-road driving under cannabis). Therefore, the first two sessions were realized in simulation conditions and the last two sessions in real driving conditions. Participants were kept under medical supervision for 8 hours after smoking. At the beginning of each experimental session, a breath alcohol test and a urine immunoassay for THC detection (ServiDrogue® THC, ServiBio) were performed. Administration of the placebo or THC cigarettes was only performed if drug and alcohol test were negative.

Cannabis stock was provided by the Forensic Science Laboratory and contained 8% THC. The THC cigarettes contained 20 mg of THC and tobacco. The placebo cigarette contained only strong tobacco (DRUM®, fine-cut hand rolling tobacco). Cigarettes had identical odor (strong tobacco in all cigarettes for masking), appearance and weight. The randomization list was performed by the department of Biometry, CIC-CPCET Marseille, using the PROC PLAN procedure (SAS® 9.2). The randomization has been stratified by recruitment center (Marseille/Toulouse). Participant were randomly allocated into 4 sequences according to the 2-treatment level, 2-cross-over of 2-period design (Placebo-THC/Placebo-THC, THC-Placebo/Placebo-THC, THC-Placebo/THC-Placebo and Placebo-THC/THC-Placebo). Both subjects and investigators were unaware of treatment allocation.

Smoking started on the morning of test days (between 9.15 and 9.45 a.m). Subjects were instructed to smoke the cigarette, under a medical supervision, according to a computerized fixed procedure: to inhale the smoke as deeply as possible, hold each inhalation for approximately 4 s and then exhale [35]. This sequence was repeated until the cigarette was smoked as completely as possible and took around 20 minutes.

Due to the importance of reporting THC dose as well as plasma concentrations in performance studies [36-38], blood samples were collected in order to have a proxy of THC plasmatic exposure. Blood samples (10 mL) were taken at baseline, at the mid-time of simulated driving (T1h45

minutes) and at the end of driving (T2h45) after onset of THC smoking. Due to the duration of real driving session (2 hours), blood samples were performed at hospital before and after the end of real driving, i.e 45 minutes and 4h30 after smoking, respectively. Blood samples were centrifuged and serum was frozen at -20°C until analyses for pharmacokinetics assessments. THC concentrations and its main metabolites, 11-hydroxy THC (OH-THC) and nor-9-carboxy-THC (THC-COOH), were determined using gas chromatography with mass spectrometric detection.

Apparatus

Driving simulation

Driving simulation sessions took place in Marseilles (Institute of Movement Sciences, Mediterranean Virtual Reality Center) and in Toulouse (Institute of Space Medicine and Physiology MEDES). We used driving simulators developed by IFSTAAR in collaboration with FAROS and OKTAL companies. This type of device (hardware and software) enables full control of driving scenarios, real-time interactive driving, visual, 3D auditory and steering-wheel force feedback. Simulated trajectories were recorded on-line for off-line analyses [39]. The simulated road environment was a precise, scale 1:1, reproduction of a 200 km portion of the A62 freeway in France. Road traffic was also simulated. Driving simulators are extensively used in basic driving behavior research [40,41], as well as in driving rehabilitation [42] and in the study of detrimental effects of drugs (Stough et al. 2012) or sleep deprivation [13] on driving fitness.

Real driving

An actual portion of the A62 freeway was used, for comparison with the simulation condition. It is a large and rather straight highway, with generally low or medium traffic intensity. During the whole experiment, a professional driving instructor monitored the driving speed and was ready to take

control of the car (equipped with dual controls) if the subject started losing control of the vehicle, such that the safety of participants was ensured. The car used for the experiment was equipped with a video camera (Continental Automotive Systems) that filmed and recorded the lateral position of the car in relation to the road's right lateral lane marker (frequency = 10 Hz). The same freeway has already been used in previous studies on sleep deprivation [43].

Task

Subjects had to drive on the separated lanes motorway for about 2 hours. This duration was considered as necessary and sufficient to assess driving impairment of THC. The rules were to maintain the vehicle in the center of the driving lane and to keep as much as possible a constant speed (between 90 and 130 km/h). They were asked not to cross the painted lines separating the lanes.

Behavioral measures

Psychological measures

During each session, before driving and after inhalation, subjects had to answer a series a question about their confidence in their ability to drive, their level of drowsiness and tiredness (using a 0-100 response scale).

Driving performance

All variables were computed with Matlab (The MathWorks ®). Volunteer subjects were not aware of their results during all the study. For simulated data, we considered the beginning of the driving task for all subject when the vehicle ran over 30 m/s speed and the end of the trial as the last time the driver released the accelerator. Driving performance was estimated through the following two variables, recorded on-line during the driving task.

i. Inappropriate line crossings

For both real and simulated driving, participants were asked to stay in their driving lane. First, inappropriate line crossings (ILC) were detected, indicating a direct violation of the subject's task. ILC is clearly related to sleep-induced traffic accidents, hence a direct risk factor [13].

An ILC was counted each time the car crossed entirely one of the lateral highway lane markers. In real driving conditions, only passing maneuvers and emergency driving actions which the car crossed entirely one of the lateral highway lane markers were excluded from analysis. In simulated driving conditions, all traffic vehicles drove faster than the subject's vehicle so that drivers did not have to voluntarily cross the lines. A line crossing in simulated driving condition was computed from the most eccentric point of the virtual car, which corresponds to the first point of the car that crossed the line, given the direction of motion of the car. The total number of ILC was recorded and then analyzed for each participant.

ii. Standard deviation of the vehicle's lateral position

The standard deviation of the vehicle's lateral position (SDLP) was calculated from the lateral deviation of the car center from the middle of the driving lane, registered continuously during the entire session. SDLP measures the stability of the trajectory, and is a validated measure of the degree of driving impairment [14].

Statistical analysis

The available data to estimate the number of subjects were very scare. To our knowledge no previous published study has investigated the effect of cannabis on the chosen point (ILC). The only published studies on carried out on the same circuit (actual driving) showed that the number of crossing lines is estimated on average 0.17 (± 0.39) [13, 45].

An *a priori* sample size estimation performed in two-tailed condition, indicated that a total sample size of n=16 was sufficient to detect a significant difference of 0.5 point between THC and placebo whatever the condition on the log-transformed line crossing criterion, assuming a standard deviation of 0.39, with a significance level of 5% and a power of 95%. Considering a rate of 20% for lost to follow-up of subjects, a total of 20 subjects had to be included in the study.

All subject's data related to the clinical trial were collected in case report forms which were reviewed by an independent trial monitor. All data were reviewed into the EpiData software version 3.1. The consistency of data entered was controlled using SAS programs, developed according to the protocol and the data management plan.

All data were reviewed and validated during the data review meeting, and the data base was locked before starting the analysis. Populations used for analyzes had to be a full simulated session.

In the multivariate analyses, we used a linear mixed model with REML estimation method and a Satterthwaite approximation for computing the denominator degrees of freedom.

The independent fixed effects tested for significance were: treatment (THC, tobacco), sequence of treatment, type of driving (simulated, real), and interaction (type of driving x treatment). Because the experiments systematically start with stimulation sessions, the session effect (1-2-3-4) was included into the model. Random intercept and subject nested within sequence was included in the model.

Model tested:

$$Outcomes = \beta_o + \beta_1.\ \text{treatment} + \beta_2.\text{sequence} + \beta_3.\text{driving} + \beta_4.\text{session} + \beta_5.(\text{driving X treatment}) + \text{subject(sequence)} + \varepsilon.$$

ILC was log-transformed for analysis. Correlations between outcomes were calculated using Spearman's coefficient. P-values <0.05 were considered significant. The consistency and analyzes were performed with SAS software version 9.2 (SAS Institute, Cary, NC).

RESULTS

A total of 21 subjects were included and 19 subjects were randomized. Due to missing data or uncompleted sessions, the analyses were performed on a total of 15 participants for simulation driving and 11 participants for real driving task (See flow chart in Figure 2)

Adverse effects

A total of 21 adverse events were reported during the study with no serious adverse events. All adverse events occurred during placebo session and THC session (Table I).

Smoking procedure and blood analysis

Mean (SD) number of puffs smoked from the placebo and THC cigarettes was 35.6 (5.6) and 35.1 (5.6) during the simulated session. For the real driving the mean (SD) number of puffs smoked from the placebo and THC cigarette was 35.8 (4.8) and 32 (6.2) respectively, that was not statistically different from the simulated session.

Average plasmatic concentrations of THC and its metabolites during simulated and actual driving are presented in Table II. No significant difference in sum of concentrations of THC and its metabolites were found between simulation and real driving conditions.

Psychological measures

Confidence in the ability to drive

On a 0-100 analog scale, participants' confidence in their ability to drive was significantly lower after THC intake, as compared to placebo ($p<.0001$). Confidence in the ability to drive (CAD) was not significantly lower in simulated driving conditions, as compared to real driving ($p=.1403$) (see figure 2A).

Drowsiness

Concerning drowsiness, we also found a significant effect of treatment (p=.0007) and no significant effect of driving conditions (p=.7021). In other words, participants felt significantly drowsier after THC intake, as compared to placebo, whatever the driving conditions (see figure 2B).

Tiredness

A pattern similar to that observed for drowsiness was observed for tiredness. Participants felt more tired after THC intake, as compared to placebo (p=.0003), whatever the driving conditions (see figure 2C).

Driving performance

Inappropriate line crossings (ILC)

Participants were asked to stay in their driving lane. Thus, any situation where the side of the car exited the lane was considered an ILC, excluding passing maneuvers and emergency situations during real driving sessions. Statistical analysis (table III) revealed a significant main effect of driving condition (p<.0001). ILCs were increased in simulation condition, as compared to real driving (figure 3A). There was no main effect of the treatment, though ILCs being more numerous after THC intake in simulated condition, as compared to placebo (p=.262). However, post-hoc analysis do not revealed significant differences.

Standard deviation of the vehicle's lateral position (SDLP)

We found a significant main effect of driving conditions (p<.0001, table III), with SDLP being higher in simulated conditions, as compared to real driving (figure 3B). There was also a main effect of

treatment, with SDLP being significantly higher after THC intake, as compared to placebo (p=0.005), whatever the driving conditions. Post-hoc analysis revealed a higher SDLP after THC intake in simulated condition (p=0.006).

Correlation of treatment effect between driving conditions

Scatterplot of the difference THC - placebo (treatment effect) suggests an agreement between simulator and real driving (figure 5). Nevertheless, Spearman's correlation coefficients were positive but not statistically significant (ILC r=0.41, p=0.201, SDLP r=0.13, p=0.70).

Correlations between outcomes under driving conditions

The two driving parameters were more strongly correlated during simulated driving (r=0.93, p<.0001) than during real driving (r=0.41, p=0.043). Psychological measures were moderately to strongly correlated between each other, whatever the driving conditions (drowsiness and tiredness r>0.83, p<.0001; driving self-confidence and drowsiness /or tiredness, r from -0.44 to -0.86, p<0.016 to p<.0001). Drowsiness and tiredness were not correlated with driving parameters (r from 0.03 to 0.27, p>0.140). In simulated driving condition, confidence was moderately correlated with ILC (r=-0.39, p=0.03) and with SDLP (r=-0.44 p=0.016), whereas in real driving condition, confidence was less correlated with SDLP (r=-0.34 p=0.097) and not correlated with ILC (r=-0.03, p=0.899).

DISCUSSION

In this study, we measured the effects of THC intake on driving performance and psychological state, as compared to placebo in a randomized study. We also evaluated differential effects of driving simulation and real driving conditions, in order to validate the driving simulator as a valuable tool for assessing drug-induced effects on car driving. We believe it was important to validate our driving

simulator with the most realistic conditions, which is why we chose smoked THC as a positive control. To evaluate specifically the simulator/real driving paradigm, we tried to include the most possible homogeneous population, controlling a wide range of confounding factors. All participants were young men, moderate tobacco users. We performed screening for illicit drug use, precession alcohol use, psychiatric disorders, abnormal body weight, heart, vision and nausea problems. Participants were requested to be well-rested before each session that was controlled by the use of actimetry. A timed smoking computerized procedure was applied to normalize numbers of inhalation and to minimize exposure variability. Finally, the simulated road environment was a high-fidelity reproduction of the same A62 freeway portion used for real driving.

Overall, the results show that THC intake had a detrimental effect on self-confidence to drive, drowsiness and tiredness on the psychological side. Moreover, driving performance is also significantly impaired, in terms of the capacity of the driver to stay in his/her driving lane, but only in simulation conditions. We analyzed the standard deviation of the lateral position (SDLP) of the vehicle, which is a validated way to precisely evaluate trajectory stability [44]. Results show that driving stability is impaired after THC intake, both in simulated and real driving conditions. Globally, these results are coherent with the results of previous studies involving simulation [46,47] or real driving conditions [16-18,28]. In Lenné et al's study, SDLP was significantly increased, as compared to placebo, after smoking cigarettes with 19 and 38 mg of THC, in occasional smokers [46]. In Ronen et al's, SDLP was also increased after 17 mg THC [47]. Comparable effects were observed in on-road studies. In Lamers & Ramaekers's study stable degradation of SDLP was observed after 40 min and 100 min of driving on a closed-course road [16]. Dose-dependent impairment was observed in Robbe's study [17].

More precisely, we found that inappropriate line crossings were significantly more numerous in driving simulation conditions, as compared to real driving. We selected ILC as our main outcome criterion to quantify driving impairment after THC intake, as several studies showed that accidents related to a diminution of awareness frequently occur after an ILC with a single car driving off the

road without reaction from the driver [43]. Other studies evaluating the effects sleepiness, alcohol or benzodiazepine intake on driving performance in simulated and real conditions reported similar results [13,24,25,27,28]. For example, Philip et al. studied the effects of sleepiness on driving performance of twelve subjects in real-life versus simulation and showed that real driving and driving simulators were comparable for measuring line crossings but that effects were magnified in the simulated condition [13]. Indeed, if ILC analysis is a pertinent and validate tool to evaluate driving performance, it is well-described that simulator could be more sensitive to detect driving impairment. Another advantage is that simulator could also provide the opportunity to measure easily other criteria such as EEG activity or muscle tone that may help to demonstrate potentially drug effects unobservable in classical real driving conditions.. Several hypotheses may be considered to explain a greater effect of the evaluated drug in the simulator compared to real driving. First, this effect might be due to the lack of sensorial (proprioceptive, vestibular) stimulation during driving on a fixed-base simulator, as compared to real driving conditions, favoring also distraction of the participant. On this point, further experimental studies should evaluate the interest of using a moving-base simulator. A second explanation might be inherent to the simulated conditions. Indeed, participants know that there is no actual risk in a driving simulator, which might also favor distraction and/or abnormal behavior. Unfamiliarity with the simulated driving experience could induce more errors, and the monotonous aspect of driving simulation could magnify drivers' loss of vigilance [27,28]. One last aspect of the difference between our simulation and real driving conditions was that, in real driving condition, a driving instructor was ready to counteract any abnormal behavior. In case of emergency situations, driving instructor had to take over control of the vehicle, so this action could induce a recovery of alertness of participants. In our study, we were unable to explore this hypothesis because information concerning the number of driving instructor interventions during the real driving sessions was not collected.

Another possible explanation was a difference of drug exposure in the simulator session compared to the real conditions. In our study, THC blood concentrations have reached a sufficient threshold related to impairment of motor control performance in both sessions. Indeed, a serum THC

range concentration at 2-5 µg/ml was related to an evident impairment in cognition [3,37]. As blood samples collections were performed at different time between the real driving conditions and the simulation driving, a detailed interpretation of these results appeared limited. However, THC and metabolites concentrations were similar to expected concentration usually described after THC consumption in occasional smokers in both sessions [48,49], so differences in THC exposure appears unlikely to explain the differential effects between simulated and real conditions. Nevertheless, this point underlines the difficulty to collect useful blood samples during real driving session that can be comparable to those collected more easily during simulated conditions. Using a Bayesian approach with population PK models that described pharmacokinetics parameters of THC could therefore represent an interesting tool allowing a more meticulous interpretation of drug concentrations [50,51].

All these factors might explain why an increase in inappropriate line crossings was only observed in simulation conditions after THC intake. On this aspect, one might speculate that driving simulation leads to abnormal behavior, as compared to real driving. We do not think this is the case. In fact, we suggest that driving simulation is a more precise evaluation of the effects of psychoactive drugs that real driving situations (these latter being moreover obviously more difficult to organize and more dangerous). Firstly, correlations between outcomes showed a similar pattern under the two driving conditions, apart from inappropriate line crossings which are poorly correlated in real driving condition possibly due to a lower range of values. Secondly, we found that similar effects of THC intake were observed when we analyzed the car's trajectory stability, which is a more precise evaluation of driving performance. With this indicator, although we still find a magnifying effect of driving simulation, the detrimental effect of THC intake becomes comparable between simulation and real driving conditions.

One limitation of these results could be the sample size of the study. Indeed, a total sample size of n=16 participants was required to detect a significant difference of 0.5 point on the line crossing criterion, with a significance level of 5% and a power of 95%. 11 participants actually

completed both the simulated and on-road driving sessions. This can be partly explained by the complexity of the study implementation and/or the constraining conditions of real driving session, resulting in uncompleted sessions for some subjects. Nevertheless, observed effects being greater than expected, it was possible to show significant results despite the small number of subjects. However, we agree that we cannot exclude a power effect for real driving session results. Another limitation could be that only men were included, in order to minimize variability, based on evidence for gender differences in the effects of THC. As our major objective was to validate a driving simulator method for assessing drugs effects on driving, we needed to include the most homogenous population as possible.

CONCLUSION

In conclusion, THC intake significantly impairs driving performance in both simulated and real conditions. Driving simulation appears to be a good qualitative predictor of driving safety after drug intake.

AUTHOR CONTRIBUTIONS

JM, JD, EJ and DM wrote the manuscript; JM, OB, OR, PP, EJ, CA, AD, JT, DM designed the research; JM, PP, CA, AD, JT, DM performed the study; BL performed and assessed all toxicological analysis, RT and EJ performed statistical analysis. All authors have revising the manuscript for intellectual content and approved the final version of the article.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Table I. Adverse events occurring during the study

Adverse events	Placebo	THC
Dry mouth	-	1
Nausea	1	-
Vomiting	-	2
Asthenia	1	2
Disturbance in attention	1	1
Dizziness	1	1
Presyncope	1	2
Somnolence	2	1
Tremor	-	1
Visual Pathway disorder	1	-
Hyperhydrosis	-	1
Hypotension	-	1
Total	8	13

Table II. Mean plasmatic concentrations (SD) of THC and its metabolites during simulator and real driving

	Time after smoking (hour)	THC (ng/mL)	THC-COOH (ng/mL)	THC-OH (ng/mL)
Simulator driving	1h45	3.6 (2.6)	6.5 (4.7)	0.5 (0.8)
	2h45	1.7 (1.6)	4.7 (2.9)	0.4 (0.7)
Real driving	0h45	10.3 (4.7)	9.6 (5.2)	2.8 (1.6)
	4h30	1 (2.3)	3 (2.1)	1 (1.1)

Table III. Least Square Mean (LSM) estimates and adjusted differences of LSM between placebo and THC of driving parameters from mixed model

		Inappropriate Line Crossings (log-transformed)		Standard Deviation of the vehicle's Lateral Position	
		Estimate [95% CI]	p-value	Estimate [95% CI]	p-value
Driving condition	Real	0.57 [0.33 ; 0.80]	<.0001	0.26 [0.23 ; 0.29]	<.0001
	Simulated	1.18 [0.97 ; 1.40]		0.32 [0.30 ; 0.35]	
Treatment	Placebo	0.80 [0.57 ; 1.02]	0.262	0.27 [0.25 ; 0.30]	0.005
	THC	0.95 [0.73 ; 1.17]		0.31 [0.28 ; 0.33]	
Driving condition x Treatment interaction	Real-Placebo	0.56 [0.25 ; 0.87]	0.316	0.25 [0.22 ; 0.28]	0.395
	Real-THC	0.58 [0.28 ; 0.88]		0.27 [0.24 ; 0.30]	
	Simulated-Placebo	1.03 [0.76 ; 1.31]		0.30 [0.27 ; 0.33]	
	Simulated-THC	1.33 [1.05 ; 1.60]		0.34 [0.31 ; 0.37]	
Sequence	THC- Placebo THC- Placebo	0.65 [0.33 ; 0.97]	0.202	0.26 [0.21 ; 0.30]	0.107
	THC- Placebo Placebo -THC	1.17 [0.76 ; 1.58]		0.33 [0.28 ; 0.38]	
	Placebo –THC THC- Placebo	0.92 [0.56 ; 1.28]		0.30 [0.26 ; 0.35]	
	Placebo –THC Placebo -THC	0.77 [0.34 ; 1.19]		0.27 [0.21 ; 0.32]	
Session	1	0.84 [0.62 ; 1.06]	0.616	0.29 [0.26 ; 0.31]	0.496
	2	0.91 [0.68 ; 1.13]		0.29 [0.27 ; 0.32]	
Differences of LSM between THC and placebo	Real	0.02 [-0.39 ; 0.43]	0.931	0.02 [-0.01 ; 0.06]	0.160
	Simulated	0.29 [-0.07 ; 0.66]	0.115	0.04 [0.01 ; 0.07]	0.006

Linear mixed model with REML estimation method, Satterthwaite approximation for computing the denominator degrees of freedom, fixed effect (treatment, driving condition, treatment x driving condition, session, sequence, random intercept and subject nested sequence.

FIGURE LEGENDS

Figure 1: Experimental schema of the study. Top: General path for a participant; Bottom: During a session.

Figure 2: flow diagram of the study through the four sessions. S1, S2, S3, S4: session 1, 2, 3, 4 respectively.

Figure 3: A. Driving self-confidence, evaluated on a 0-100 analog scale, after treatment intake and before driving. Data are averaged values across participants, with standard deviation, as a function of driving condition (simulated/driving) and treatment (THC/Placebo); B. Same graph for drowsiness scale; C. Same graph for tiredness scale.

Figure 4: A. Number of inappropriate line crossings (ILC, log scale), during the 2 hours driving period, as a function of driving condition (simulated/driving) and treatment (THC/Placebo); B. Same graph for standard deviation of lateral position on the road (SDLP).

Figure 5: Scatterplot of individual THC-Placebo differences of ILC (A) and SDLP (B) between driving conditions