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Effect of cannabinol, tetrahydrocannabivarin and cannabidiol on voluntary alcohol consumption

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ABSTRACT

Aims: **Previous studies have demonstrated that the endocannabinoid system plays a significant role in the development of alcohol use disorder (AUD), and CB1 receptor antagonists/inverse agonists show promise as a novel AUD pharmacotherapy. However, these compounds failed in clinical trials due to the severe psychiatric side effects. Non-psychoactive phytocannabinoids may have a better safety profile and could be used as an alternative approach to treat AUD. The aim of this study was to test the potential of three phytocannabinoids in reducing alcohol consumption: CB1 receptor partial agonist cannabinol (CBN), neutral antagonist tetrahydrocannabivarin (THCV) and negative allosteric modulator cannabidiol (CBD).**

Methods: **Male Wistar rats were subjected to a long-term voluntary alcohol drinking procedure that lasted for several months. Thereafter, rats were given three once daily administrations of CBN, THCV, or CBD. Their side-effect profile was examined by recording changes in water consumption, body weight and locomotor activity. Ultrasonic vocalisations were recorded in alcohol-naïve group-housed rats to monitor if treatment induced discomfort, distress, or other changes in emotional states.**

Results: **Our data demonstrated that all phytocannabinoids reduced voluntary alcohol consumption; however, the compounds differed in their effectiveness and side-effect profile. Treatment with CBN and THCV reduced alcohol intake and alcohol preference and had a mild sedative effect. CBD had a minor effect on alcohol consumption, did not affect alcohol preference, reduced the locomotor activity and lowered the positive emotional states of rats. None of the compounds caused discomfort or distress.**

Conclusions: **We conclude that CBN and THCV may have potential in treating AUD.**

Keywords alcohol use disorder, long-term voluntary alcohol drinking rats, cannabinol, tetrahydrocannabivarin, cannabidiol.

Introduction

The endogenous cannabinoid system is the most abundant system in the brain, modulating nearly all physiological processes including mood, pain perception, sleep–wake cycle and appetite (for a review see Kano et al. 2009, Ligresti et al. 2016, Aizpurua-Olaizola et al. 2017). Accordingly, it has been demonstrated that this system may be involved in many known pathological conditions, including several neurological and neurodegenerative disorders, as well as many psychiatric disorders like anxiety, depression and post-traumatic stress disorder (Ligresti et al. 2009, 2016, Basavarajappa et al. 2017). It has also been established that the endocannabinoid system is involved in the development of substance use disorders (SUD, Maldonado et al. 2006). Furthermore, multiple preclinical studies demonstrated that a reduction in CB1 receptor signalling was effective in reducing substance self-administration and relapse-related behaviours (Maldonado et al. 2006, Vengeliene et al. 2008), suggesting a good rationale for targeting the CB1 receptor to treat SUD. Hence, several CB1 antagonists/inverse agonists have been tested clinically; however, due to their adverse effects, they have not been found to be a suitable treatment option for patients suffering from SUD (Robinson et al. 2018, Nguyen et al. 2019).

Cannabis plants have long been used both medicinally and recreationally, mainly due to the psychoactive compound delta9-tetrahydrocannabinol (THC, a partial agonist of the CB1 receptor). However, the health benefits of these plants may be attributable to over a hundred of other, non-psychoactive compounds or their metabolites, collectively termed phytocannabinoids (Gülck and Møller 2020). Phytocannabinoids act on the CB1 receptor either as neutral antagonists, partial agonists, or as allosteric modulators (Gülck and Møller 2020), and may have a better safety profile

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than CB1 antagonists/inverse agonists that suppress constitutive receptor activity (e.g. Manning et al. 2021). The full therapeutic potential of phytocannabinoids is not yet known, and the majority of the available studies have focused on studying one specific major phytocannabinoid, cannabidiol (CBD, a negative allosteric modulator of the CB1 receptor). Besides the cannabinoidergic system, CBD affects several other brain systems: opioidergic, serotonergic, TRP channels, PPAR nuclear receptors and others (Pertwee 2008, De Petrocellis et al. 2011, McPartland et al. 2015). It has been demonstrated that CBD has the potential to treat a variety of medical conditions, such as anxiety, epilepsy and chronic pain (Mechoulam et al. 2002).

Targeting the endocannabinoid system by the use of non-psychoactive phytocannabinoids have recently been suggested as a potential strategy for clinical development of SUD pharmacotherapies (e.g. Spanagel 2020, Karimi-Haghighi et al. 2022). For instance, preclinical studies demonstrated that CBD may be protective against neurotoxicity induced by binge drinking (Liput et al. 2013, Hamelink et al. 2005). CBD have also shown to alleviate nicotine withdrawal symptoms (Smith et al. 2021) and attenuate cognitive deficits and neuroinflammation induced by early alcohol exposure (García-Baos et al. 2021), and reduce alcohol seeking and self-administration (Turna et al. 2019). Recent clinical trials using CBD to treat alcohol use disorder (AUD) suggest that CBD may be effective in reducing alcohol craving (Kirkland et al. 2025, Mueller et al. 2025, Pfisterer et al. 2025, Zimmermann et al. 2025).

Many other phytocannabinoids are less studied but have partly overlapping pharmacodynamic characteristics with CBD, including interaction with CB1 and CB2 receptors and TRP channels (Pertwee 2008, De Petrocellis et al. 2011, McPartland et al. 2015, Gülck and Møller 2020). A combination therapy using additively or synergistically acting phytocannabinoids (Christensen et al. 2023) may lead to better clinical outcomes than CBD monotherapy (e.g. Redonnet et al. 2025). However, more research is needed to evaluate therapeutic potential of other phytocannabinoids in treating SUD.

In the present study we explored, for the first time, the impact of cannabinol (CBN) and tetrahydrocannabivarin (THCV) on voluntary alcohol consumption in long-term drinking rats. We also tested CBD under the same experimental conditions as a positive control, since it has already been demonstrated that this compound reduced alcohol consumption in rodents (Viudez-Martínez et al. 2018). CBN is a partial agonist of the CB1 receptor and activates the receptor but with a much lower potency than the psychoactive compound THC (Rhee et al. 1997). CBN also acts on several other targets, e.g. CB2 receptors and TRP channels (Rhee et al. 1997, De Petrocellis et al. 2011). CBN is mildly sedative and may have a potential in treating sleep disorders (Lavender et al. 2023, Bonn-Miller et al. 2024, Arnold et al. 2025). Preclinical studies suggest that restoration of the circadian activity and sleep quality in chronically drinking animals could contribute to a reduced likelihood of alcohol consumption (Lawrence et al. 2006, Vengeliene et al. 2020). THCV is a neutral antagonist of the CB1 receptor, however, at higher doses it may behave as an 'indirect agonist' by inhibiting elimination of endocannabinoids. THCV also acts at several other targets, such as the CB2 and the 5-HT1A receptor and TRP channels (Pertwee 2008, McPartland et al. 2015). It has been suggested that THCV and other neutral antagonists may have potential in treating obesity and SUD (Le Foll et al. 2009, Abioye et al. 2020, Mendoza 2025).

To achieve the study goals, we used a long-term voluntary alcohol drinking model in male Wistar rats. This model is used to study changes in the maintenance of drinking behaviour and has good predictive validity (i.e. voluntary drinking in rats has been reduced by clinically used drugs acamprosate and naltrexone, e.g. Cowen et al. 2005, Waeiss et al. 2019). We also explored the side-effect profile of CBN, THCV and CBD by measuring treatment-induced changes in water consumption, body weight and home-cage locomotor activity in long-term voluntary alcohol drinking rats. To measure if compounds caused a state of discomfort or distress and other changes in the emotional state of rats, we recorded ultrasonic vocalisations (USVs) in alcohol naïve group-housed male and female rats. Two major subtypes of USVs were counted: 50 kHz vocalizations expressing positive emotional states (i.e. simple contact calls, trills emitted in highly emotional situations expressing arousal state and step calls emitted in rewarding situations) and 22 kHz vocalizations expressing negative emotional states (i.e. long calls emitted during exposure to a dangerous situation and short calls expressing a state of discomfort or distress) (Wright et al. 2010, Brudzynski 2015, Poceviciute et al. 2023). Alcohol naïve group-housed rats were used, as our pilot studies showed that long-term alcohol drinking single-housed rats emit few, or no USVs at all.

Materials and methods

Animals

Fifty-one 8-week-old outbred male Wistar rats were used in the alcohol consumption study and 32 eight-week-old outbred male Wistar rats and 24 eight-week-old outbred female rats were used to record ultrasonic vocalisations (from our own breeding colony at the Vilnius University, Lithuania). All animals were group-housed throughout their adolescence into adulthood. For the alcohol study, adult animals were housed individually in standard rat cages and, for the recording of vocalisations, all animals remained grouped-housed throughout the entire course of the experiment. Animals were kept under a 12/12-hour artificial light/dark cycle (lights on at 7:00 a.m.) and constant room temperature ($22 \pm 1°C$). Standard laboratory rat food (4RF21-GLP, Mucedola srl, Milan, Italy) and tap water were provided *ad libitum* throughout the experimental period.

Drugs

For the repeated administration of phytocannabinoids in the alcohol consumption study, CBN and THCV (generously provided by Sanobiotec, Lithuania) were dissolved in tween 80 (Sigma-Aldrich, Germany) and then diluted with 0.9% saline to a final tween concentration of 4% and 5%, respectively, and injected intraperitoneally (IP) as a volume of 2 ml/kg. CBD (THC Pharm GmbH, Germany) was suspended in 0.9% saline containing 0.5% methylcellulose (Sigma-Aldrich, Germany) and injected as a volume of 3 ml/kg IP. For a single administration of phytocannabinoids in alcohol naïve rats, all compounds were dissolved in tween 80 and then diluted with 0.9% saline to a final tween concentration of 20% and injected IP as a volume of 2 ml/kg (please note that one common control group was used and 20% tween was used in order to dissolve CBD and ensure

its fast absorption, as the USV recordings lasted for 5 minutes). Control animals received an equal volume of respective vehicles.

Effect of phytocannabinoid treatment on alcohol consumption and locomotor activity

Voluntary alcohol consumption

After two weeks of habituation to the experimental room, all rats were given *ad libitum* access to water and to 5% ethanol solution (v/v) for one week, after which alcohol concentration was increased to 8% (v/v) for another week, and finally, increased to 10% (v/v) for the remainder of the experiment. Alcohol drinking solutions were prepared from the absolute (99.8%) ethanol (Honeywell, Germany) and then diluted with tap water. The positions of bottles were changed weekly to avoid location preferences. Alcohol and water intake were measured either weekly or daily, and from these data, alcohol consumption (in g of pure ethanol/kg of body weight per day, g/kg/day), water consumption (in ml/kg/-day) and alcohol preference (the proportion of alcohol consumed relative to total fluid intake) were calculated.

Locomotor activity recordings

To measure the effect of the treatment on locomotor activity, rats were placed in rectangular acrylic cages (floor: 40 × 40 cm, height: 50 cm) to ensure enough moving space and to avoid recording interruptions caused by a rat moving under the bottle/food area. Cages were equipped with ports for food and 2 bottles (placed on the outer surface of the cage), and rats were given *ad libitum* access to food, tap water and to 10% ethanol solution. Passive infrared (PIR) sensors (SEN0171, DFRobot, Shanghai, China) were used for measuring 24-hour locomotor activity of rats. The sensors were activated by infrared light radiating from moving objects in its field of view. Each PIR sensor was placed inside of a 3D printed casing, placed above the cage and had a 60° detection angle. The sensors were connected to the digital pins of an Arduino Uno R3 microcontroller, programmed to send a timestamp and pin ID whenever a change of state (ON or OFF) was detected (the microcontroller code is available at https://github.com/MartynasArba/motionsensors). Since the Arduino Uno R3 has no clock module, the output was set to display milliseconds from turning the device on. This output was sent to a computer via a serial port, for which the baud rate was set to 9600. Data was captured using the Realterm 3.0.1.44 terminal program, which allowed writing the output to a .txt file with an added system timestamp. To evaluate the locomotor activity of rats, the sum movement duration was calculated by subtracting the time of every ON event (when motion was detected, i.e. a moving event) from the time of the subsequent OFF event.

Pharmacological intervention study

Only those rats that had an average baseline alcohol intake of ∼2 g/kg/day or more were used for the pharmacological intervention study (please note that these intake levels are typical for outbred male Wistar rats, Vengeliene et al. 2014; and rats consuming ∼1 g/kg/day of ethanol or lower were excluded from the study to avoid the 'floor effect'). All experiments were done in two parallel subsets of rats (n = 25–26) kept under slightly different experimental conditions – drinking in the conventional rat home-cages and drinking in the rectangular cages (see the chapter above 'Locomotor activity recordings'). Locomotor activity was measured in one subset of rats, starting at least 3 days before the drug treatment procedure and was continued for several post-treatment days. In order to study the effect of phytocannabinoid treatment on baseline alcohol and water drinking, and on the locomotor activity, rats from both subsets were divided into groups (n = 6 per group in each subset and n = 12 per treatment condition) so that mean alcohol and water intake were matched, and ensuring random assignment to the groups for each subsequent treatment to avoid carry-over effects from the previous treatment. Before each drug treatment, stable baseline drinking was monitored for at least 3 days.

Pharmacological intervention was done approximately once per month starting at the 6th month of continuous alcohol consumption. After the last day of baseline measurements, each animal was subjected to a daily injection of vehicle, 5 mg/kg of CBN or 10 mg/kg of CBN over the course of the three subsequent days, given at ∼6:00 p.m. (n = 12 per treatment condition). Thereafter, animals were left undisturbed until baseline drinking levels recovered. As it was found that CBN produced a delayed effect on drinking, this treatment was repeated in home-cage drinking rats using either vehicle or 5 mg/kg of CBN (n = 8 per treatment condition) injected at the beginning of the light phase (∼8:00 a.m.) to examine if delayed effect of CBN was related to its accumulation in the body or if the acute pharmacological effect of CBN was necessary to induce a long-lasting change in animal behaviour. Similarly, with 1-month of recovery in-between treatment periods, vehicle, 5 mg/kg of THCV or 20 mg/kg of THCV (n = 12 per treatment condition); and later, vehicle, 20 mg/kg of CBD or 40 mg/kg of CBD (n = 12 per treatment condition), were administered once every 24 hours for three consecutive days at ∼6:00 p.m. THCV and CBD were administered just before the animal's active phase. The doses (Rock et al. 2013, Wargent et al. 2013, Viudez-Martínez et al. 2018, Maccioni et al. 2022, Arnold et al. 2025) and treatment regimen (Deiana et al. 2012, Gonzalez-Cuevas et al. 2018, Moore et al. 2023) were chosen based on published reports and our pilot studies showing that a single administration of lower doses of all three compounds did not induce sedation in alcohol naïve rats in the open field test. Brain/plasma elimination half-life in rats after a single administration of either compound is ∼4–5 hours and they can be detected beyond 24 hours following repeated administration (Deiana et al. 2012, Gonzalez-Cuevas et al. 2018, Moore et al. 2023).

Body weights were measured just before pharmacological intervention experiments and the next day after the last drug administration. The person responsible for animal handling was blind to the treatment assignment throughout the experiment.

Effect of phytocannabinoid treatment on ultrasonic vocalisations in alcohol naïve rats

Acoustic data acquisition took place in an Eurostandard type IVS cage placed in a sound-attenuated cubicle. For acoustic data acquisition, an electret ultrasound microphone with a frequency response range of 0 to 125 kHz (Avisoft Bioacoustics, Germany), securely positioned through a small opening on the top of the

cubicle, and the Avisoft-SASLab software Pro (Avisoft Bioacoustics, Germany) were used.

During USV recordings, rats were ∼7-month-old. Prior to recordings, all rats were habituated to the testing conditions 4 times: 3 times (every second/third day) by placing all cage-mates together into the testing cage and once individually (one day prior to the test) for 5 min. For acoustic data acquisition prior to drug treatment (baseline, BL), each rat was separated from the group and gently placed into the testing cage for 5 min. Approximately 2 weeks later, the effect of phytocannabinoid treatment on ultrasonic vocalisations was tested. For this purpose, rats were divided into four groups (male rats n = 8 and female rats n = 6 per group, please note that males and females were not tested in parallel) in such a way that the mean number of USVs during the BL was matched, and maintaining grouping randomisation within each cage. One group of rats received a single administration of vehicle (see 'Drugs'), and the other three groups received 5 mg/kg of CBN, 5 mg/kg of THCV, or 20 mg/kg of CBD 30 min prior to the test (please note that these doses were chosen as they demonstrated better safety profile than higher doses in the long-term alcohol drinking rats and had no effect on the locomotor activity in the open field test in the pilot studies). For data analysis, all calls were manually selected from spectrograms by a trained observer blind to the experimental design. The USVs were classified into two major subtypes: (i) 50 kHz USVs (i.e. vocalizations expressing positive emotional states, such as simple calls, trills, frequency-modulated calls with attached trills and all types of step calls), and (ii) 22 kHz USVs (i.e. vocalizations expressing negative emotional states, such as long and short 22 kHz calls).

Statistics and data analysis

Data derived from home-cage drinking (alcohol intake and preference, water intake) and locomotor activity (for data analysis the time spent moving and the number of moving events during 12-hour post-injection intervals of the animals' active (dark) phase was used) was analysed using a two-way analysis of variance (ANOVA) with repeated measures [factors were: treatment group and time (days)]. Data analysis regarding the effects of treatment on the change in the animals' body weight (%) was performed using a one-way ANOVA [factor – treatment group]. Data derived from acoustic data acquisition was analyzed using a two-way ANOVA with repeated measures [factors were: treatment group and session (baseline vs. treatment)]. Whenever significant differences were found, post-hoc Student Newman Keuls tests were performed. The chosen level of significance was $P < .05$.

Results

Effect of phytocannabinoid treatment on alcohol consumption and locomotor activity

CBN treatment effect

A two-way repeated measures ANOVA revealed that treatment with CBN had a significant effect on alcohol drinking in rats [factor treatment group × time interaction effect: $F(16,323) = 2.76$, $P < .001$ and $F(16,323) = 2.11$, $P < .01$ for alcohol intake and preference, respectively]. Post hoc analysis demonstrated that both doses of CBN caused a significant reduction in alcohol consumption compared to vehicle treatment and reduced the alcohol intake below baseline drinking levels (Fig. 1A, B). Lower alcohol intake and preference in rats treated with CBN persisted for 3 post-treatment days. Water intake data analysis demonstrated that CBN treatment caused a long-lasting increase in water intake compared to both vehicle treatment and baseline water consumption [factor treatment group × day interaction effect: $F(16,323) = 3.07$, $P < .001$] (Fig. 1C), demonstrating that this treatment was selective towards lowering alcohol consumption. Post hoc analysis revealed that the strongest effect on water intake was seen during post-treatment days, suggesting that CBN might be accumulating in the body. However, 5 mg/kg of CBN administered on 3 consecutive days during the onset of the rat's inactive phase had no effect on alcohol and water intake (data not shown), suggesting that the acute pharmacological effect of CBN is necessary to lower alcohol consumption in rats.

Analysis of the locomotor activity data by use of a two-way repeated measures ANOVA revealed that treatment with CBN slightly but not significantly reduced the time spent moving during the active phase when compared to vehicle treatment [factor treatment group: $P = .07$ and factor treatment group × time interaction effect: $P = .26$] (Fig. 2A). However, the number of moving events was lower in groups treated with CBN [factor treatment group: $F(2,107) = 4.39$, $P < .05$ and factor treatment group × time interaction effect: $F(10,107) = 2.02$, $P < .05$]. Further analysis demonstrated that CBN treated rats increased locomotor activity during post-treatment days (Fig. 2B). The lower dose of CBN did not have a significant impact on the body weight; however, treatment of rats with the higher dose caused a small but significant loss ($-1.3 \pm 0.4\%$) of body weight [factor treatment group: $F(2,23) = 5.15$, $P < .05$]. These data suggest that CBN had a mild sedative effect and the 10 mg/kg dose affected food intake and/or metabolism.

THCV treatment effect

A two-way repeated measures ANOVA demonstrated that THCV treatment caused a significant reduction in alcohol intake compared to vehicle treatment and reduced alcohol consumption below baseline drinking levels [factor treatment group × day interaction effect: $F(16,323) = 3.93$, $P < .001$ and $F(16,323) = 3.37$, $P < .001$ for alcohol intake and preference, respectively]. However, a subsequent post hoc analysis showed that only the higher THCV dose had a significant impact on alcohol consumption (Fig. 1D, E). Analysis of the water consumption data revealed that THCV treatment also had a significant effect on water intake [factor treatment group × day interaction effect: $F(16,323) = 1.78$, $P < .05$]. Post hoc analysis revealed that 20 mg/kg of THCV increased water consumption above baseline levels during two post-treatment days (Fig. 1F).

Both locomotor activity and body weight were affected by repeated THCV administration. The total time spent moving during the active phase was slightly lower during treatment days but recovered immediately as drug administration was terminated [factor treatment group: $P = .71$ and factor treatment group × time interaction effect: $F(10,107) = 2.19$, $P < .05$]. The number of moving events was also lower [factor treatment group: $P = .98$ and factor treatment group × time interaction effect: $F(10,107) = 2.53$, $P < .05$]. Subsequent post hoc analysis revealed that THCV treated

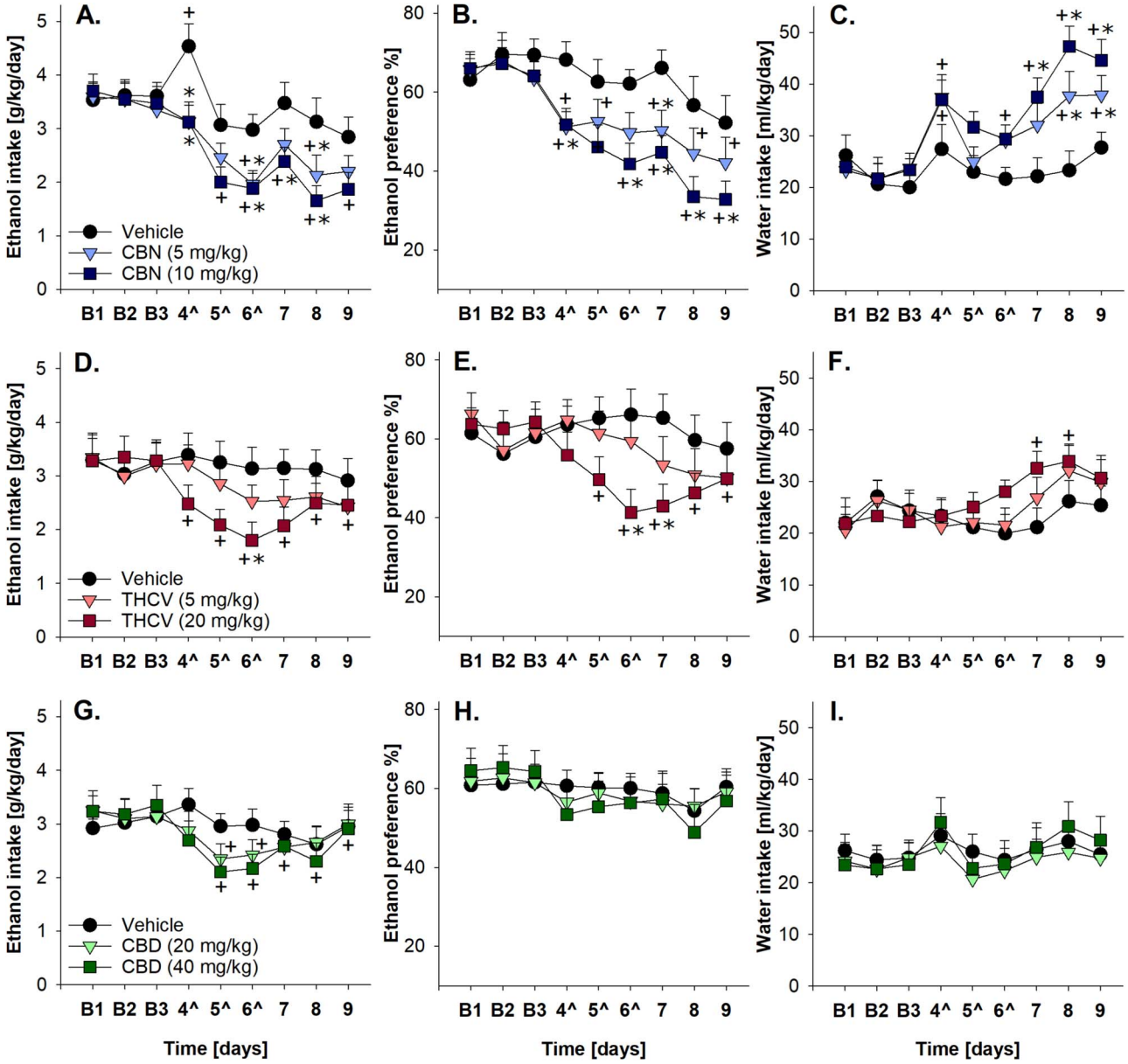

Figure 1 Total daily alcohol intake (calculated in g of pure ethanol per kg of body weight per day, g/kg/day, A, D, G), alcohol preference (calculated as percentage of total fluid intake, B, E, H) and water intake (ml/kg/day, C, F, I) in long-term drinking rats measured prior to (days B1-B3), during (days 4^-6^) and after (days 7–9) three once daily administrations (just before the animal's active phase) of (A, B, C) vehicle, 5 mg/kg of CBN or 10 mg/kg of CBN; (D, E, F) vehicle, 5 mg/kg of THCV, or 20 mg/kg of THCV; and (G, H, I) vehicle, 20 mg/kg of CBD or 40 mg/kg of CBD (n = 12 per treatment condition). Data are presented as means ± S.E.M. ∗ indicates significant differences from the vehicle group and + indicates significant difference from all 3 baseline days, $P < .05$.

groups were slightly more active during post-treatment days (Fig. 2C, D). Similarly to CBN, the higher dose of THCV caused small but significant loss (−1.2 ± 0.3%) of body weight [factor treatment group: F(2,23) = 11.24, $P < .001$].

CBD treatment effect

Drinking data analysis revealed a significant effect of CBD treatment on alcohol intake [factor treatment group × day interaction effect: F(16,323) = 2.24, $P < .01$]. Post hoc analysis showed that both CBD doses reduced alcohol consumption below baseline drinking levels; however, the differences in alcohol intake between treatment groups were not significant (Fig. 1G). Administration of CBD had no effect on either alcohol preference [factor treatment group × day interaction effect: $P = .822$] or water consumption [factor treatment group × day interaction effect: $P = .91$] (Fig. 1H, I).

CBD treatment had a significant effect on the time spent moving [factor treatment group: F(2,107) = 4.39, $P < .05$ and factor treatment group × time interaction effect: F(10,107) = 2.64, $P < .01$] and the number of moving events [factor treatment group: F(2,107) = 3.98, $P < .05$ and factor treatment group × time interaction effect: F(10,107) = 3.40, $P < .001$]. Post hoc analysis demonstrated that both CBD doses had a significant impact on

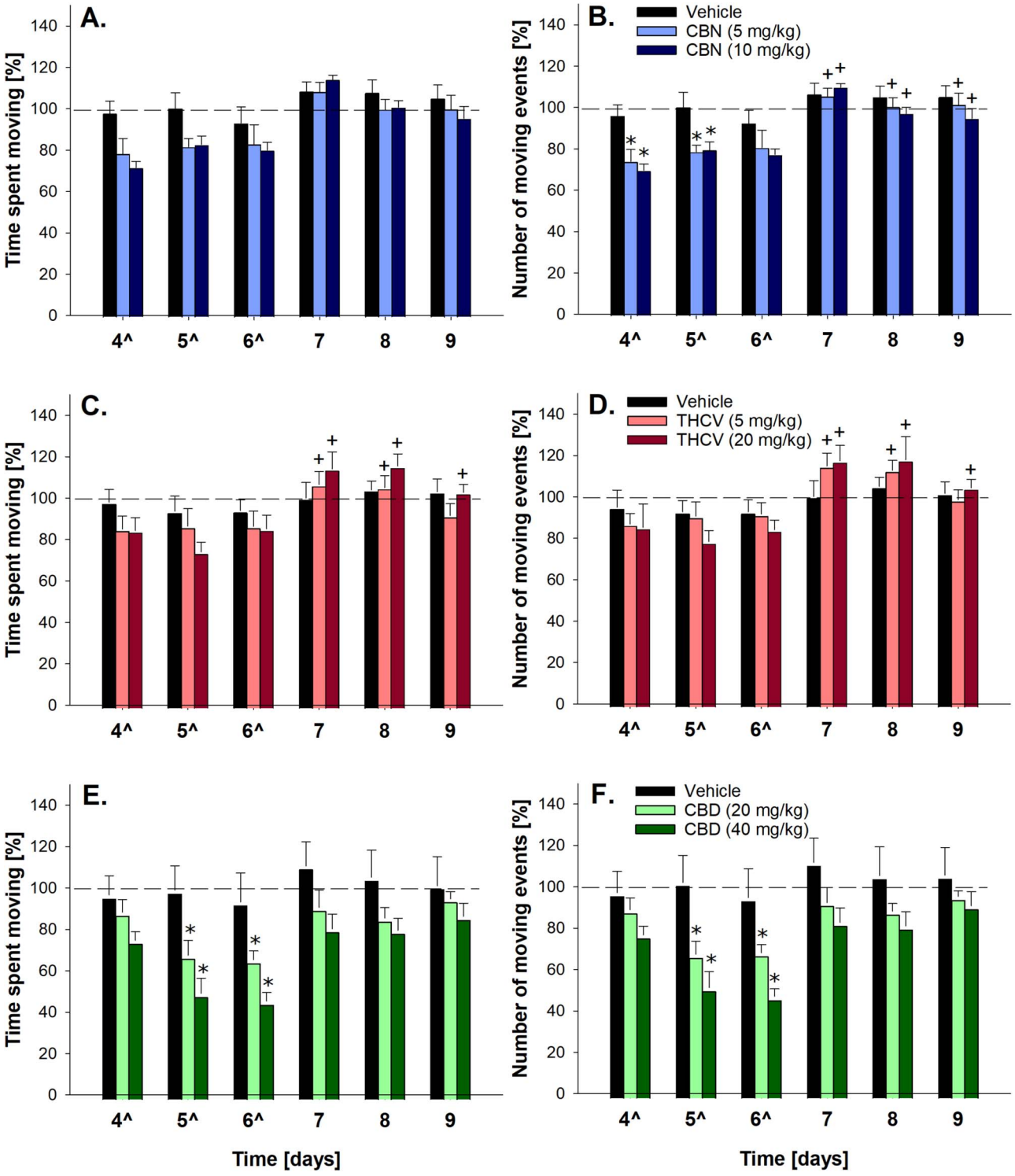

Figure 2 Locomotor activity in (A, B) vehicle, 5 mg/kg of CBN or 10 mg/kg of CBN, (C, D) vehicle, 5 mg/kg of THCV or 20 mg/kg of THCV, and (E, F) vehicle, 20 mg/kg of CBD or 40 mg/kg of CBD treated long-term drinking rat groups (n = 6 per treatment condition). Locomotor activity is shown as (A, C, E) the time spent moving and (B, D, F) the number of moving events during 12-hour postinjection intervals of the animals' active phase. The percentage of each rat's locomotor activity during (days 4^–6^) and after (days 7–9) treatment was calculated with respect to the basal activity prior to treatment (dashed line). Data are presented as means ± S.E.M. ∗ indicates significant differences from the vehicle group, + indicates significant difference from all 3 treatment days, $P < .05$.

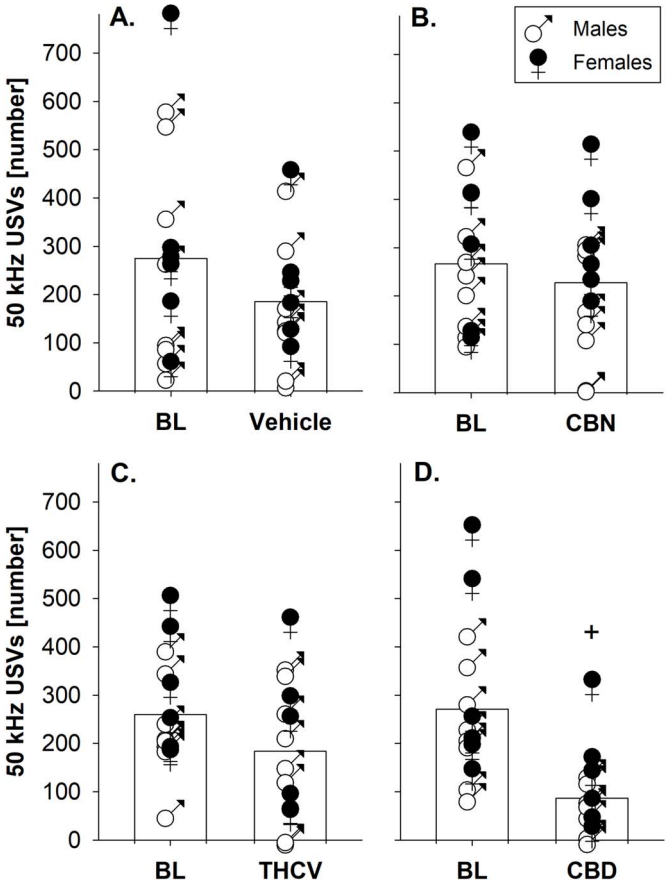

Figure 3 The number of 50 kHz ultrasonic vocalisations (USVs) in alcohol naïve male (n = 8 per treatment condition) and female (n = 6 per treatment condition) rats emitted during 5-min acoustic recordings before (BL) and 30 min after a single administration of (A) vehicle, (B) 5 mg/kg of CBN, (C) 5 mg/kg of THCV or (D) 20 mg/kg of CBD. Data are presented as the mean number of USVs (bars) and as individual data points. + indicates significant differences from baseline condition, $P < .05$.

the locomotor activity (Fig. 2E, F). The activity recovered during the first post-treatment days showing that the sedative effect of CBD was short-lasting. The higher dose of this treatment also caused a slight reduction ($-0.5 \pm 0.5\%$) in body weight [factor treatment group: $F(2,23) = 4.33$, $P < .05$].

Effect of phytocannabinoid treatment on ultrasonic vocalisations in alcohol naïve rats

A two-way repeated measures ANOVA revealed that administration of test compounds lowered the number of 50 kHz USVs compared to the baseline recording session prior to treatment in both male and female rats [factor session: $F(1,63) = 9.64$, $P < .01$ and $F(1,47) = 12.25$, $P < .01$ for male and female rats, respectively]. Post hoc analysis revealed that this reduction was statistically significant only in the 20 mg/kg of CBD treatment groups (Fig. 3). Neither compound reduced the number of 50 kHz USVs below that recorded in the vehicle-treated group [factor treatment group × session interaction effect: $P = .58$ and $P = .14$ for male and female rats, respectively]. Selective effect on a specific subtype of 50 kHz vocalizations was not observed (data not shown).

Rats did not emit any 22 kHz calls, neither during baseline nor during post-treatment recording session, demonstrating that a single administration of either 5 mg/kg of CBN, 5 mg/kg of THCV, or 20 mg/kg of CBD did not cause discomfort or distress to the animals.

Discussion

Our study demonstrated that repeated administration of all three phytocannabinoids reduced voluntary alcohol consumption in long-term drinking male Wistar rats. However, the compounds differed in their effectiveness. Administration of CBN caused a significant dose-dependent reduction in alcohol intake, and it was accompanied by a marked increase in water intake, demonstrating the selectiveness of this treatment towards lowering alcohol consumption. This effect was observed during three consecutive days following treatment completion. The long-lasting effect of CBN was not caused by its accumulation, since CBN administered during the onset of the rat's inactive phase had no effect on alcohol or water intake, suggesting that the acute pharmacological effect of CBN is necessary to induce a long-lasting change in animal behaviour. Treatment with the highest dose of THCV had a similar but somewhat weaker effect on alcohol and water consumption. Finally, CBD only had a modest effect on alcohol intake and did not affect water intake or alcohol preference. All phytocannabinoids had a transient sedative effect on chronically drinking animals. However, the reduction in the

locomotor activity was stronger during treatment with either dose of CBD than with the other compounds. The higher dose of all compounds led to a small but significant decrease in body weight, demonstrating that they may cause unselective effects in long-term voluntary alcohol drinking male rats. The lower dose of all three compounds had no effect on body weight in chronically drinking rats and did not induce a state of discomfort or distress in alcohol naïve male and female rats. However, the lower dose of CBD reduced the expression of positive emotional state of rats measured as fewer 50 kHz vocalizations. These data suggest that CBN and THCV, at the doses used in the present study, demonstrate a better safety profile than CBD.

It is known that endocannabinoid signalling contributes to the primary rewarding effects of alcohol due to its modulatory function on the mesocorticolimbic dopaminergic system; and CB1 receptor blockade attenuates voluntary alcohol intake and several other alcohol-related behaviours (Maldonado et al. 2006, Basavarajappa 2007). Therefore, it is surprising that in our study, CBN, a low potency partial agonist, was more effective in lowering alcohol consumption than THCV and CBD. It has been demonstrated that chronic alcohol exposure may lead to increased extracellular levels of endocannabinoids and down-regulation of CB1 receptor expression and function (Basavarajappa 2007). This suggests that under the present experimental conditions (rats had continuous access to alcohol for at least 6 months), endocannabinoid levels may have been elevated and CBN acted as an indirect CB1 receptor antagonist by interfering with the endogenous agonist binding. Furthermore, it has been shown that endocannabinoid signalling may be reversed by alcohol abstinence (Pava and Woodward 2012, Kleczkowska et al. 2016); hence, response to CB1 receptor partial agonists following a short alcohol exposure or in abstinent rats may be different than that of chronically drinking rats. Indeed, it has been demonstrated that administration of a partial agonist of the CB1 receptor, THC, promoted relapse-like behaviour in alcohol abstinent rats (McGregor et al. 2005). However, the study of McMillan and Snodgrass (1991) showed that acute administration of THC reduced maintenance of alcohol consumption in rats that had been trained to consume alcohol in an operant setting for 2 months.

It has been demonstrated that CB1 antagonists/inverse agonists were effective in both body weight reduction (Murphy and Le Foll 2020) and self-administration of many abused substances (Maldonado et al. 2006). A neutral antagonist of the CB1 receptor THCV has also been effective in appetite suppression and increased the energy expenditure (Mendoza 2025), and thus, it could be expected that this compound would produce similar effect on alcohol consumption as CB1 antagonists/inverse agonists. Our study supports this notion, demonstrating that 20 mg/kg of THCV reduced alcohol intake and alcohol preference.

The effectiveness of CBD, a negative allosteric modulator of the CB1 receptor, on alcohol-related behaviours has already been demonstrated in several preclinical studies. Both acute and repeated daily administration of CBD has been shown to reduce voluntary alcohol consumption, self-administration and have a long-lasting effect on relapse-related behaviours (Gonzalez-Cuevas et al. 2018, Viudez-Martínez et al. 2018, Maccioni et al. 2022). In our study, CBD only had a small and short-lasting effect on alcohol intake and had no effect on alcohol preference. Increasing the dose of CBD may have had a stronger impact on alcohol consumption; however, as mentioned above, the highest dose of CBD caused a significant loss of body weight, indicating the occurrence of unwanted effects. In addition, both doses of CBD caused a marked reduction in home-cage activity. Compared to earlier studies, our rats were not only older (during CBD treatment they were ∼1 year old) but also had been drinking alcohol for a long period of time. This could have contributed to lower effectiveness of the CBD treatment on alcohol consumption and higher sensitivity to unwanted effects. However, CBD had the effect of lowering 50 kHz vocalizations, and recent clinical trials have shown that CBD was effective in reducing alcohol craving but not drinking (Kirkland et al. 2025, Mueller et al. 2025, Pfisterer et al. 2025, Zimmermann et al. 2025). We therefore conclude that CBD may not be an effective option to lower the hedonic effect of alcohol, and may need to be used in conjunction with other treatments that have greater direct effect on alcohol consumption (see also Redonnet et al. 2025). Lowering the dosing regimen in a combination therapy could potentially mitigate unwanted side effects.

Furthermore, as mentioned above, besides their direct action on the cannabinoidergic receptors, all three compounds interact with several other receptors and ion channels that may have additional beneficial therapeutic effects in the treatment of AUD. The neurotoxic effect of chronic alcohol consumption on cortical neurons could be of particular importance (Charlton et al. 2019). Phytocannabinoids may have an anti-inflammatory and neuroprotective effect on the nervous system (Hamelink et al. 2005, Liput et al. 2013, Gojani et al. 2023) that could be attributed to both their cannabinoidergic activity and their interactions with TRP channels (De Petrocellis et al. 2011, Ligresti et al. 2016). Multiple other targets have also been identified in the case of CBD that may contribute to its anti-inflammatory and neuroprotective effect, including adenosine, glycine and PPAR nuclear receptors (Carrier et al. 2006, Ahrens et al. 2009, Esposito et al. 2011). However, more research is needed to fully identify the pharmacological targets of the minor phytocannabinoids CBN and THCV (Walsh et al. 2021).

In summary, the present study demonstrated that CBN and THCV were more effective in reducing the maintenance of voluntary alcohol consumption and had a better safety profile compared to CBD. The effect of all three phytocannabinoids on alcohol consumption may be related to their action on the CB1 receptor. However, all three compounds have multiple other molecular targets that may have contributed to their effect on rat behaviour. Because of their different mechanisms of action, phytocannabinoids, such as those used in the present study, could be used to treat different aspects of AUD (i.e. maintenance, withdrawal, or relapse prevention) and thus, contribute to the development of personalized treatment strategies. For instance, CBN may not be effective in relapse prevention; however, it might be effective as a substitution therapy in chronically drinking individuals. CBD may not be effective as a substitution therapy but may have beneficial effects on prevention of reinstatement of alcohol seeking (Gonzalez-Cuevas et al. 2018).

While these results are promising, a few limitations need to be considered. Findings from the study on the effect of phytocannabinoid treatment on alcohol consumption in male Wistar rats might not apply to female rats. Second, CBD-treated animals had alcohol exposure for ∼4 months longer than CBN-treated animals. Longer duration of alcohol drinking could have had an impact on the lower effectiveness of the CBD treatment. Further, USV recordings

were done in group-housed alcohol naïve rats. Long-term alcohol consumption history could have an impact on the affective states of rats and consequently drinking rats may have responded differently to phytocannabinoid treatment with respect to changes in USVs. Finally, considering that individual phytocannabinoids may work additively, or synergistically with one another, the future research should test the effect of combination treatments on voluntary alcohol consumption.

Author contributions

Ieva Poceviciute (Data curation [equal], Formal analysis [equal], Investigation [equal], Writing—original draft [equal]), Martynas Arbaciauskas (Methodology [equal], Resources [equal]), Rokas Buisas (Methodology [equal], Resources [equal]), Osvaldas Ruksenas (Funding acquisition [lead], Writing—review & editing [supporting]), Valentina Vengeliene (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Project administration [equal], Supervision [equal], Writing—review & editing [equal]).

Conflict of interest

The authors declare no conflict of interest.

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Data availability

The data supporting the conclusions of this article will be made available by the authors upon reasonable request. The Arduino Uno R3 microcontroller code is available at https://github.com/MartynasArba/motionsensors.

Ethical approval

All experimental procedures were approved by the State Food and Veterinary Service of the Republic of Lithuania and were carried out in accordance with the local Animal Welfare Act and the European Communities Council Directive of 22 September 2010 (2010/63/EU).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use generative AI or AI-assisted technologies in scientific writing.

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