Drug and Alcohol Dependence 247 (2023) 109888

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Ethanol enhances JWH-018-induced impairment of sensorimotor and memory functions in mice: From preclinical evidence to forensic implication in Driving Under the Influence of Drugs

Giorgia Corli [a,1], Micaela Tirri [a,1], Sabrine Bilel [a], Arianna Giorgetti [b], Tatiana Bernardi [c], Federica Boccuto [a], Martina Borsari [a], Raffaele Giorgetti [d], Matteo Marti [a,e,*]

[a] Department of Translational Medicine, Section of Legal Medicine, LTTA Center and University Center of Gender Medicine, University of Ferrara, Ferrara, Italy
[b] Department of Medical and Surgical Sciences, Unit of Legal Medicine, University of Bologna, Via Irnerio 49, Bologna, 40126, Italy
[c] Department of Environmental Sciences and Prevention, University of Ferrara, Ferrara, 44121, Italy
[d] Department of Excellence of Biomedical Science and Public Health, Faculty of Medicine, Polytechnic University of Marche, Ancona, Italy
[e] Collaborative Center for the Italian National Early Warning System, Department of Anti-Drug Policies, Presidency of the Council of Ministers, Italy

ARTICLE INFO

Keywords:
NPS
Synthetic cannabinoid
JWH-018
Ethanol
Novel Object Recognition
DUID

ABSTRACT

Background: Several new Synthetic Cannabinoids have appeared each year since their introduction into the illicit drug market as recreational drugs. Among these, naphtalen-1-yl-(1-pentylindol-3-yl) methanone (JWH-018) is one of the most detected compounds in biological samples from patients involved in intoxication or death cases. Furthermore, consumption of JWH-018 has been linked to several cases of Driving Under the Influence of Drugs (DUID) suggesting that effects induced by this compound can affect individuals' ability to drive.
Methods: Given the high spread of polydrug consumption and the wide number of alcohol-related traffic accidents, this study aims to investigate the acute effects induced by co-administration of JWH-018 with ethanol on sensorimotor and motor responses, grip strength and memory functions in CD-1 male mice. Acute impairments induced by JWH-018 and ethanol alone have also been investigated, in order to compare their effects with that induced by their concurrent administration.
Results: In vivo behavioral experiments revealed a worsening of the cognitive and sensorimotor disruption after the co-administration of JWH-018 with ethanol compared to single compounds.
Conclusions: These animal-based findings suggest a potential increased impairment on psychomotor performances which could be related to driving abilities posed by poly-drug consumption involving SCs and ethanol.

1. Introduction

Over the last years, emerging Novel Psychoactive Substances (NPS) in the illicit drugs market have been steadily on rise (Luethi and Liechti, 2020). A large number of NPS is seized every year in European countries and, among these, Synthetic Cannabinoids (SCs) currently dominate seizures, continuing to emerge and resulting in severe risks for public health and regulatory systems (EMCDDA, 2022). Those are usually consumed for their cannabis-like effects, owed to their action on cannabinoid receptors. Nevertheless, they display greater affinity for such receptors with respect to the main psychoactive component of *Cannabis sativa* plant delta-9-tetrahydrocannabinol (Δ^9-THC; Alves et al., 2020). Therefore, their consumption has been linked to high potential risk of intoxication due to their pharmacological effects (Cohen and Weinstein, 2018; Adamowicz et al., 2019; Tamama and Lynch, 2020; Giorgetti et al., 2021). SCs or synthetic cannabinoid receptors agonists (SCRAs) are usually sold online under different brand names such as 'Spice', 'K2' or 'Magic gold', and include a wide range of compounds (Poklis et al., 2012; EMCDDA, 2017). They have been first identified as recreational drugs in 2008 (Auwärter et al., 2009) and the

Abbreviations: JWH-018, Naphtalen-1-yl-1-pentylindol-3-ylmethanone; Δ^9-THC, Δ9-tetrahydrocannabinol; SCs, Synthetic Cannabinoids; NPS, Novel Psychoactive Substances; DUID, Driving Under the Influence of Drugs.

* Correspondence to:Department of Translational Medicine, Section of Legal Medicine, LTTA Center and University Center of Gender Medicine, University of Ferrara, via Fossato di Mortara 17-19, Ferrara, 44121, Italy.
E-mail address: matteo.marti@unife.it (M. Marti).
[1] Authors equally contributed to the manuscript.

https://doi.org/10.1016/j.drugalcdep.2023.109888
Received 6 March 2023; Received in revised form 3 April 2023; Accepted 14 April 2023
Available online 18 April 2023

well-known JWH-018 is considered as the first SC detected in herbal smoking products in Europe (Fattore and Fratta, 2011; EMCDDA, 2017). JWH-018 is a napthoylindole that retains nanomolar affinity for CB_1 cannabinoid receptors (Ki=9.5±4.5 nM; Huffman and Padgett, 2005). It is still present on the illicit market (Vučinić et al., 2018; Oberenko et al., 2019) and has been involved in several intoxication and death cases. Noteworthy, recent *in vitro* (Fietzke et al., 2016) and *in vivo* (Tirri et al., 2022) metabolic studies have shown it as active metabolite of the synthetic cannabinoid JWH-175. Alone or with other psychoactive substances JWH-018 has been related to adverse effects, among which anxiety, palpitations, tachycardia, convulsions, and death (Simmons et al., 2011; Schneir and Baumbacher, 2012; EMCDDA, 2017; Darke et al., 2020; Giorgetti et al., 2020).

Moreover, it has been pointed out that intoxication by this substance can lead to cognitive and psychomotor impairment (Theunissen et al., 2021; Orazietti et al., 2022). In fact, JWH-018 has been detected in biological samples of drivers involved in cases of Driving Under the Influence of Drugs (DUID) (Musshoff et al., 2014; Tuv et al., 2014; Karinen et al., 2015).

Driving under the influence of psychoactive substances has been among the most significant issues for road safety over the decade (EMCDDA, 2012; Ji Kwon and Han, 2019). Particularly, SCs were mainly reported in DUID cases registered in several European countries between 2013 and 2018 (Jaenicke et al., 2014; Tuv et al., 2014; Ji Kwon and Han, 2019) and JWH-018 has been one of the most detected compounds (Tuv et al., 2014). On the other hand, driving under the influence of alcohol or alcohol combined with other psychoactive substances have been considered as the leading causes of road accidents in the past years (WHO, 2018; Ji Kwon and Han, 2019). Already in the early 2000 s, the constantly increasing number of fatal events due to polydrugs consumption was pointed out and alcohol and cannabinoids accounted for more than the 50% of reported combinations of drugs used for recreational purposes on the same night (EMCDDA, 2002). In line with this, the recent report states that poly-consumption is a currently common behavior. However, the wide-ranging trends of consumption (from occasional to chronic) make the evaluation of this phenomenon a challenging issue (EMCDDA, 2021). Moreover, previous findings highlighted the significant spread of poly-drug consumption, which can lead to an increased risk of suffering driving-related severe injuries or death (Wille et al., 2018). Together with other psychoactive drugs , ethanol has been notably identified in biological samples from drivers who tested positive for SCs (Musshoff et al., 2014; Tuv et al., 2014; Karinen et al., 2015).

However, experimental data regarding the effects of SCs on psychomotor performances relevant for driving and, especially, on the impact of the co-consumption of SCs and other drugs are still lacking (Orazietti et al., 2022). Human studies are mostly limited to case reports and case series, and often scientific evidence has to be drawn, with all the possible limitations, from preclinical studies (Orazietti et al., 2022), Effects and adverse effects typically induced by SCs are also depending on their metabolic features. To date, JWH-018 metabolites which retains high activity for both cannabinoid CB_1 (Brents et al., 2011) and CB_2 (Rajasekaran et al., 2013) receptors have been identified. On the other hand, Seely and colleagues demonstrated that a major glucuronidated metabolite of JWH-018 antagonizes parent drug activity on CB_1 receptors (Seely et al., 2012). Thus, it could be relevant to better evaluate the potential toxicity of these compounds alone or combined with other psychoactive substances since further studies have also shown SCs interaction with drug-metabolizing enzyme CYP450 and transporters activity (Kong et al., 2018; Kim et al., 2020). Along with these findings, preclinical studies have previously pointed out that JWH-018 and its halogenated derivatives impaired sensorimotor and motor responses, as well as affected physiological conditions and short- and long-term working memory in mice (Ossato et al., 2015; Barbieri et al., 2016; Bilel et al., 2020). Despite this, the lack of information on how SCs and poly-drug consumption possibly impact on road safety should be considered. Moreover, global epidemiological data concerning the Alcohol Use Disorder (AUD) state a higher average previous year and lifetime prevalence for male (3.6% and 14.1% respectively) than female (0.9% and 3.4% respectively) for all countries (Glantz et al., 2020). Again, the lifetime prevalence of use of SCs estimated in surveys among young adults in Europe is higher for men (3.5%) than women (2.7%; ESPAD, 2019). In line with these findings, previous reports reveal that male patients (78%) have required emergency room assistance in the past decades more frequently than female patients (22%) after suffering from SCs-related intoxications (Bush and Woodwell, 2014). Indeed, a recent review article has assessed a higher prevalence for Driving Under the Influence (DUI) of cannabinoids among male (3.5%) respect to female drivers (1.6%; Pelletti et al., 2022). Noteworthy, this agrees with a significant different gender distribution highlighted in cases of DUID registered by a case control study concerning Norway. In particular, 88.6% of the total cases considered involved men (Jamt et al., 2019). Relying on these findings the risk of suffering from intoxication or injury related to the use of these compounds appears greater for men. Thereby, since 35% of recent fatal road accidents have been associated with alcohol consumption (WHO, 2018), the purpose of this study is the investigation of acute effects induced by combined administration of JWH-018 and ethanol on sensorimotor and motor responses, grip strength and short- and long-term working memory of adult male mice. Acute impairments induced by JWH-018 and ethanol alone have been investigated, in order to compare them with that induced by their concurrent administration.

2. Materials and methods

2.1. Animals

Male ICR (CD-1®) mice, 3–4 months old, weighing 25–30 gr (ENVIGO Harlan Italy, Italy), were group-housed (8–10 mice per cage; floor area/animal: 80 cm2; minimum enclosure height: 12 cm) on a 12:12-h light-dark cycle (light on at 6:30 AM), the temperature of 20–22 °C, the humidity of 45–55% and were provided *ad libitum* access to food (Diet 4RF25 GLP; Mucedola, Settimo Milanese, Milan, Italy) and water. Experimental protocols were performed in accordance with the European Communities Council Directive of September 2010 (2010/63/EU) a revision of the Directive 86/609/EEC were approved by the Ethics Committee of the University of Ferrara and by Italian Ministry of Health (license n° 223/2021-PR and extension CBCC2.46.EXT.21). Adequate measures were taken to reduce the number of employed animals and their pain and discomfort. In the analysis of behavioral (sensorimotor and motor) responses for each treatment [vehicle, EtOH (0.1 g/kg), and co-administration of 2 different JWH-018 doses (0.01 and 0.1 mg/kg) with ethanol: 8 mice; 2 different dosages of JWH-018 (0.01 and 0.1 mg/kg): 6 mice] and memory performance for each treatment [vehicle, EtOH (0.1 g/kg), 2 different dosages of JWH-018 (0.01 and 0.1 mg/kg), and co-administration of 2 different JWH-018 doses (0.01 and 0.1 mg/kg) with ethanol: 8 mice] a total number of 92 mice were used.

2.2. Drug preparation and dose selection

Ethanol was purchased from BioUltra (ethanol for molecular biology, ≥99.8%; Sigma-Aldrich) and was diluted with saline solution (0.9% NaCl; Eurospital, S.p.A, Italy) to obtain a dose of 0.1 g/kg (De Giorgio et al., 2021) and administered by using oral gavage (o.g.) needles at a volume of 4 µl/gr (Arfè et al., 2021). JWH-018 was purchased from LGC Standards (LGC Standards, Milan, Italy). The compound was initially dissolved in absolute ethanol (final concentration: 2%) and Tween 80 (2%) and brought to the final volume with saline (0.9% p/v NaCl) and the solution made with ethanol, Tween 80 and saline was also used as vehicle. JWH-018 was administered by intraperitoneal injection (i.p.) at a volume of 4 µl/gr. In order to compare the effect of treatments, doses of JWH-018 (0.01 and 0.1 mg/kg; i.p.) and ethanol (0.1 g/kg; o.g.) were chosen using interspecies dose scaling (Nair and Jacob, 2016) and

basing on our previous studies (Ossato et al., 2015; Bilel et al., 2020; De-Giorgio et al., 2021). Mice treated with co-administration of JWH-018 and ethanol received an oral gavage dosing and, 10 min later, an intraperitoneal injection. Likewise, the vehicle group received saline by oral gavage and, 10 min later, saline by intraperitoneal injection. The following regimen was tested: saline alone, ethanol alone at a dosage of 0.1 g/kg, JWH-018 alone at dosages of 0.01 and 0.1 mg/kg, 0.1 g/kg ethanol + 0.01 mg/kg JWH-018, and 0.1 g/kg ethanol + 0.1 mg/kg JWH-018.

2.3. Behavioral tests

In the present study, effect induced by JWH-018 and ethanol on sensorimotor responses was investigated using a battery of behavioral tests widely used in studies of "safety-pharmacology" and routinely adopted in our laboratory for the preclinical characterization of new molecules in rodents (Ossato et al., 2015; Bilel et al., 2020; Arfè et al., 2021). Voluntary and involuntary motor responses of the animal to different visual, acoustic, and tactile stimuli were evaluated according to the procedure previously described by Ossato et al., 2015. To reduce the number of animals used, mice were evaluated in functional observational tests carried out in a consecutive manner according to the following time scheme: observation of visual object responses (frontal and lateral view), acoustic response, tactile response (vibrissae, corneal, and pinnae reflexes) and visual placing response. Behavioral tests were conducted in a thermostated (temperature: 20–22 °C, humidity: 45–55%) and light (150 lux) controlled room with a background noise of 40 ± 4 dB. The apparatus for the visual object, acoustic and tactile sensorimotor tests consisted of an experimental chamber (350 ×350×350 mm) with black methacrylate walls and a transparent front door. During the week before the experiment, each mouse was placed in the box and handled (once a day) every other day, i.e. 3 times, to get used to both the environment and the experimenter. To avoid mice olfactory cues, cages were carefully cleaned with a dilute (5%) ethanol solution and rinsed with water. All experiments were performed between 8:30 AM to 2:00 PM and conducted blindly by trained observers working in pairs (Ossato et al., 2015). The behavior of mice was videotaped by a camera (B/W USB Camera day&night with varifocal lens; Ugo Basile, Italy) placed at the top or on one side of the box and analyzed off-line by a different trained operator.

2.3.1. Evaluation of the visual response

Visual response was verified by two behavioral tests which evaluated the ability of the animal to capture visual information when the animal is stationary (the visual object response) or moving (the visual placing response).

Visual object response test was performed to evaluate the ability of the mouse to see an object approaching from the front (frontal view) or the side (lateral view) that typically induces the animal to shift or turn the head or retreat from it. For the frontal visual response, a white horizontal bar was moved frontally to the mouse head and the maneuver was repeated 3 times. For the lateral visual response, a small dentist's mirror was moved into the mouse's field of view in a horizontal arc, until the stimulus was between the mouse's eyes. The procedure was conducted bilaterally and was repeated 3 times (Ossato et al., 2015). The score assigned was 1 if there was a reflection in the mouse movement or 0 if it was not present. The total value was calculated by adding the scores obtained in the frontal with those obtained in the lateral visual object response test (overall score: 9). Sensorimotor tests were performed at 10, 30, 60, 120, 180, 240, 300 min, and 24 hrs after administrations.

Visual Placing response test was performed using a tail suspension modified apparatus able to bring down the mouse towards the floor at a constant speed of 10 cm/sec (Ossato et al., 2015). The downward movement of the mouse was videotaped by a camera (B/W USB Camera day&night with varifocal lens; Ugo Basile, Italy) placed at the base of the tail suspension apparatus. Movies were analyzed off-line by a trained operator who was unaware of the drug treatments performed. The analysis frame by frame allows evaluating the beginning of the reaction of the mouse while it was approaching the floor. The first movement of the mouse when it perceives the floor is the extension of the front legs. When the mouse started the reaction, an electronic ruler evaluated the perpendicular distance in millimeters between the eyes of the mice to the floor. Untreated control mice typically perceive the floor and prepare to contact at a distance of about 23.6 ± 4.8 mm. Sensorimotor tests were performed at 10, 30, 60, 120, 180, 240, 300 min, and 24 hrs after the administrations.

2.3.2. Evaluation of acoustic response

Acoustic response measures the reflex of the mouse in response to an acoustic stimulus produced behind the animal (Ossato et al., 2015). In particular, four acoustic stimuli of different intensity and frequency were tested. A snap of the fingers (four snaps repeated in 1.5 sec), a sharp click (produced by a metal instrument; four clicks repeated in 1.5 sec), an acute sound (produced by an audiometer; frequency: 5.0–5.1 kHz) and a severe sound (produced by an audiometer; frequency: 125–150 Hz). Each test was repeated 3 times. The score assigned was 1 if there was a response or 0 if it was not present, for a total score of 3 for each sound. The acoustic total score was calculated by adding the scores obtained in the four tests (overall score: 12). The background noise (about 40 ± 4 dB) and the sound from the instruments were measured with a digital sound level meter. Sensorimotor tests were performed at 10, 30, 60, 120, 180, 240, 300 min, and 24 hrs after administrations.

2.3.3. Evaluation of tactile response

Tactile response in the mouse was verified through vibrissae, corneal and pinnae reflexes (Ossato et al., 2015). Data is expressed as the sum of the three above-mentioned parameters. *Vibrissae reflex* was evaluated by touching vibrissae (right and left) with a thin hypodermic needle once for side giving a value of 1 if there was a reflex (turning of the head to the side of touch or vibrissae movement) or 0 if not present (overall score: 2). *Corneal reflex* was assessed by gently touching the cornea of the mouse with a thin hypodermic needle and evaluating the response: the score assigned was 1 if the mouse moved only the head, 2 if it only closed the eyelid, 3 if it closed the lid and moved the head. The procedure was conducted bilaterally (overall score: 6). *Pinna reflex* was assessed by touching pavilions (left and right) with a thin hypodermic needle: first the interior pavilions and then the external. This test was repeated twice for side giving a score of 1 if a reflex was present and 0 if it was not present (overall score: 4). Sensorimotor tests were performed at 10, 30, 60, 120, 180, 240, 300 min, and 24 hrs after administrations.

2.3.4. Motor activity

Motor activity alterations were measured performing the *Drag test* and the *Accelerod test* (Ossato et al., 2015).

The *Drag test* measures the ability of the animal to balance the body posture with the front legs in response to an externally dynamic stimulus (Marti et al., 2005). The mouse was lifted by the tail, leaving the front paws on the table and dragged backward at a constant speed of about 20 cm/s for a fixed distance (100 cm). The number of steps performed by each paw was recorded by two different observers. For each animal from five to seven measurements were collected. The drag test was performed at 45, 70, 105, 160, 220, 280, 340 min, and 24 hrs after administrations.

The *Accelerod test* measures different motor parameters, such as motor coordination, locomotive ability (akinesia/bradykinesia), balance ability, muscular tone and motivation to run. The animals were placed on a rotating cylinder that increases velocity automatically in a constant manner (0–60 rotations/min in 5 min). The time spent on the cylinder was measured. The accelerod test was performed at 40, 65, 95, 150, 210, 270, 330 min, and 24 hrs after administrations.

2.3.5. Evaluation of skeletal muscle strength (grip strength)

Grip strength test was used to evaluate the skeletal muscle strength of mice (Arfè et al., 2021). The grip strength apparatus (ZP-50 N, IMADA) is consisted of a wire grid (5 × 5 cm) connected to an isometric force transducer (dynamometer). Mice were held by their tails and allowed to grasp the grid with their forepaws, to perform the test. Then, mice were gently pulled backward by the tail until the grid was released. The average force exerted by each mouse before losing its grip was recorded. The mean average force was then determined calculating the mean of three measurements for each animal. The skeletal muscle strength is expressed as gram force (gf), recorded and processed using IMADA ZP-Recorder software. Grip strength was measured at 0, 15, 35, 70, 125, 185, 245, 305 min, and 24 hrs after administrations.

2.3.6. Novel Object Recognition test

The NOR test represents a ‘‘pure’’ working memory task entirely based on the spontaneous exploratory behavior of rodents towards objects (Ennaceur and Meliani, 1992; Ennaceur et al., 1997). Procedure consists of three phases defined as habituation, familiarization and choice (Antunes and Biala, 2012; Barbieri et al., 2016). During the habituation phase, each animal was placed into the NOR chamber (a square open field 60 cm×60 cm x 40 cm, dark PVC plastic box) located in a dimly lit (50 lux), sound-attenuated and acclimatized room. Mice were allowed to freely explore the box for 20 min/day for three days and no objects were placed in during this trial. Twenty-four hours after the last habituation trial, the familiarization phase was performed by placing the mouse in the field in which two identical objects (A, A) were disposed on adjacent corners approximately 6 cm from the walls. Mice were placed at the mid-point of the wall opposite to the objects and allowed to explore them for 15 min. After 15 min from the familiarization phase, mice were injected with vehicle or drugs and two consecutive choice sections were performed 2 hrs (short-term memory) and 24 hrs (long-term memory) after the administration. During the choice trial performed 2 hrs after the injection, one of the two familiar objects (A) was replaced with a new one (novel; B), which was different in shape, dimension and color. Each mouse was then placed in the apparatus and allowed to freely explore the objects (A and B) for 5 min. During the choice trial performed 24 hrs after the injection, the mice explored the open field for 5 min in the presence of one familiar (A) and one novel object (C, different from B). Exploration was defined as the time (sec) during which the mouse nose was in touch with the object or directed toward it at a close distance (~2 cm). Turning around the object was not considered as exploratory behavior. All experimental trials were performed using the ANY-maze video tracking system (Ugo Basile, application version 4.99 g Beta) and subsequently analyzed by an observer blind to the mouse treatment and to which object was the novel or displaced one (Barbieri et al., 2016). Exploration time of familiar (A) and novel (B) object was detected. The novel object preference was quantified as Recognition Index (RI) calculated as: (novel B - familiar A)/(novel B - familiar A). Scores approaching zero reflects no preference (impairment of recognition memory), positive values reflect preference for the novel object (good recognition memory) while negative numbers reflect preference for the familiar one (impairment of recognition memory). Moreover, the total exploration time (sec) spent by the animal during the choice phase performed 2 hrs (familiar A - novel B) and 24 hrs (familiar A - novel C) after the injection was calculated to investigate the effect of drugs on object exploration. During both choice trials, executed 2 and 24 hrs after administration, spatial memory has been also evaluated. After each above-mentioned choice sections, identical objects previously employed in the familiarization phases were placed in the open-field arena, but one of them was displaced from the original position (Williams et al., 2007). Mice were then tested, and displaced object preference and total exploration time were quantified as described for novel object recognition. Seven sets of novel and familiar objects of different material (plastic, glass or ceramic), shape (cube, parallelepiped and cylinder), dimension (height: 3×8 cm; width: 6×8) and color (light yellow, red and blue) were employed. To prevent object material from interfering with mouse preference, objects of different material (plastic, glass or ceramic) were randomly used and the use of plastic, glass or ceramic objects were balanced among the different groups (doses and drugs). The set of objects used in the familiarization phase (two identical A, A objects) was used in the subsequent vehicle/drug conditions 2 and 24 hrs after the administration. The choice of object for novel or familiar was counter balanced and the position of each object was also alternated between trials to avoid any misinterpretation of data. Objects weight enough to not be displaced by mice. To avoid mice olfactory cues, objects and apparatus were carefully cleaned with a dilute (5%) ethanol solution and rinsed with water between each animal trials and also between familiarization and choice (executed 2 and 24 hrs after the familiarization phase) phases. Animals that spent less than 10 s exploring both objects were excluded from the study and replaced by other animals.

2.4. Statistical analysis

In behavioral response experiments data are expressed in arbitrary units (visual objects response, acoustic response, overall tactile reflex) and percentage of baseline (visual placing response, drag, rotarod and grip strength tests). All data are shown as mean ± SEM of 6 or 8 independent experimental replications. Statistical analysis of the effects of each compound at different concentrations over time (Figs. 1A, C,2A, C,3A, C and D) was performed by two-way ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons. Analysis of the total average effect induced between 0 and 110–135 min (T1), and between 145 and 350 min (T2) by treatments (Figs. 1B, D, 2C, D and 3B) was performed with one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons. Statistical analyses were performed using the program Prism software (GraphPad Prism, San Diego CA).

3. Results

3.1. Evaluation of the visual object response

Visual object response did not change in vehicle-treated mice (Fig. 1A) and effect was similar to that observed in naïve untreated animals (data not shown). Ethanol alone did not affect the visual response of mice (Fig. 1A and B). Systemic administration of JWH-018 (0.01 and 0.1 mg/kg; i.p.) and its co-administration with ethanol (0.1 g/kg; o.g.) reduced the visual object response in mice (Fig. 1A; significant effect of treatment ($F_{40,360}$=5.977, $p<0.0001$), time (F_{8360}=133.1, $p<0.0001$) and time x treatment interaction (F_{5360}=89.52, $p<0.0001$)). Specifically, 0.01 mg/kg of JWH-018 induced an inhibitory effect during the first 30 min, while the effect induced by the dose of 0.1 mg/kg persisted up to 60 min. Similarly, co-administration of 0.01 mg/kg of JWH-018 with ethanol produced a significant impairment up to 60 min. Otherwise, effects induced by the co-administration of the high dose of JWH-018 with ethanol persisted up to 180 minutes. Noteworthy, the effect did not persist up to 24 hrs. Total average effect across fixed time periods (T1: from 0 to 110–135 min; T2: from 145 to 350 min) were then considered, to evaluate the duration of effect. High dose of JWH-018 (0.1 mg/kg; i.p.) alone and JWH-018 (0.01 and 0.1 mg/kg; i.p.) co-administration with ethanol decreased the visual object response, during T1 (Fig. 1B; significant effect of treatment ($F_{5,40}$=34.47, $p<0.0001$)). Only co-administration of the high dose tested of JWH-018 (0.1 mg/kg; i.p.) with ethanol impaired visual response, during T2 (Fig. 1B, significant effect of treatment ($F_{5,40}$=8.538, $p<0.0001$)).

3.2. Evaluation of the visual placing response

Visual placing response did not change in vehicle-treated mice (Fig. 1C) and effect was similar to that observed in naïve untreated

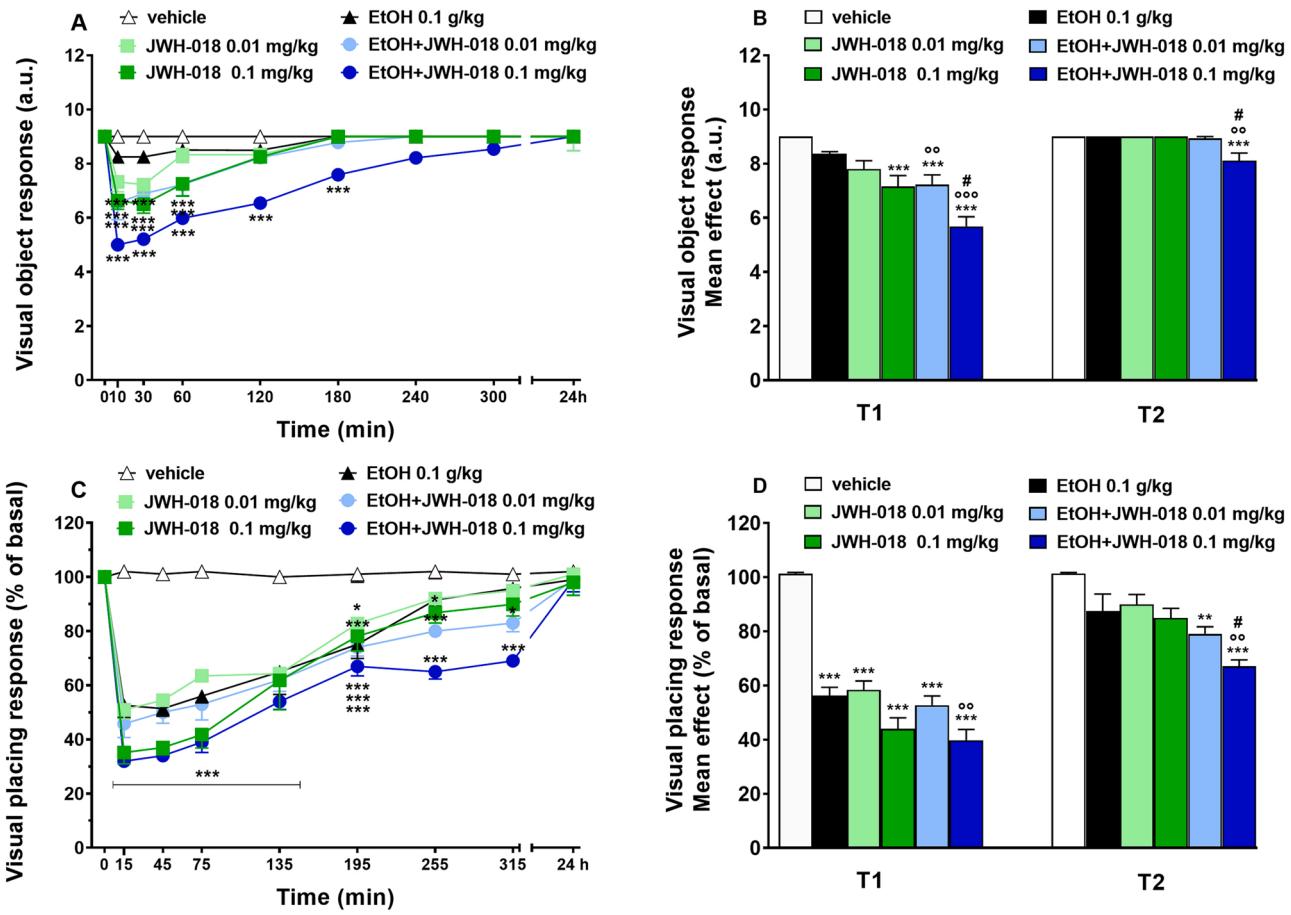

Fig. 1. Effect of ethanol (0.1 g/kg), JWH-018 (0.01 and 0.1 mg/kg) and co-administration of the two substances on the visual object test (A) and visual placing test (C) in mice; total average effect of ethanol, JWH-018 and co-administration of the two substances on the visual object test (B) and visual placing test (D), between 0 and 110–135 min (T1), and between 145 and 350 min (T2). Data are expressed as arbitrary units (a.u.; visual object test) or percentage of the baseline (visual placing test) and represent the mean ± SEM of 6 or 8 determinations for each treatment. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test (A and C) for multiple comparison for the dose-response curve of each compound at different time-points. The analysis of the mean overall effect induced by each compound and substances co-administration were performed with one-way ANOVA followed by Tukey's test (B and D). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle; °°$p < 0.01$, °°°$p < 0.001$ versus JWH-018 alone; #$p < 0.05$ versus ethanol + JWH-018 (0.01 mg/kg).

animals (data not shown). JWH-018 (0.01 and 0.1 mg/kg; i.p.), ethanol (0.1 g/kg; o.g.) and their co-administration long lastingly inhibited the visual placing response in mice up to 315 min (Fig. 1C; significant effect of treatment ($F_{40.360}$=5.882, p<0.0001), time (F_{8360}=64.73, p<0.0001) and time x treatment interaction (F_{5360}=58.48, p<0.0001)). Noteworthy, the effect did not persist up to 24 hrs.

Ethanol (0.1 g/kg), JWH-018 (0.01 and 0.1 mg/kg; i.p.) and their co-administration long lastingly inhibited the visual placing response of mice, during T1 (Fig. 1D; significant effect of treatment ($F_{5,40}$=13.46, p<0.0001)). Co-administration of JWH-018 (0.01 and 0.1 mg/kg; i.p.) and ethanol induced impaired visual response, during T2 (Fig. 1D; significant effect of treatment ($F_{5,40}$=8.700, p<0.0001)).

3.3. Evaluation of the acoustic response

Acoustic response did not change in vehicle-treated mice (Fig. 2A) and effect was similar to that observed in naïve untreated animals (data not shown). Ethanol alone did not affect the acoustic response of mice (Fig. 2A and B). Systemic administration of JWH-018 (0.01 and 0.1 mg/kg; i.p.) and its co-administration with ethanol (0.1 g/kg; o.g.) transiently inhibited the acoustic response in mice (Fig. 2A, significant effect of treatment ($F_{40,360}$=5.796, p<0.0001), time (F_{8360}=47.88, p<0.0001) and time x treatment interaction (F_{5360}=32.33, p<0.0001)). In particular, JWH-018 alone and co-administration of 0.01 mg/kg of JWH-018 with ethanol reduced responses up to 60 min. Otherwise, effects induced by the co-administration of high dose of JWH-018 (0.1 mg/kg; i.p.) with ethanol persisted up to 120 min. Noteworthy, the effect did not persist up to 24 hrs.

Both JWH-018 (0.01 and 0.1 mg/kg; i.p.) alone and its co-administration with ethanol impaired acoustic response of mice, during T1 (Fig. 2B, significant effect of treatment ($F_{5,40}$=16.82, p<0.0001)). On the other hand, JWH-018 and its co-administration with ethanol did not affect the acoustic response of mice during T2.

3.4. Evaluation of the overall tactile reflex

Overall tactile reflex did not change in vehicle-treated mice (Fig. 2C) and effect was similar to that observed in naïve untreated animals (data not shown). Ethanol alone did not alter overall tactile responses of mice (Fig. 2A and B). Systemic administration of JWH-018 (0.01 and 0.1 mg/kg; i.p.) and its co-administration with ethanol (0.1 g/kg; o.g.) promptly and transiently inhibited overall tactile reflexes (Fig. 2C; significant effect of treatment ($F_{40,360}$=7.102, p<0.0001), time (F_{8360}=49.81, p<0.0001) and time x treatment interaction (F_{5360}=49.87, p<0.0001)). In particular, JWH-018 alone and co-administration of 0.01 mg/kg of JWH-018 with ethanol reduced responses up to 60 min. Otherwise, effects induced by the co-administration of high dose of JWH-018 (0.1 mg/kg; i.p.) with ethanol persisted up to 180 min. Noteworthy, the effect did not persist up to 24 hrs.

High dose of JWH-018 (0.1 mg/kg; i.p.) and its co-administration

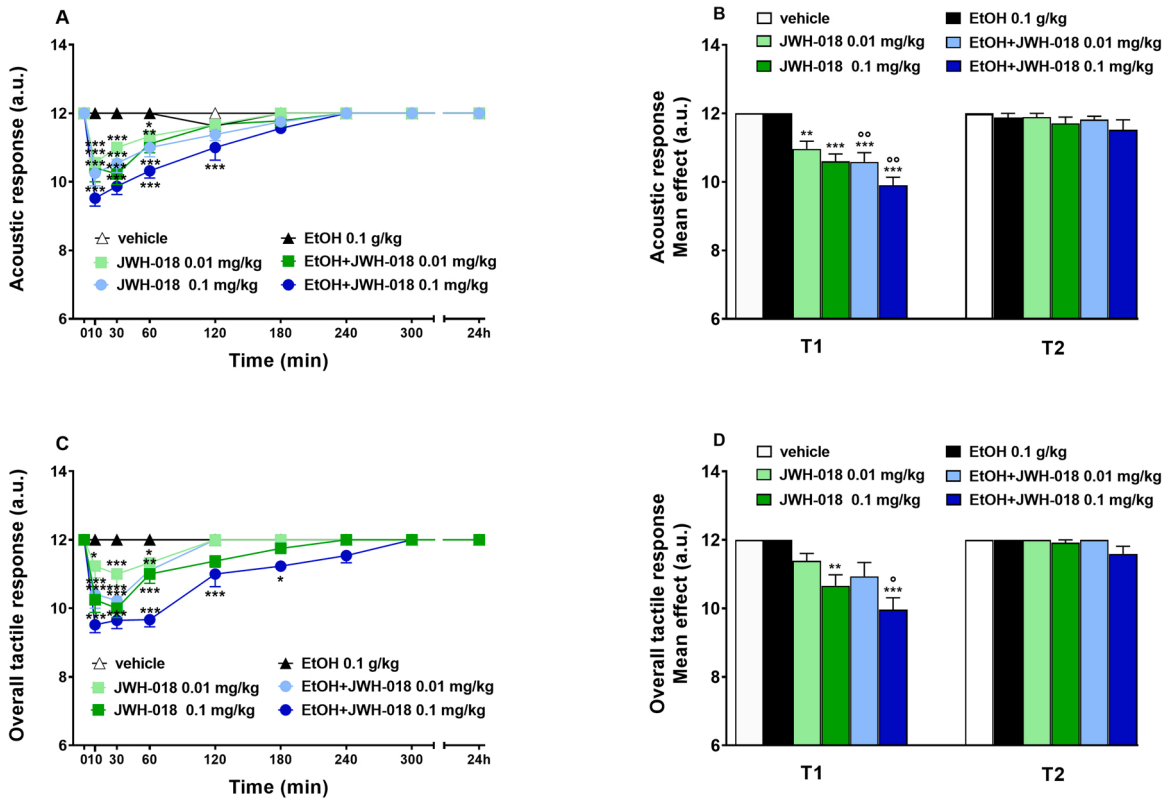

Fig. 2. Effect of ethanol (0.1 g/kg), JWH-018 (0.01 and 0.1 mg/kg) and co-administration of the two substances on the acoustic (A) and overall tactile response (C) in mice; total average effect of ethanol, JWH-018 and co-administration of the two substances on the acoustic (B) and overall tactile response (D), between 0 and 110–135 min (T1), and between 145 and 350 min (T2). Data are expressed as arbitrary units (a.u.) and represent the mean ± SEM of 6 or 8 determinations for each treatment. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test (A and C) for multiple comparison for the dose-response curve of each compound at different time-points. The analysis of the mean overall effect induced by each compound and substances co-administration were performed with one-way ANOVA followed by Tukey's test (B and D). *p < 0.05, **p < 0.01, ***p < 0.001 versus vehicle; °p < 0.05, °°p < 0.01 versus JWH-018 alone.

with ethanol impaired responses of mice, during T1 (Fig. 2D; significant effect of treatment ($F_{5,40}$=7.990, p<0.0001)). On the other hand, JWH-018 and its co-administration with ethanol did not alter the overall tactile response during T2 (Fig. 2B, significant effect of treatment ($F_{5,40}$=2.662, p=0.0361)).

3.5. Evaluation of number of steps

Number of steps remains unchanged in vehicle-treated mice over the 5 hrs observation (Fig. 3A) and effect was similar to that observed in naïve untreated animals (data not shown). Ethanol alone, JWH-018 alone and co-administration of 0.01 mg/kg of JWH-018 with ethanol did not alter the number of steps (Fig. 3A and B). Systemic administration of the high dose of JWH-018 (0.1 mg/kg; i.p.) with ethanol (0.1 g/kg; o.g.) reduced the number of steps between 50 and 110 min (Fig. 3A; significant effect of treatment ($F_{40,360}$=0.6530, p<0.9497), time (F_{8360}=3.140, p=0.0019) and time x treatment interaction (F_{5360}=3.655, p=0.0031)). Noteworthy, the effect did not persist up to 24 hrs.

Co-administration of the high dose of JWH-018 (0.1 mg/kg; i.p.) with ethanol induced a significant decrease of the number of steps during T1 (Fig. 3B, significant effect of treatment ($F_{5,40}$=6.782, p=0.0001)), but the effect did not persist up to T2.

3.6. Evaluation of grip strength

Grip strength did not change in vehicle-treated mice (Fig. 3C) and effect was similar to that observed in naïve untreated animals (data not shown). Ethanol (0.1 g/kg; o.g.) alone, JWH-018 (0.01 and 0.1 mg/kg; i.p.) or their co-administration did not affect the muscular strength of mice (Fig. 3C).

3.7. Evaluation of time on rod

Time on rod did not change in vehicle-treated mice over the 5 hrs observation (Fig. 3D) and effect was similar to that observed in naïve untreated animals (data not shown). Ethanol (0.1 g/kg; o.g.) alone, JWH-018 (0.01 and 0.1 mg/kg; i.p.) alone and its co-administration did not affect time on rod (Fig. 3D).

3.8. Evaluation of Novel Object Recognition

In order to evaluate the effect on the object and spatial memory retention in mice, Novel Object Recognition (NOR) test was performed (Barbieri et al., 2016). During the familiarization phase, time spent by mice investigating the objects did not change (data not shown). There was no difference between vehicle-treated and control mice in the NOR test (data not shown). As indicated by the Recognition Index (RI) values, object memory was affected both 2 (Fig. 4A; significant effect of treatment ($F_{5,42}$=25.82, p<0.0001)) and 24 hrs (Fig. 4A; significant effect of treatment ($F_{5,42}$=20.97, p<0.0001)) after the administration of the high dose of JWH-018 (0.1 mg/kg; i.p.) alone or JWH-018 (0.01 and 0.1 mg/kg; i.p.) co-administration with ethanol (0.1 g/kg). Similarly, spatial memory was affected both 2 (Fig. 4C; significant effect of treatment ($F_{5,42}$=25.78, p<0.0001)) and 24 hrs (Fig. 4C; significant effect of treatment ($F_{5,42}$=13.56, p<0.0001)) from the administration of the high dose of JWH-018 or co-administration of JWH-018 (0.01 and 0.1 mg/kg; i.p.) with ethanol (0.1 g/kg).

The Total Object Exploration (TOE) time was then calculated to investigate the effects of treatment on mice ability to explore the objects

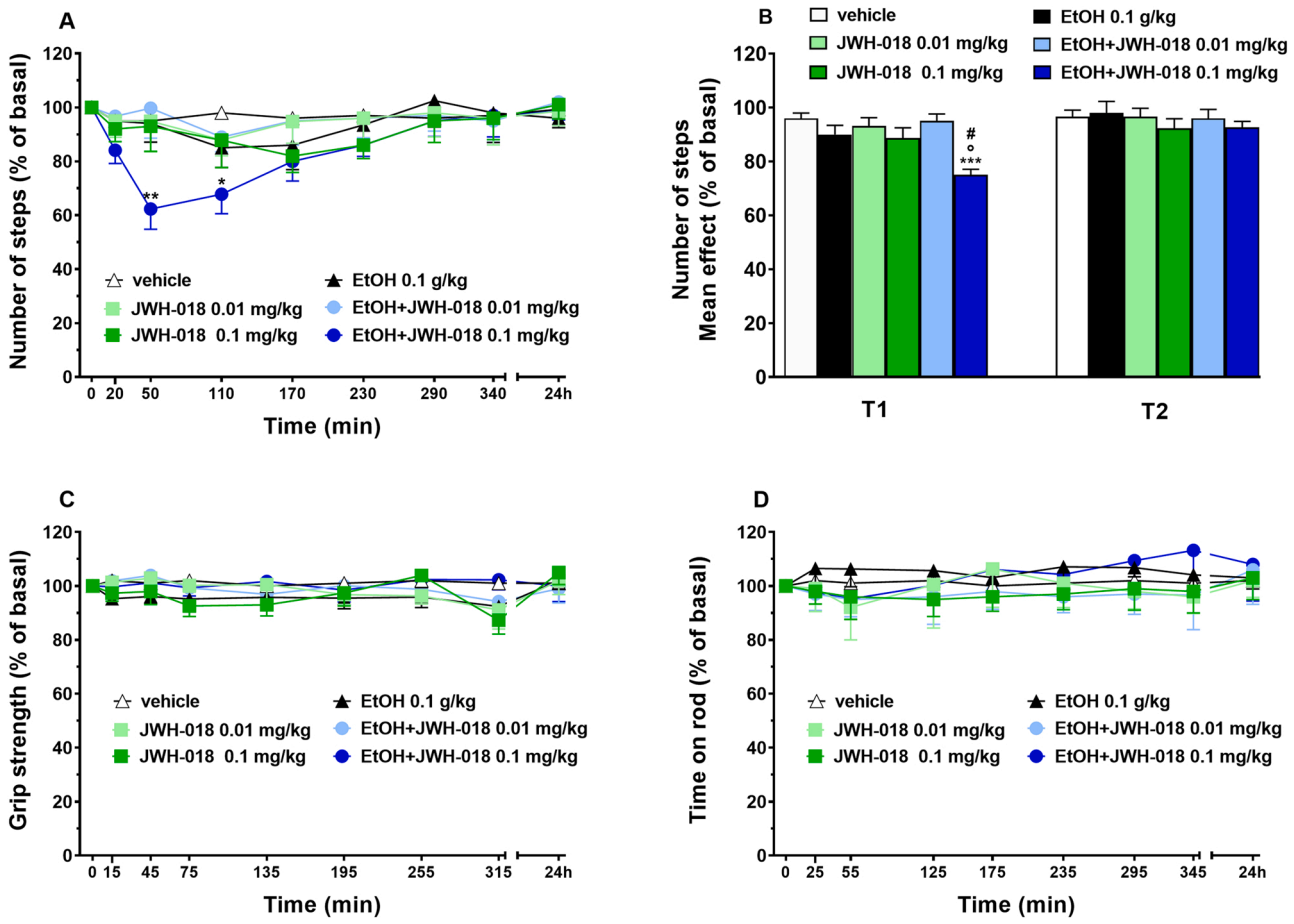

Fig. 3. Effect of ethanol (0.1 g/kg), JWH-018 (0.01 and 0.1 mg/kg) and co-administration of the two substances on number of steps (A) and grip strength (C) of mice; total average effect of ethanol, JWH-018 and co-administration of the two substances on number of steps (B) and grip strength (D) of mice, between 0 and 110–135 min (T1), and between 145 and 350 min (T2). Data are expressed as percentage of the baseline and represent the mean ± SEM of 6 or 8 determinations for each treatment. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's test (A and C) for multiple comparison for the dose-response curve of each compound at different time-points. The analysis of the mean overall effect of each compound and their co-administration were performed with one-way ANOVA followed by Tukey's test (B and D). *p < 0.05, **p < 0.01, ***p < 0.001 versus vehicle; °p < 0.05 versus JWH-018 alone; #p < 0.05 versus ethanol + JWH-018 (0.01 mg/kg).

in the NOR test (Barbieri et al., 2016). TOE did not change in vehicle treated mice and effect was similar to that observed in naïve untreated animals (data not shown). Concerning the evaluation of the object memory, ethanol alone, JWH-018 alone and their co-administration did not affect the TOE time 2 and 24 hrs after administration (Fig. 4B). Similarly, tested compounds and their co-administration did not affect TOE time related to spatial memory 2 and 24 hrs after administration (Fig. 4D).

4. Discussion

The present study shows that a low ethanol dose, ineffective by itself on most of the behavioral parameters used, enhanced the effects of JWH-018 on sensorimotor and motor responses, and short- and long-term working memory of mice, in a dose-dependent manner. Although it is challenging to translate the results of animal experimental studies to the humans, both in terms of doses and of psychomotor performances, the present research provides evidence of the detrimental effect induced by the consumption of SCs combined with other psychoactive substances on sensory, motor, and cognitive skills, highlighting their potential burden on human health and road safety. JWH-018 and other SCs have been linked to impaired driving (Yeakel and Logan, 2013; Karinen et al., 2015) and previous studies have shown that SCs might act on Central Nervous System (CNS) possibly affecting reaction time, as well as judgment and processing skills (Walsh et al., 2004; Orazietti et al., 2022). Furthermore, increased frequency of confusion, disorientation and incoherence have been noted in drivers after the intake of these illicit drugs (Logan et al., 2017), as well as performance impairments in driving and non-driving tasks following the intake of both THC and ethanol (Ronen et al., 2008, 2010). It is well known that alcohol impairs the handling of the vehicle starting from levels lower than 0.05% of Blood Alcohol Concentration (BAC; Howat et al., 1991; Moskowitz and Fiorentino, 2000; Martin et al., 2013). Specifically, it has been linked to its effect on driving-related skills such as information processing, memory, response time, divided attention, and spatial perception (Finnigan and Hammersley, 1992; Kerr and Hindmarch, 1998; Liu and Ho, 2010). Since ethanol has been identified in biological samples from drivers involved in cases of driving under the influence of SCs (Musshoff et al., 2014; Tuv et al., 2014; Karinen et al., 2015), a better understanding of the potentially dangerous role played by the co-administration of these compounds with ethanol is currently needed.

4.1. Sensorimotor and motor responses

JWH-018 alone inhibited visual, acoustic, and tactile responses in mice. This is in line with our previous studies, showing that low doses of JWH-018 (Ossato et al., 2015) and its halogenated derivatives (Bilel et al., 2020) impaired sensorimotor functions in mice possibly via

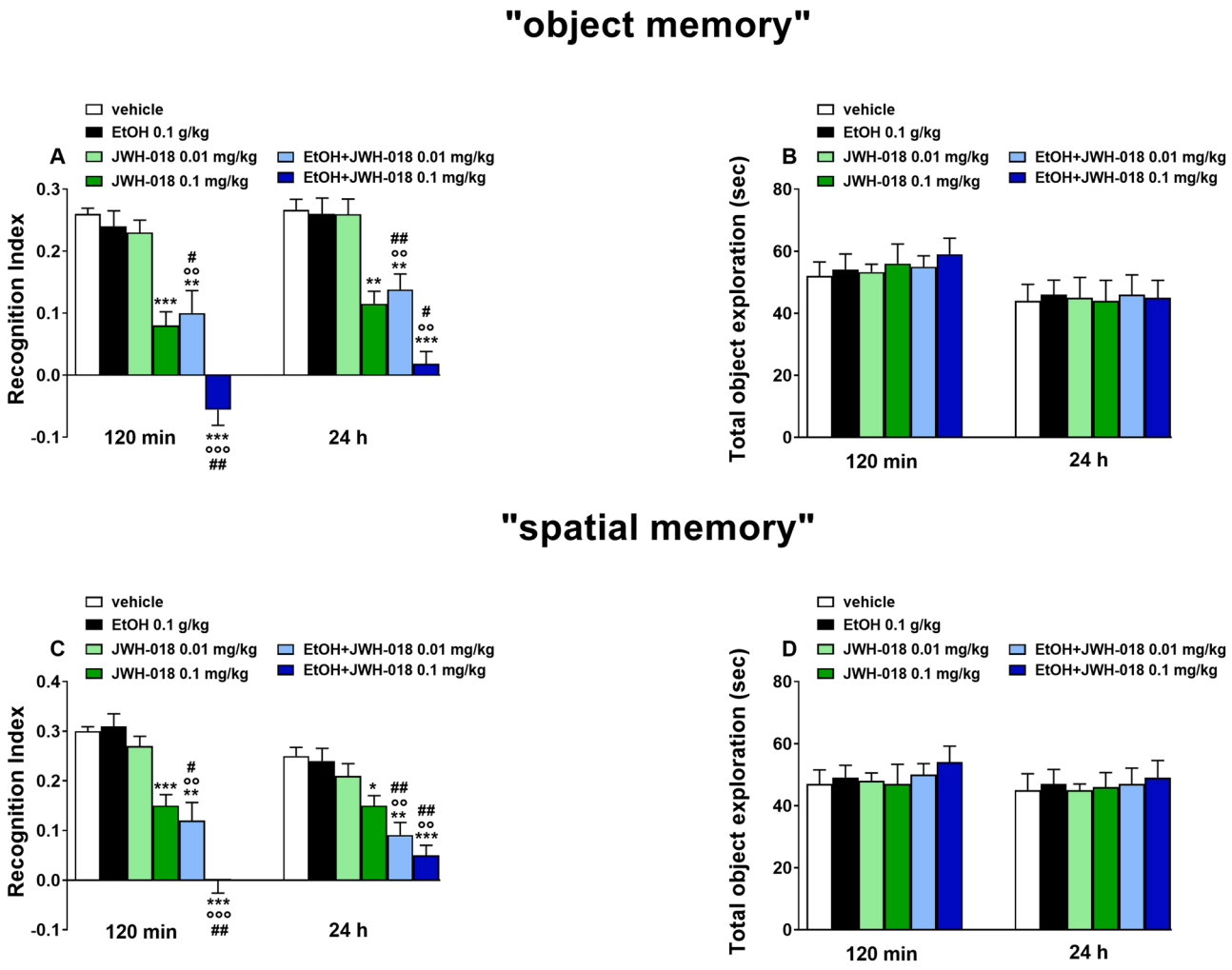

Fig. 4. Effect of ethanol (0.1 g/kg), JWH-018 (0.01 and 0.1 mg/kg) and co-administration of the two substances on Recognition Index (RI), concerning object (A) and spatial memory (C), in the NOR test in mice. Compounds given 15 min after the familiarization phase impaired the short- (at 2 hrs) and long- (24 hrs) memory recognition in mice. Data are expressed as RI (see materials and methods) and represent the mean ± SEM of 8 determinations for each treatment. Statistical analysis was performed with one-way ANOVA followed by Tukey's test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle; °°$p < 0.01$, °°°$p < 0.001$ versus JWH-018 alone; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ versus ethanol + JWH-018 (0.01 mg/kg). Effect of ethanol (0.1 g/kg), JWH-018 (0.01 and 0.1 mg/kg) and co-administration of the two substances on Total Object Exploration (TOE), concerning object (B) and spatial memory (D), in the NOR test in mice. Compounds given 15 min after the familiarization phase impaired the short- (at 2 hrs) and long- (24 hrs) memory recognition in mice. Data are expressed as absolute values (sec) and represent the mean ± SEM of 8 determinations for each treatment. Statistical analysis was performed with one-way ANOVA followed by Tukey's test.

stimulating CB_1 receptors located in circuitries designated for sensory responsiveness (Price et al., 2003; Tzounopoulos et al., 2007; Hemelt and Keller., 2008; Gómez-Nieto et al., 2014; Reig and Silberberg., 2014).

It has been demonstrated that CB_1 receptors are located in the dorsomedial striatum of the basal ganglia (Tsou et al., 1998; Marsicano and Lutz, 1999), in which visual information is processed in mice (Reig and Silberberg, 2014). However, it has been demonstrated that CB_1 receptors are also localized in retina of humans (Straiker et al., 1999a; Le Boisselier et al., 2017) and rodents (Straiker et al., 1999b; Yazulla et al., 1999). Therefore, their potential contribution to the SC-induced effect on ocular functions and vision itself should be considered (Järvinen et al., 2002; Le Boisselier et al., 2017). This is also in line with the identification of affected visuospatial functions in cases of DUI of SCs (Orazietti et al., 2022).

Similarly, Gòmez-Nieto and colleagues have pointed out that acoustic startle reflex in mice is induced by the activation of three serially connected structures that involve the dorsal cochlear nucleus (Gòmez-Nieto et al., 2014), in which CB_1 receptors are located (Tzounopoulos et al., 2007). Furthermore, it has been revealed that endogenous cannabinoids (Tzounopoulos et al., 2007; Zhao et al., 2011), as well as exogeneous WIN-55,212–2 (Tzounopoulos et al., 2007) can modulate short term synaptic plasticity in dorsal cochlear nucleus of mice.

Likewise, both endogenous cannabinoidergic neurotransmission (Patel et al., 2002; Ho et al., 2010) and administration of exogenous cannabinoids like Δ^9-THC (Pietr et al., 2010) and WIN-55,212–2 (Bereiter et al., 2002) have been shown to modulate responses to sensory stimulation in rodents. In line with these findings, further studies have stated that CB_1 receptors are expressed in the inferior olive, somatosensory cortex, superior colliculus (Tsou et al., 1998; Cristino et al., 2006; Hemelt and Keller, 2008) and trigeminal structures (Herkenham et al., 1991; Tsou et al., 1998; Price et al., 2003).

Although ethanol alone appeared to be ineffective in altering almost all sensorimotor responses, a prompt and deep inhibition of visual placing responses were observed after its administration. This agrees with previous studies showing altered visual placing reflexes in ethanol-treated neonates (Ciociola and Gautieri, 1988), suggesting the toxic effect induced by ethanol in brain areas involved in processing these stimuli. This different response may be due to the different experimental conditions among these behavioral tests, since in the visual placing test

in mice requires the integration of the visual, tactile (from vibrissae stimulation), and vestibular functions in order to prepare the correct extension movement of the muscles for contact with the ground (Lambert et al., 2016). Specifically, vestibule–spinal pathways play a crucial role in the control of posture and movement in rodents (Clarac et al., 1998; Cullen, 2012; Tosolini and Morris, 2012; Lambert et al., 2016). This assumption is supported by already pointed out inhibitory effects induced by ethanol in vestibular nucleus neurons of rats (Sasa et al., 1987; Ishihara et al., 1998). Moreover, both SCs (Barbieri et al., 2016; Funada et al., 2020) and ethanol (Costardi et al., 2015) administration in rodents have been linked to depressive effects on CNS. In fact, SCs (Ossato et al., 2015; Bilel et al., 2020) as well as ethanol (Martin et al., 1985; Cronise et al., 2005; Wu et al., 2014) induces similar *in vivo* effects such as impaired sensory information processing, hypolocomotion and hypothermia. Particularly, Wu and colleagues have shown that ethanol disrupts sensory information processing via activating presynaptic CB_1 receptors in cerebellar Purkinje cells of mice (Wu et al., 2014) possibly explaining the potential interaction between these substances. This is in line with our findings (Fig. 5). Moreover, a depressant effect on the CNS has been shown in cases of co-consumption of ethanol and SCs in humans, with delayed pupillary reaction, slurred speech, bradypsychia and somnolence (Giorgetti et al., 2023).

It is worth noting that co-administration of the high dose of JWH-018 with ethanol also provoked a transient impairment of the motor functions (*Drag test*) in mice. This observation agrees with a recent study showing that synthetic cannabinoids as JWH-018 enhanced ethanol-induced disruption of the motor activity in mice (Funada et al., 2020). However, neither JWH-018 and ethanol alone nor their concurrent administration have exerted a detrimental action on time on rod and grip strength at any dose in the present study. This may confirm that these psychoactive substances can impair sensorimotor (visual, acoustic and tactile responses) and cognitive (working memory) responses at dosages that do not induce altered motor activity in mice (Ossato et al., 2015) and considered as "threshold dosages" by users (Psychonautwiki, 2023). Thus, the present results suggest that the consumption of ethanol, SCs and even more of their combination may contribute to the general impairment typically observed in drivers involved in DUID cases (Theunissen et al., 2021; Orazietti et al., 2022).

It is also plausible that higher doses of ethanol and SCs might affect motor abilities although not primarily motor strength. Indeed, impaired motor coordination and slow movement, similar to that induced by Δ^9-THC (Weinstein et al., 2007), are seen in humans after SCs consumption (Theunissen et al., 2021) and in DUID cases (Orazietti et al., 2022), and impaired motor coordination is reported in cases of co-consumption of ethanol and SCs (Giorgetti et al., 2023).

4.2. Memory functions

High dose of JWH-018 alone, as well as its co-administration with ethanol, altered short- (2 hrs) and long-term (24 hrs) spatial and non-spatial working memory in mice. This is in agreement with the detrimental effect of JWH-018, its halogenated derivatives (Barbieri et al., 2016) on memory functions, as previously ascertained in mice. Further studies have shown that ethanol impairs both spatial (Melchior et al., 1993; Givens, 1995; White et al., 1997) and non-spatial (Givens, 1996; Givens and McMahon, 1997) working memory in rodents. Effects induced by both SCs (Sticht et al., 2015; Ito et al., 2019) and ethanol (White et al., 2000) on acquisition and retention of memory may be due to their pharmacological action on specific brain regions. Indeed, hippocampus and contiguous cortical area, including perirhinal cortex, have been shown to be involved in normal memory functions (Baxter, 2010). Specifically, the deleterious effects of both SCs (Basavarajappa and Subbanna, 2014) and ethanol (Blitzer et al., 1990; Givens and McMahon, 1995; Givens, 1995; Ludvig et al., 1995) on hippocampal functions in rodents has been highlighted, suggesting the potential interaction between these psychoactive substances in altering cognitive functioning.

Administration of the high dose of JWH-018 and its co-administration with ethanol altered RI value in the NOR test 2 and 24 hrs after the administrations. Therefore, it should be ruled out the

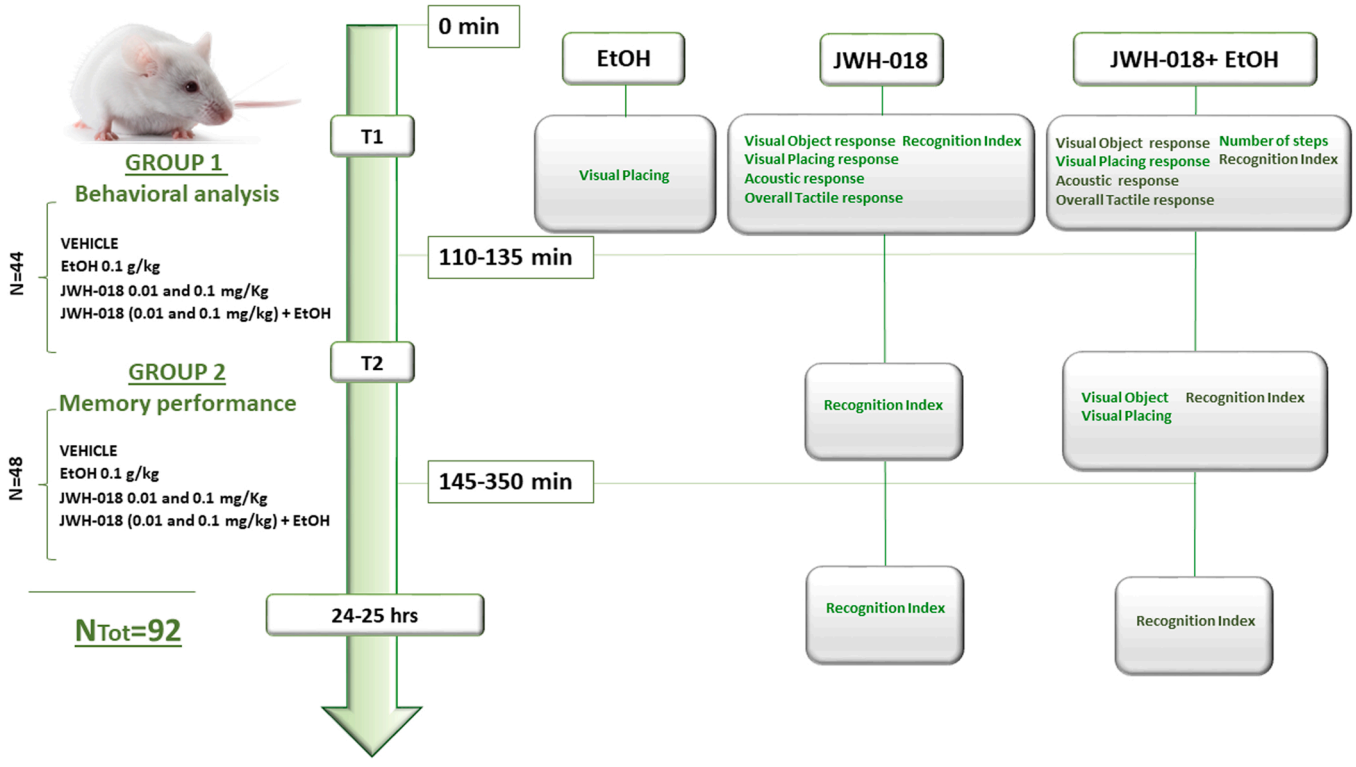

Fig. 5. Schematic comparison of the progressive appearance of behavioral effects induced by ethanol (1 g/kg), JWH-018 (0.01 and 0.1 mg/kg) and their co-administration in CD-1 male mice.

assumption that these effects are related to a reduction in locomotor activity. Indeed, only the co-administration of the high dose of the tested synthetic cannabinoid with ethanol affected the motor performance of mice inducing a brief and transient inhibitory effect (starting from 60 up to 110 min). Likewise, administration of JWH-018, ethanol, and their co-administration provoked sensorimotor impairment in mice, which however completely disappeared 24 hrs after the administration. Furthermore, neither compounds nor their co-administration affected the TOE time. Thus, these data may confirm that cognitive deficits induced by ethanol and JWH-018 are likely related to their effects on processes involved in memory formation and retention.

Noteworthy, co-administration of the high dose of JWH-018 with ethanol caused a greater exploration of the familiar object respect to the new one (RI reversion) 2 hrs after the injections. Tested compounds were administered at a sufficient time (15 min) to acquire memory of the objects (A, A) during the familiarization phase. Therefore, this could be linked to a drug-induced impairment in the already acquired memory (Ennaceur, 2010). As previously assumed for JWH-018 and its halogenated derivatives (Barbieri et al., 2016), it cannot be ruled out the possibility that this effect could be due to the alterations induced by these compounds on sensorimotor responses and further studies could be required to investigate this point.

Long-lasting cognitive and sensorimotor impairments have been observed in the NOR and visual response tests, after the concurrent administration of JWH-018 and ethanol (Fig. 5). These are likely due to the pharmacokinetic features of the compounds. In line with this assumption, a recent study investigating new metabolites of 5 F-MDMB-PINACA, 5 F-MMB-PINACA, and MMB-FUBINACA in the presence of ethanol has suggested the potential augmented toxicity linked to co-abuse of SCs and alcohol (Wang et al., 2021).

5. Conclusions

The present study showed for the first time a worsening of the cognitive and sensorimotor impairment following the concurrent administration of JWH-018 and ethanol. Tested compounds provoke a disruption of sensory information processing, motor activity and short- and long-term working memory of mice, that may possibly result in impaired driving skills. Taken together, despite the many limitations that reside in the translation from animal models to humans, these findings suggest that polydrug consumption involving SCs may affect psychomotor performances which are related to driving abilities and may pose an increased risk for road accidents. Further studies on animals, testing more recent compounds pertaining to the class of SCs and combinations with other psychoactive substances, as well as driving-simulation studies on humans are needed to confirm this hypothesis. Ultimately, the gender influence on the pharmacodynamic and pharmacokinetic profile of psychoactive substances such as ethanol (reviewed in Agabio et al., 2017 and Flores-Bonilla and Richardson, 2020) and SCs (reviewed in Fattore et al., 2020) should be considered for further investigation.

Funding

This research has been funded by the Drug Policies Department, Presidency of the Council of Ministers, Italy (project: “Effects of NPS: development of a multicentric research for the information enhancement of the Early Warning System” to MM), by local funds from the University of Ferrara (FAR 2020 and FAR 2021 to MM). All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Ethical statements

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Project activated in collaboration with the Presidency of the Council of Ministers-DPA Anti-Drug Policies (Italy).

CRediT authorship contribution statement

MM, GC and MT designed the studies, with refinements contributed by SB, AG, and RG. MT, GC and SB performed the research, conducted initial data analysis and TB, FB and MB created figures. MM, GC and MT conducted statistical analysis of data and wrote the major drafts of the paper. All authors approved of the submitted version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

Adamowicz, P., Meissner, E., Maślanka, M., 2019. Fatal intoxication with new synthetic cannabinoids AMB-FUBINACA and EMB-FUBINACA. Clin. Toxicol. 57 (11), 1103–1108. https://doi.org/10.1080/15563650.2019.1580371.

Agabio, R., Pisanu, C., Gessa, G.L., Franconi, F., 2017. Sex differences in alcohol use disorder. Curr. Med. Chem. 24 (24), 2661–2670. https://doi.org/10.2174/0929867323666161202092908.

Alves, V.L., Gonçalves, J.L., Aguiar, J., Teixeira, H.M., Câmara, J.S., 2020. The synthetic cannabinoids phenomenon: from structure to toxicological properties. A review. Crit. Rev. Toxicol. 50 (5), 359–382. https://doi.org/10.1080/10408444.2020.1762539.

Antunes, M., Biala, G., 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn. Process. 13 (2), 93–110. https://doi.org/10.1007/s10339-011-0430-z.

Arfè, R., Bilel, S., Tirri, M., Frisoni, P., Serpelloni, G., Neri, M., Boccuto, F., Bernardi, T., Foti, F., De-Giorgio, F., Marti, M., 2021. Comparison of N-methyl-2-pyrrolidone (NMP) and the "date rape" drug GHB: behavioral toxicology in the mouse model. Psychopharmacology 238 (8), 2275–2295. https://doi.org/10.1007/s00213-021-05852-5.

Auwärter, V., Dresen, S., Weinmann, W., Müller, M., Pütz, M., Ferreirós, N., 2009. ’Spice’ and other herbal blends: harmless incense or cannabinoid designer drugs? J. Mass Spectrom. 44 (5), 832–837. https://doi.org/10.1002/jms.1558.

Barbieri, M., Ossato, A., Canazza, I., Trapella, C., Borelli, A.C., Beggiato, S., Rimondo, C., Serpelloni, G., Ferraro, L., Marti, M., 2016. Synthetic cannabinoid JWH-018 and its halogenated derivatives JWH-018-Cl and JWH-018-Br impair novel object recognition in mice: behavioral, electrophysiological and neurochemical evidence. Neuropharmacology 109, 254–269. https://doi.org/10.1016/j.neuropharm.2016.06.027.

Basavarajappa, B.S., Subbanna, S., 2014. CB1 receptor-mediated signaling underlies the hippocampal synaptic, learning, and memory deficits following treatment with JWH-081, a new component of spice/K2 preparations. Hippocampus 24 (2), 178–188. https://doi.org/10.1002/hipo.22213.

Baxter, M.G., 2010. "I’ve seen it all before": explaining age-related impairments in object recognition. Theoretical comment on Burke et al. (2010). Behav. Neurosci. 124 (5), 706–709. https://doi.org/10.1037/a0021029.

Bereiter, D.A., Bereiter, D.F., Hirata, H., 2002. Topical cannabinoid agonist, WIN55,212-2, reduces cornea-evoked trigeminal brainstem activity in the rat. Pain 99 (3), 547–556. https://doi.org/10.1016/S0304-3959(02)00271-3.

Bilel, S., Tirri, M., Arfè, R., Ossato, A., Trapella, C., Serpelloni, G., Neri, M., Fattore, L., Marti, M., 2020. Novel halogenated synthetic cannabinoids impair sensorimotor functions in mice. Neurotoxicology 76, 17–32. https://doi.org/10.1016/j.neuro.2019.10.002.

Blitzer, R.D., Gil, O., Landau, E.M., 1990. Long-term potentiation in rat hippocampus is inhibited by low concentrations of ethanol. Brain Res. 537 (1-2), 203–208. https://doi.org/10.1016/0006-8993(90)90359-j.

Brents, L.K., Reichard, E.E., Zimmerman, S.M., Moran, J.H., Fantegrossi, W.E., Prather, P.L., 2011. Phase I hydroxylated metabolites of the K2 synthetic cannabinoid JWH-018 retain in vitro and in vivo cannabinoid 1 receptor affinity and activity. PLoS One 6 (7), e21917. https://doi.org/10.1371/journal.pone.0021917.

Bush, D.M., Woodwell, D.A., 2014. Update: drug-related emergency department visits involving synthetic cannabinoids. The CBHSQ Report. Substance Abuse and Mental Health Services Administration, Rockville, MD, US, pp. 1–10.

Ciociola, A.A., Gautieri, R.F., 1988. Teratogenic and behavioral anomalies induced by acute exposure of mice to ethanol and their possible relation to fetal brain DNA synthesis. Pharm. Res. 5 (7), 447–452. https://doi.org/10.1023/a:1015996603418.

Clarac, F., Vinay, L., Cazalets, J.R., Fady, J.C., Jamon, M., 1998. Role of gravity in the development of posture and locomotion in the neo natal rat. Brain Res. Rev. 28 (1–2), 35–43. https://doi.org/10.1016/s0165-0173(98)00024-1.

Cohen, K., Weinstein, A.M., 2018. Synthetic and non-synthetic cannabinoid drugs and their adverse effects-a review from public health prospective. Front. Public Health 6, 162. https://doi.org/10.3389/fpubh.2018.00162.

Costardi, J.V., Nampo, R.A., Silva, G.L., Ribeiro, M.A., Stella, H.J., Stella, M.B., Malheiros, S.V., 2015. A review on alcohol: from the central action mechanism to chemical dependency. Rev. Assoc. Med. Bras. 61 (4), 381–387. https://doi.org/10.1590/1806-9282.61.04.381 (1992).

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., Di Marzo, V., 2006. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139 (4), 1405–1415. https://doi.org/10.1016/j.neuroscience.2006.02.074.

Cronise, K., Finn, D.A., Metten, P., Crabbe, J.C., 2005. Scheduled access to ethanol results in motor impairment and tolerance in female C57BL/6J mice. Pharmacol. Biochem. Behav. 81 (4), 943–953. https://doi.org/10.1016/j.pbb.2005.07.005.

Cullen, K.E., 2012. The vestibular system: multimodal integration and encoding of self-motion for motor control. Trends Neurosci. 35 (3), 185–196. https://doi.org/10.1016/j.tins.2011.12.001.

Darke, S., Duflou, J., Farrell, M., Peacock, A., Lappin, J., 2020. Characteristics and circumstances of synthetic cannabinoid-related death. Clin. Toxicol. 58 (5), 368–374. https://doi.org/10.1080/15563650.2019.1647344.

De-Giorgio, F., Bergamin, E., Bilel, S., Tirri, M., Arfè, R., Marchetti, B., Corli, G., Serpelloni, G., Marti, M., 2021. Ethanol enhanced MDPV- and cocaine-induced aggressive behavior in mice: forensic implications. Drug Alcohol Depend. 229 (Pt A), 109125 https://doi.org/10.1016/j.drugalcdep.2021.109125.

EMCDDA, 2021. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2021: Trends and Developments. Publications Office of the European Union, Lisbon.

EMCDDA, 2022. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2022: Trends and Developments. Publications Office of the European Union, Lisbon.

EMCDDA, 2012. European Monitoring Centre for Drugs and Drug Addiction Driving under the Influence of Drugs, Alcohol and Medicines in Europe, Lisbon.

EMCDDA, 2002. European Monitoring Centre for Drugs and Drug Addiction. Polydrug Use, Lisbon.

EMCDDA, 2017. European Monitoring Centre for Drugs and Drug Addiction Synthetic cannabinoids in Europe (Perspectives on Drugs), Lisbon.

Ennaceur, A., 2010. One-trial object recognition in rats and mice: methodological and theoretical issues. Behav. Brain Res. 215 (2), 244–254. https://doi.org/10.1016/j.bbr.2009.12.036.

Ennaceur, A., Meliani, K., 1992. A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. Behav. Brain Res. 51 (1), 83–92. https://doi.org/10.1016/s0166-4328(05)80315-8.

Ennaceur, A., Neave, N., Aggleton, J.P., 1997. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Exp. Brain Res. 113 (3), 509–519. https://doi.org/10.1007/pl00005603.

ESPAD, 2019. The European School Survey Project on Alcohol and Other Drugs. ESPAD Report 2019. (http://espad.org/espad-report-2019).

Fattore, L., Fratta, W., 2011. Beyond THC: the new generation of cannabinoid designer drugs. Front. Behav. Neurosci. 21 (5), 60. https://doi.org/10.3389/fnbeh.2011.00060.

Fattore, L., Marti, M., Mostallino, R., Castelli, M.P., 2020. Sex and gender differences in the effects of novel psychoactive substances. Brain Sci. 10 (9), 606. https://doi.org/10.3390/brainsci10090606.

Fietzke, M., Thomas, A., Beike, J., et al., 2016. In vitro elucidation of the metabolic profile of the synthetic cannabinoid receptor agonists JWH-175 and JWH-176. Forensic Toxicol. 34, 353–362. https://doi.org/10.1007/s11419-016-0322-0.

Finnigan, F., Hammersley, R., 1992. The effects of alcohol on performance. In: Smith, A. P., Jones, D.M. (Eds.), Handbook of Human Performance, Vol. 1. The Physical Environment; Vol. 2. Health and Performance; Vol. 3. State and Trait. Academic Press, pp. 73–126.

Flores-Bonilla, A., Richardson, H.N., 2020. Sex differences in the neurobiology of alcohol use disorder. Alcohol Res. 40 (2), 04. https://doi.org/10.35946/arcr.v40.2.04.

Funada, M., Takebayashi-Ohsawa, M., Tomiyama, K.I., 2020. Synthetic cannabinoids enhanced ethanol-induced motor impairments through reduction of central glutamate neurotransmission. Toxicol. Appl. Pharmacol. 408, 115283 https://doi.org/10.1016/j.taap.2020.115283.

Giorgetti, A., Busardò, F.P., Tittarelli, R., Auwärter, V., Giorgetti, R., 2020. Post-mortem toxicology: a systematic review of death cases involving synthetic cannabinoid receptor agonists. Front. Psychiatry 11, 464. https://doi.org/10.3389/fpsyt.2020.00464.

Giorgetti, A., Orazietti, V., Busardò, F.P., Giorgetti, R., 2023. Psychomotor performances relevant for driving under the combined effect of ethanol and synthetic cannabinoids: a systematic review. Front. Psychiatry 14, 1131335. https://doi.org/10.3389/fpsyt.2023.1131335.

Giorgetti, A., Pascali, J.P., Fais, P., Pelletti, G., Gabbin, A., Franchetti, G., Cecchetto, G., Viel, G., 2021. Molecular mechanisms of action of novel psychoactive substances (NPS). A new threat for young drug users with forensic-toxicological implications. Life 11 (5), 440. https://doi.org/10.3390/life11050440.

Givens, B., 1996. Behavioral correlates of single units in the medial septal area: the effect of ethanol. Neuroscience 71 (2), 417–427. https://doi.org/10.1016/0306-4522(95)00443-2.

Givens, B., 1995. Low doses of ethanol impair spatial working memory and reduce hippocampal theta activity. Alcohol Clin. Exp. Res. 19 (3), 763–767. https://doi.org/10.1111/j.1530-0277.1995.tb01580.x.

Givens, B., McMahon, K., 1997. Effects of ethanol on nonspatial working memory and attention in rats. Behav. Neurosci. 111 (2), 275–282. https://doi.org/10.1037//0735-7044.111.2.275.

Givens, B., McMahon, K., 1995. Ethanol suppresses the induction of long-term potentiation in vivo. Brain Res. 688 (1-2), 27–33. https://doi.org/10.1016/0006-8993(95)00499-g.

Glantz, M.D., Bharat, C., Degenhardt, L., et al., 2020. The epidemiology of alcohol use disorders cross-nationally: findings from the World Mental Health Surveys [published correctionappears in Addict Behav. 2020 Jul;106:106381]. Addict. Behav. 102, 106128 https://doi.org/10.1016/j.addbeh.2019.106128.

Gómez-Nieto, R., Jde, Horta-J.únior, Castellano, A., Millian-Morell, O., Rubio, L., López, D.E, M.E., 2014. Origin and function of short-latency inputs to the neural substrates underlying the acoustic startle reflex. Front. Neurosci. 8, 216. https://doi.org/10.3389/fnins.2014.00216.

Hemelt, M.E., Keller, A., 2008. Superior colliculus control of vibrissa movements. J. Neurophysiol. 100 (3), 1245–1254. https://doi.org/10.1152/jn.90478.2008.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11 (2), 563–583. https://doi.org/10.1523/JNEUROSCI.11-02-00563.1991.

Ho, W.S., Patel, S., Thompson, J.R., Roberts, C.J., Stuhr, K.L., Hillard, C.J., 2010. Endocannabinoid modulation of hyperaemia evoked by physiologically relevant stimuli in the rat primary somatosensory cortex. Br. J. Pharmacol. 160 (3), 736–746. https://doi.org/10.1111/j.1476-5381.2010.00772.x.

Howat, P., Sleet, D., Smith, I., 1991. Alcohol and driving: is the 0.05% blood alcohol concentration limit justified? Drug Alcohol Rev. 10 (2), 151–166. https://doi.org/10.1080/09595239100185211.

Huffman, J.W., Padgett, L.W., 2005. Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes. Curr. Med.Chem. 12 (12), 1395–1411. https://doi.org/10.2174/0929867054020864.

Ishihara, K., Ujihara, H., Akaike, A., Sasa, M., Takaori, S., 1998. Ethanol reduces spontaneous firing and potentiates GABA-induced currents in acutely dissociated rat medial vestibular nucleus neurons. Nihon Arukoru Yakubutsu Igakkai Zasshi 33 (3), 252–262.

Ito, S., Deyama, S., Domoto, M., Zhang, T., Sasase, H., Fukao, A., Esaki, H., Hinoi, E., Kaneko, S., Kaneda, K., 2019. Effects of the synthetic cannabinoid 5F-AMB on anxiety and recognition memory in mice. Psychopharmacology 236 (7), 2235–2242. https://doi.org/10.1007/s00213-019-05222-2.

Jaenicke, N.J., Pogoda, W., Paulke, A., Wunder, C., Toennes, S.W., 2014. Retrospective analysis of synthetic cannabinoids in serum samples–epidemiology and consumption patterns. Forensic Sci. Int. 242, 81–87. https://doi.org/10.1016/j.forsciint.2014.06.010.

Jamt, R.E.G., Gjerde, H., Romeo, G., Bogstrand, S.T., 2019. Association between alcohol and drug use and arrest for driving under the influence after crash involvement in a rural area of Norway: a case-control study. BMJ Open 9 (1), e023563. https://doi.org/10.1136/bmjopen-2018-023563.

Järvinen, T., Pate, D.W., Laine, K., 2002. Cannabinoids in the treatment of glaucoma. Pharmacol. Ther. 95 (2), 203–220. https://doi.org/10.1016/s0163-7258(02)00259-0.

Ji Kwon, N., Han, E., 2019. A review of drug abuse in recently reported cases of driving under the influence of drugs (DUID) in Asia, USA, and Europe. Forensic Sci. Int. 302, 109854 https://doi.org/10.1016/j.forsciint.2019.06.012.

Karinen, R., Tuv, S.S., Øiestad, E.L., Vindenes, V., 2015. Concentrations of APINACA, 5F-APINACA, UR-144 and its degradant product in blood samples from six impaired drivers compared to previous reported concentrations of other synthetic cannabinoids. Forensic Sci. Int. 246, 98–103. https://doi.org/10.1016/j.forsciint.2014.11.012.

Kerr, J.S., Hindmarch, I., 1998. The effects of alcohol alone or in combination with other drugs on information processing, task performance and subjective responses. Hum. Psychopharmacol. Clin. Exp. 13.

Kim, S., Kim, D.K., Shin, Y., Jeon, J.H., Song, I.S., Lee, H.S., 2020. In vitro interaction of AB-FUBINACA with human cytochrome P450, UDP-glucuronosyltransferase enzymes and drug transporters. Molecules 25 (19), 4589. https://doi.org/10.3390/molecules25194589.

Kong, T.Y., Kim, J.H., Kim, D.K., Lee, H.S., 2018. Synthetic cannabinoids are substrates and inhibitors of multiple drug-metabolizing enzymes. Arch. Pharm. Res. 41 (7), 691–710. https://doi.org/10.1007/s12272-018-1055-x.

Lambert, F.M., Bras, H., Cardoit, L., Vinay, L., Coulon, P., Glover, J.C., 2016. Early postnatal maturation in vestibulospinal pathways involved in neck and forelimb motor control. Dev. Neurobiol. 76, 1061–1077. https://doi.org/10.1002/dneu.22375.

Le Boisselier, R., Alexandre, J., Lelong-Boulouard, V., Debruyne, D., 2017. Focus on cannabinoids and synthetic cannabinoids. Clin. Pharmacol. Ther. 101 (2), 220–229. https://doi.org/10.1002/cpt.563.

Liu, Y.C., Ho, C.H., 2010. Effects of different blood alcohol concentrations and post-alcohol impairment on driving behavior and task performance. Traffic Inj. Prev. 11 (4), 334–341. https://doi.org/10.1080/15389581003747522.

Logan, B.K., Mohr, A.L.A., Friscia, M., Krotulski, A.J., Papsun, D.M., Kacinko, S.L., Ropero-Miller, J.D., Huestis, M.A., 2017. Reports of adverse events associated with use of novel psychoactive substances, 2013-2016: a review. J. Anal. Toxicol. 41 (7), 573–610. https://doi.org/10.1093/jat/bkx031.

Ludvig, N., Altura, B.T., Fox, S.E., Altura, B.M., 1995. The suppressant effect of ethanol, delivered via intrahippocampal microdialysis, on the firing of local pyramidal cells

in freely behaving rats. Alcohol 12 (5), 417–421. https://doi.org/10.1016/0741-8329(95)00012-g.
Luethi, D., Liechti, M.E., 2020. Designer drugs: mechanism of action and adverse effects. Arch. Toxicol. 94 (4), 1085–1133. https://doi.org/10.1007/s00204-020-02693-7.
Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur. J. Neurosci. 11 (12), 4213–4225. https://doi.org/10.1046/j.1460-9568.1999.00847.x.
Marti, M., Mela, F., Fantin, M., Zucchini, S., Brown, J.M., Witta, J., Di Benedetto, M., Buzas, B., Reinscheid, R.K., Salvadori, S., Guerrini, R., Romualdi, P., Candeletti, S., Simonato, M., Cox, B.M., Morari, M., 2005. Blockade of nociceptin/orphanin FQ transmission attenuates symptoms and neurodegeneration associated with Parkinson's disease. J. Neurosci. 25 (42), 9591–9601. https://doi.org/10.1523/JNEUROSCI.2546-05.2005.
Martin, P.R., Majchrowicz, E., Tamborska, E., Marietta, C., Mukherjee, A.B., Eckardt, M. J., 1985. Response to ethanol reduced by past thiamine deficiency. Science 227 (4692), 1365–1368. https://doi.org/10.1126/science.3975622.
Martin, T.L., Solbeck, P.A., Mayers, D.J., Langille, R.M., Buczek, Y., Pelletier, M.R., 2013. A review of alcohol-impaired driving: the role of blood alcohol concentration and complexity of the driving task. J. Forensic Sci. 58 (5), 1238–1250. https://doi.org/10.1111/1556-4029.12227.
Melchior, C.L., Glasky, A.J., Ritzmann, R.F., 1993. A low dose of ethanol impairs working memory in mice in a win-shift foraging paradigm. Alcohol 10 (6), 491–493. https://doi.org/10.1016/0741-8329(93)90071-u.
Moskowitz, H., Fiorentino, D., 2000. A Review of the Literature on the Effects of Low Doses of Alcohol on Driving Related Skills. National Technical Information Service, Springfield, Virginia, p. 22161.
Musshoff, F., Madea, B., Kernbach-Wighton, G., Bicker, W., Kneisel, S., Hutter, M., Auwärter, V., 2014. Driving under the influence of synthetic cannabinoids ("Spice"): a case series. Int. J. Leg. Med. 128 (1), 59–64. https://doi.org/10.1007/s00414-013-0864-1.
Nair, A.B., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. J. Basic Clin. Pharm. 7 (2), 27–31. https://doi.org/10.4103/0976-0105.177703.
Oberenko, A.V., Kachin, S.V., Sagalakov, S.A, 2019. Types of synthetic cannabinoids seized from illicit trafficking in the territory of the Siberian Federal District (Russia) between 2009-2018. Forensic Sci. Int. 302, 109902 https://doi.org/10.1016/j.forsciint.2019.109902.
Orazietti, V., Basile, G., Giorgetti, R., Giorgetti, A., 2022. Effects of synthetic cannabinoids on psychomotor, sensory and cognitive functions relevant for safe driving. Front. Psychiatry 13, 998828. https://doi.org/10.3389/fpsyt.2022.998828.
Ossato, A., Vigolo, A., Trapella, C., Seri, C., Rimondo, C., Serpelloni, G., Marti, M., 2015. JWH-018 impairs sensorimotor functions in mice. Neuroscience 300, 174–188. https://doi.org/10.1016/j.neuroscience.2015.05.021.
Patel, S., Gerrits, R., Muthian, S., Greene, A.S., Hillard, C.J., 2002. The CB1 receptor antagonist SR141716 enhances stimulus-induced activation of the primary somatosensory cortex of the rat. Neurosci. Lett. 335 (2), 95–98. https://doi.org/10.1016/s0304-3940(02)01170-9.
Pelletti, G., Boscolo-Berto, R., Barone, R., Giorgetti, A., Fiorentini, C., Pascali, J.P., Fais, P., Pelotti, S., 2022. Gender differences in driving under the influence of psychoactive drugs: evidence mapping of real case studies and meta-analysis. Forensic Sci. Int. 341, 111479 https://doi.org/10.1016/j.forsciint.2022.111479.
Pietr, M.D., Knutsen, P.M., Shore, D.I., Ahissar, E., Vogel, Z., 2010. Cannabinoids reveal separate controls for whisking amplitude and timing in rats. J. Neurophysiol. 104 (5), 2532–2542. https://doi.org/10.1152/jn.01039.2009.
Poklis, J.L., Amira, D., Wise, L.E., Wiebelhaus, J.M., Haggerty, B.J., Poklis, A., 2012. Detection and disposition of JWH-018 and JWH-073 in mice after exposure to "Magic Gold" smoke. Forensic Sci. Int. 220 (1-3), 91–96. https://doi.org/10.1016/j.forsciint.2012.02.003.
Price, T.J., Helesic, G., Parghi, D., Hargreaves, K.M., Flores, C.M., 2003. The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. Neuroscience 120 (1), 155–162. https://doi.org/10.1016/s0306-4522(03)00333-6.
Psychonautwiki, 2023. JWH-018. (JWH-018 - PsychonautWiki). [Last access on 31st January 2023].
Rajasekaran, M., Brents, L.K., Franks, L.N., Moran, J.H., Prather, P.L., 2013. Human metabolites of synthetic cannabinoids JWH-018 and JWH-073 bind with high affinity and act as potent agonists at cannabinoid type-2 receptors. Toxicol. Appl. Pharmacol. 269 (2), 100–108. https://doi.org/10.1016/j.taap.2013.03.012.
Reig, R., Silberberg, G., 2014. Multisensory integration in the mouse striatum. Neuron 83 (5), 1200–1212. https://doi.org/10.1016/j.neuron.2014.07.033.
Ronen, A., Gershon, P., Drobiner, H., Rabinovich, A., Bar-Hamburger, R., Mechoulam, R., Cassuto, Y., Shinar, D., 2008. Effects of THC on driving performance, physiological state and subjective feelings relative to alcohol. Accid. Anal. Prev. 40 (3), 926–934. https://doi.org/10.1016/j.aap.2007.10.011.
Ronen, A., Chassidim, H.S., Gershon, P., Parmet, Y., Rabinovich, A., Bar-Hamburger, R., Cassuto, Y., Shinar, D., 2010. The effect of alcohol, THC and their combination on perceived effects, willingness to drive and performance of driving and non-driving tasks. Accid. Anal. Prev. 42 (6), 1855–1865. https://doi.org/10.1016/j.aap.2010.05.006.
Sasa, M., Shimizu, H., Yoshida, S., Takaori, S., 1987. Effects of ethanol on vestibular nucleus neurons responding to sinusoidal rotation. Alcohol Alcohol. Suppl. 1, S731–S735.
Schneir, A.B., Baumbacher, T., 2012. Convulsions associated with the use of a synthetic cannabinoid product. J. Med. Toxicol. 8 (1), 62–64. https://doi.org/10.1007/s13181-011-0182-2.
Seely, K.A., Brents, L.K., Radominska-Pandya, A., Endres, G.W., Keyes, G.S., Moran, J.H., Prather, P.L., 2012. A major glucuronidated metabolite of JWH-018 is a neutral antagonist at CB1 receptors. Chem. Res. Toxicol. 25 (4), 825–827. https://doi.org/10.1021/tx3000472.
Simmons, J., Cookman, L., Kang, C., Skinner, C., 2011. Three cases of "spice" exposure. Clin. Toxicol. 49 (5), 431–433. https://doi.org/10.3109/15563650.2011.584316.
Sticht, M.A., Jacklin, D.L., Mechoulam, R., Parker, L.A., Winters, B.D., 2015. Intraperirhinal cortex administration of the synthetic cannabinoid, HU210, disrupts object recognition memory in rats. Neuroreport 26 (5), 258–262. https://doi.org/10.1097/WNR.0000000000000338.
Straiker, A.J., Maguire, G., Mackie, K., Lindsey, J., 1999a. Localization of cannabinoid CB1 receptors in the human anterior eye and retina. Investig. Ophthalmol. Vis. Sci. 40 (10), 2442–2448.
Straiker, A., Stella, N., Piomelli, D., Mackie, K., Karten, H.J., Maguire, G., 1999b. Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc. Natl. Acad. Sci. USA 96 (25), 14565–14570. https://doi.org/10.1073/pnas.96.25.14565.
Tamama, K., Lynch, M.J., 2020. Newly emerging drugs of abuse. Handb. Exp. Pharmacol. 258, 463–502. https://doi.org/10.1007/164_2019_260.
Theunissen, E.L., Reckweg, J.T., Hutten, N.R.P.W., Kuypers, K.P.C., Toennes, S.W., Neukamm, M.A., Halter, S., Ramaekers, J.G., 2021. Intoxication by a synthetic cannabinoid (JWH-018) causes cognitive and psychomotor impairment in recreational cannabis users. Pharm. Biochem. Behav. 202, 173118 https://doi.org/10.1016/j.pbb.2021.173118.
Tirri, M., Arfè, R., Bilel, S., Corli, G., Marchetti, B., Fantinati, A., Vincenzi, F., De-Giorgio, F., Camuto, C., Mazzarino, M., Barbieri, M., Gaudio, R.M., Varani, K., Borea, P.A., Botrè, F., Marti, M., 2022. In vivo bio-activation of JWH-175 to JWH-018: pharmacodynamic and pharmacokinetic studies in mice. Int. J. Mol. Sci. 23 (14), 8030. https://doi.org/10.3390/ijms23148030.
Tosolini, A.P., Morris, R., 2012. Spatial characterization of the motor neuron columns supplying the rat forelimb. Neuroscience 200, 19–30. https://doi.org/10.1016/j.neuroscience.2011.10.054.
Tsou, K., Brown, S., Sañudo-Peña, M.C., Mackie, K., Walker, J.M., 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83 (2), 393–411. https://doi.org/10.1016/s0306-4522(97)00436-3.
Tuv, S.S., Krabseth, H., Karinen, R., Olsen, K.M., Øiestad, E.L., Vindenes, V., 2014. Prevalence of synthetic cannabinoids in blood samples from Norwegian drivers suspected of impaired driving during a seven weeks period. Accid. Anal. Prev. 62, 26–31. https://doi.org/10.1016/j.aap.2013.09.009.
Tzounopoulos, T., Rubio, M.E., Keen, J.E., Trussell, L.O., 2007. Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. Neuron 54 (2), 291–301. https://doi.org/10.1016/j.neuron.2007.03.026.
Vučinić, S., Kilibarda, V., Đorđević, S., Đorđević, D., Perković-Vukčević, N., Vuković-Ercegović, G., Antonijević, B., Ćurčić, M., Antonijević, E., Brajković, G., 2018. Clinical and analytical experience of the National Poison Control Centre with synthetic cannabinoids. Arh. Hig. Rada Toksikol. 69 (2), 178–185. https://doi.org/10.2478/aiht-2018-69-3096.
Walsh, J.M., de Gier, J.J., Christopherson, A.S., Verstraete, A.G., 2004. Drugs and driving. Traffic Inj. Prev. 5 (3), 241–253. https://doi.org/10.1080/15389580490465292.
Wang, Z., Fong, C.Y., Goh, E., Moy, H.Y., Chan, E., 2021. Transesterification of indazole-3-carboxamide synthetic cannabinoids: identification of metabolite biomarker for diagnosing co-abuse of 5F-MDMB-PINACA and alcohol. J. Anal. Toxicol. https://doi.org/10.1093/jat/bkab121.
Weinstein, A., Brickner, O., Lerman, H., Greemland, M., Bloch, M., Lester, H., Freedman, N., Mechoulam, R., Bar-Hamburger, R., Chisin, R., Even-Sapir, E., 2007. Brain imaging study of the acute effects of Δ-9 THC on attention and motor coordination in regular users of marijuana. Psychopharmacology 196 (1), 119–131. https://doi.org/10.1007/s00213-007-0940-7.
White, A.M., Matthews, D.B., Best, P.J., 2000. Ethanol, memory, and hippocampal function: a review of recent findings. Hippocampus 10 (1), 88–93. https://doi.org/10.1002/(SICI)1098-1063(2000)10:1<88::AID-HIPO10>3.0.CO;2-L.
White, A.M., Simson, P.E., Best, P.J., 1997. Comparison between the effects of ethanol and diazepam on spatial working memory in the rat. Psychopharmacology 133 (3), 256–261. https://doi.org/10.1007/s002130050399.
WHO (World Health Organization), 2018. Global Status Report on Road Safety 2018, Geneva.
Wille, S.M.R., Richeval, C., Nachon-Phanithavong, M., Gaulier, J.M., Di Fazio, V., Humbert, L., Samyn, N., Allorge, D., 2018. Prevalence of new psychoactive substances and prescription drugs in the Belgian driving under the influence of drugs population. Drug Test. Anal. 10 (3), 539–547. https://doi.org/10.1002/dta.2232.
Williams, M.T., Herring, N.R., Schaefer, T.L., Skelton, M.R., Campbell, N.G., Lipton, J.W., McCrea, A.E., Vorhees, C.V., 2007. Alterations in body temperature, corticosterone, and behavior following the administration of 5-methoxy-diisopropyltryptamine ('foxy') to adult rats: a new drug of abuse. Neuropsychopharmacology 32 (6), 1404–1420. https://doi.org/10.1038/sj.npp.1301232.
Wu, G., Liu, H., Jin, J., Hong, L., Lan, Y., Chu, C.P., Qiu, D.L., 2014. Ethanol attenuates sensory stimulus-evoked responses in cerebellar granule cells via activation of GABA (A) receptors in vivo in mice. Neurosci. Lett. 561, 107–111. https://doi.org/10.1016/j.neulet.2013.12.049.
Yazulla, S., Studholme, K.M., McIntosh, H.H., Deutsch, D.G., 1999. Immunocytochemical localization of cannabinoid CB1 receptor and fatty acid amide hydrolase in rat

retina. J. Comp. Neurol. 415 (1), 80–90. https://doi.org/10.1002/(sici)1096-9861(19991206)415:1<80::aid-cne6>3.0.co;2-h.

Yeakel, J.K., Logan, B.K., 2013. Blood synthetic cannabinoid concentrations in cases of suspected impaired driving. J. Anal. Toxicol. 37 (8), 547–551. https://doi.org/10.1093/jat/bkt065.

Zhao, Y., Rubio, M., Tzounopoulos, T., 2011. Mechanisms underlying input-specific expression of endocannabinoid-mediated synaptic plasticity in the dorsal cochlear nucleus. Hear Res. 279 (1-2), 67–73. https://doi.org/10.1016/j.heares.2011.03.007.