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Acute effect of vaporized Cannabis on sleep and electrocortical activity

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ABSTRACT

The use of Cannabis for medical purposes is rapidly expanding and is usually employed as a self-medication for the treatment of insomnia disorder. However, the effect on sleep seems to depend on multiple factors such as composition of the Cannabis, dosage and route of administration. Vaporization is the recommended route for the administration of Cannabis for medical purposes; however, there is no published research about the effects of vaporized Cannabis on sleep, neither in laboratory animals, nor in humans. Because previous reports suggested that low doses of THC have sedating effects, the aim of the present study was to characterize in rats, the acute effects on sleep induced by the administration of low doses of THC by means of vaporization of a specific type of Cannabis (THC 11.5% and negligible amounts of other cannabinoids).

For this purpose, polysomnographic recordings in chronically prepared rats were performed during 6 h in the light and dark phases. Animals were treated with 0 (control), 40, 80 and 200 mg of Cannabis immediately before the beginning of recordings; the THC plasma concentrations with these doses were low (up to 6.7 ng/mL with 200 mg). A quantitative EEG analyses by means of the spectral power and coherence estimations was also performed for the highest Cannabis dose.

Compared to control, 200 mg of Cannabis increased NREM sleep time during the light phase, but only during the first hour of recording. Interestingly, no changes on sleep were observed during the dark (active) phase or with lower doses of Cannabis.

Cannabis 200 mg also produced EEG power reductions in different cortices, mainly for high frequency bands during W and REM sleep, but only during the light phase. On the contrary, a reduction in the sleep spindles intra-hemispheric coherence was observed during NREM sleep, but only during the dark phase.

In conclusion, administration of low doses of THC by vaporization of a specific type of Cannabis produced a small increment of NREM sleep, but only during the light (resting) phase. This was accompanied by subtle modifications of high frequency bands power (during the light phase) and spindle coherence (during the dark phase), which are associated with cognitive processing. Our results reassure the importance of exploring the sleep-promoting properties of Cannabis.

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1. Introduction

Cannabis is the most frequently used illicit recreational drug around the globe, with an annual prevalence of 3.8% of the adult population that consumed Cannabis in the past year (UNODC, 2017). Nowadays, the consumption of Cannabis has been legalized in different countries, both for recreational and medical uses (de la Hoz Schilling, 2015).

The plant yields > 538 chemicals of various classes, being phytocannabinoids, terpenes and phenolic compounds the most important (Andre et al., 2016). The two major phytocannabinoids are Δ^9-tetrahydrocannabinol (THC), the main psychoactive compound, and cannabidiol (CBD) (Hložek et al., 2017). It is accepted that most cellular and behavioral effects caused by phytocannabinoids are produced by the modulation of the endocannabinoid system (Murillo-Rodríguez et al., 2011). This system is constituted by two G protein-coupled cannabinoid receptors, CB1 and CB2, as well as by two endogenous ligands, anandamide and 2-arachidonylglycerol (Di Marzo and Piscitelli, 2015; Grotenhermen, 2006; Maccarrone et al., 2015). Endocannabinoids play a regulatory role in a variety of physiological processes including appetite, pain-sensation, perception, mood, memory, inflammation, energy homeostasis and sleep (Di Marzo and Piscitelli, 2015; Grotenhermen, 2006; Prospéro-García et al., 2016).

The use of Cannabis for medical purposes is rapidly expanding (Han et al., 2018; Paschall et al., 2017), and one of the main motivations for its use is to manage insomnia disorders; i.e., to induce and maintain a refreshing sleep (American-Academy-of-Sleep-Medicine, 2014; Babson et al., 2017; Belendiuk et al., 2015). However, the effects on sleep both of Cannabis or its compounds are still not clear. While sedative effect have been reported with low doses of THC by smoking cannabis cigarettes (Chait, 1990; Halikas et al., 1985), and by taking oral cannabis extracts (Cousens and DiMascio, 1973; Gorelick et al., 2015; Pivik et al., 1972), high-doses of THC tends to have an activating action (Babson and Bonn-Miller, 2014). The same happens with CBD. An activating effect was demonstrated following intracerebroventricular CBD administration in rats (Murillo-Rodríguez et al., 2006), and after the application of an oromucosal spray with a combination of CBD and THC in humans (Nicholson et al., 2004). However, intraperitoneal injection of CBD in rats had an hypnotic effect (Monti, 1977). Therefore, it seems that those effects are influenced by dosage, ratio of cannabinoids, timing and route of administration (Babson et al., 2017).

Smoking is the most predominant route of Cannabis administration in drug users (Russell et al., 2018). This route has a rapid and good absorption, while oral is slow, unpredictable, and erratic (Lanz et al., 2016; Shiplo et al., 2016). However, smoking of cannabis is potentially harmful for the consumer, and probably for the passive smokers as with tobacco (Borchers et al., 2013; Morioka et al., 2018). Hence, this route is not acceptable for therapeutic purposes (Lanz et al., 2016).

An emerging alternative inhalation-based route of Cannabis administration is vaporization. Vaporization provides delivery characteristics that are similar to smoking, without the toxicants that are present due to combustion (Shiplo et al., 2016). While large amount of research has focused on the administration of smoked, oral or injected cannabinoids (Feinberg et al., 1976; Monti, 1977; Murillo-Rodríguez et al., 2006; Russo et al., 2007), at the moment, there is no published research about the effects of vaporized Cannabis on sleep, either in laboratory animals or in humans.

Psychoactive drugs have been shown to modify the intrinsic electric oscillatory activity of the brain (Blain-Moraes et al., 2014; Dafters et al., 1999; Knott, 2000; Schartner et al., 2017). Furthermore, this changes have been correlated with the subjective reports after drug experience (Koukkou and Lehmann, 1976; Stuckey et al., 2005). In fact, oral administration of THC extracts (Koukkou and Lehmann, 1976), or smoking marihuana cigarettes (Böcker et al., 2010; Struve et al., 1999) modify power and coherence of the alpha, theta and beta bands of the electroencephalogram (EEG). Nonetheless, only few studies have analyzed the effect of Cannabis on high frequency bands of the EEG, such as gamma and high frequency oscillations (HFO) (Cortes-Briones et al., 2015; Holderith et al., 2011; Skosnik et al., 2012). The functional role of those oscillations has been linked to a diverse range of higher-order brain function such as working-memory, perception and consciousness (Bosman et al., 2014; Llinás et al., 1998; Rodriguez et al., 1999).

With the hypothesis that acute Cannabis vaporization **would modify sleep and electrocortical activity,** the aim of the present study was to characterize in rats, the effects of Cannabis on these variables. As a first step, based on previous reports that demonstrated a sedative effect of low doses of THC and a higher efficiency on reducing the frequency of use of sleep medications by high THC Cannabis varieties, we utilized low doses of Cannabis with high content of THC (11.5%) and negligible amounts of CBD. We were also particularly interested in this chemotype of Cannabis (high THC, low CBD) because it has become increasingly popular with the passage of time (ElSohly et al., 2017; Mehmedic et al., 2010).

2. Material and methods

2.1. Cannabis

Fresh flowers of *Cannabis sativa* L. were obtained from the Institute of Regulation and Control of Cannabis ("*Instituto de Regulación y Control de Cannabis, IRCCA*"), grounded, homogenized and preserved in a protection plastic bag at −18 °C until its use. Cannabinods (THC and CBD) content was determined by gas chromatography (GC) (AHP, 2014). The quantification of cannabinoids was performed with an external calibration, using the average values of three sets of standards containing target compounds at concentrations ranging from 1 to 250 μg/mL in MeOH. The limit of quantification for both compounds was 1.5 μg/mL, and the correlation coefficients were ≥0.995. The cannabinoid content in the grounded material was: THC = 11.5% and CBD < 0.05%.

2.2. Experimental animals

Twelve Wistar, male, adult rats (270–300 g) were used for polysomnography recordings. Furthermore, 12 additional naive rats were used for THC titration in plasma. The animals were obtained from the Animal Care Facility of the School of Medicine, Universidad de la República (Uruguay), and determined to be in good health by veterinarians of the institution. All experimental procedures were conducted in agreement with the National Animal Care Law (#18611) and with the "Guide to the care and use of laboratory animals" (8th edition, National Academy Press, Washington D. C., 2010). Furthermore, the Institutional Animal Care Committee approved the experimental procedures (protocol number: 070153-001077-15). Adequate measures were taken to minimize pain, discomfort or stress of the animals, and all efforts were made to use the minimal number of animals necessary to obtain reliable scientific data. Animals were maintained on a 12-h light/dark cycle (lights on at 06.00 h) and housed four to six per cage before behavioral testing. Food and water were freely available.

2.3. Surgical procedures

The rats used for polysomnography studies (n = 12) were submitted to stereotaxic surgery. We employed surgical procedures similar to those used in our previous studies (Benedetto et al., 2013; Cavelli et al., 2015, 2017a). Anesthesia was induced with a mixture of ketamine-xylazine (90 mg/kg, 5 mg/kg i/p., respectively). Rats were positioned in a stereotaxic frame and the skull was exposed. In order to record the EEG, seven stainless steel screw electrodes (1.0 mm of diameter) were placed on the skull with their tips touching the dura matter. As is illustrated in Fig. 1A, six electrodes were located on the neocortex forming two anterior-posterior consecutive squares centered with respect to the midline, and the frontal square centered with respect to Bregma (all

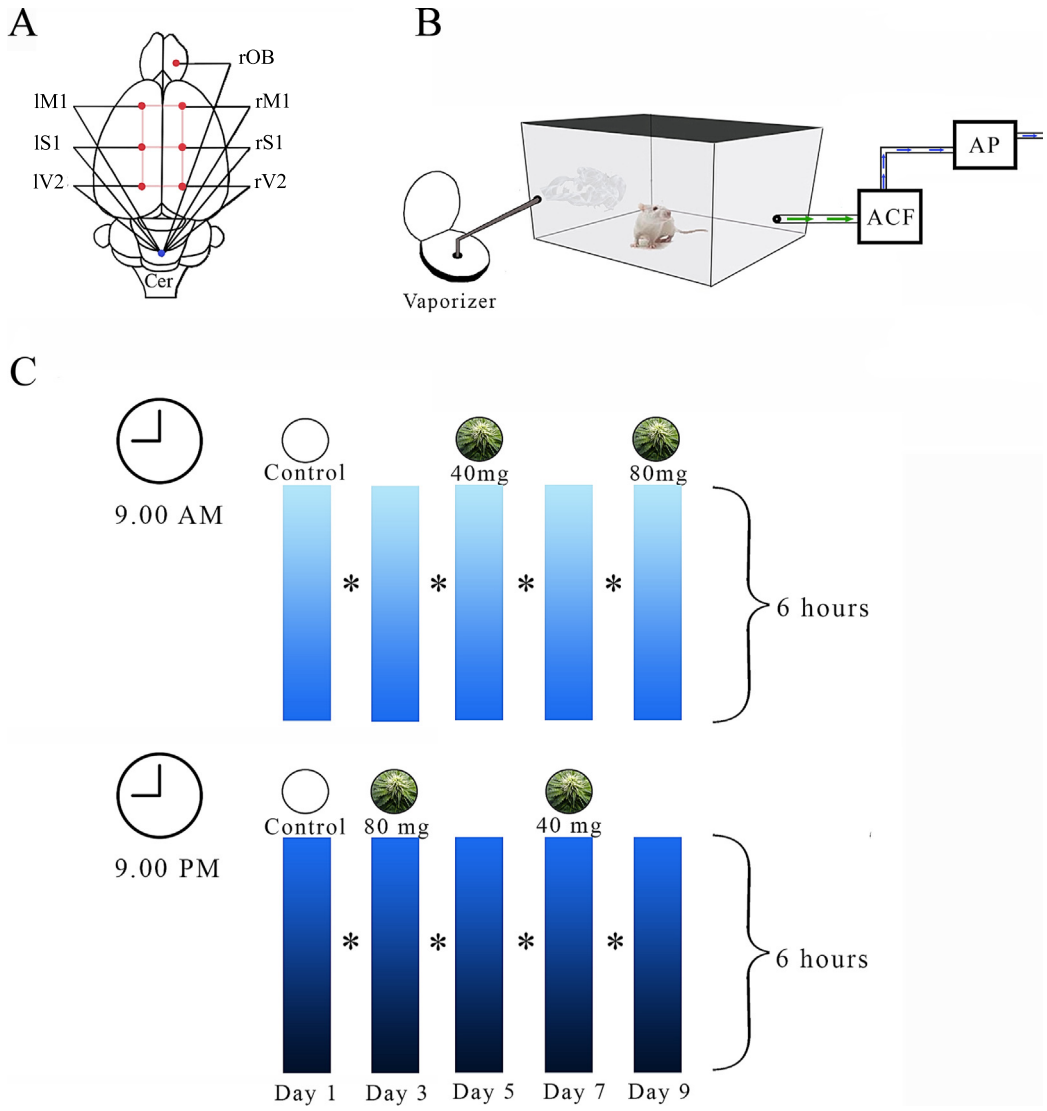

Fig. 1. A. Position of recording electrodes (red dots). The electrodes were referred to a common electrode that was located over the cerebellum (Cer) (blue dot). Electrodes form two virtual anterior-posterior consecutive squares, centered with respect to the midline and with the frontal square centered with respect to Bregma. OB, olfactory bulb; M1, primary motor cortex; S1, primary somatosensory cortex; V2, secondary visual cortex; r, right; l, left. **B.** Schematic representation of the vaporizing chamber. The vaporizer was connected to a 4.6 L capacity plastic box, where the animals were placed during 10 min. The vaporizer was set at 180 °C. After vaporization, a pump was activated to pass the air with Cannabis through an activated carbon filter. ACF, activated carbon filter; AP, air pump. Green arrows, air with Cannabis vapor; blue arrows, filtered air. **C.** Experimental design for 40 mg and 80 mg Cannabis administration. Vaporization during light phase was done at 9.00 AM, while vaporization during dark phase was done at 9.00 PM. The polysomnographic recordings began immediately after vaporization and lasted 6 h. The order of the treatments was done counterbalanced in different animals. *: day free of treatment. A similar design was employed for C_{200} experiments.

nearby neocortical electrodes were separated by the same distance, 5 mm) (Cavelli et al., 2017a). The electrodes were located bilaterally in primary motor cortex (M1: L ± 2.5 mm, AP +2.5 mm), primary somato-sensory cortex (S1: L ± 2.5 mm, AP −2.5 mm) and secondary visual cortex (V2: L ± 2.5 mm, AP −7.5 mm). The other electrode was located over the right olfactory bulb (OB) (L: +1.25 mm, AP +7.5 mm). A reference electrode was in the cerebellum (Fig. 1A). In order to record the electromyogram (EMG), two electrodes were inserted into the neck muscle. The electrodes were soldered into a 12-pin socket and fixed onto the skull with acrylic cement. At the end of the surgical procedures, an analgesic (Ketoprofen, 1 mg/kg, subcutaneously) was administered. Incision margins were kept clean and a topical antibiotic was applied on daily basis. After the animals recovered from the preceding surgical procedures, they were adapted to the recording chamber for one week.

2.4. Experimental sessions

2.4.1. Administration of Cannabis

Animals were housed individually in transparent cages (40 × 30 × 20 cm) containing wood shaving material in a temperature-controlled (21–24 °C) room, with water and food ad libitum.

In order to study the effect of Cannabis on sleep, two experimental series were performed ($n = 6$ each). In the first one, lower doses of Cannabis were used. At the beginning of the recordings each rat was placed in a plastic box where 0 mg (C_0, control or sham experiment), 40 mg (C_{40}) and 80 mg (C_{80}) of Cannabis flowers were vaporized at 180 °C for 10 min (Fig. 1B). In the second experimental series, Cannabis was vaporized, utilizing the same procedure, with C_0 and C_{200}. A vaporizer (Herbalizer HA, Clovershield, Inc., CA, USA) connected to the hermetic box by a plastic tube was utilized (Fig. 1B). Following vaporization and before opening the box, the air was cleaned flowing the vapor through an activated carbon trap.

In both experimental series, the doses were administered in different days and times (9 A.M. or 9 P.M.) in a counterbalance order. A day off was left between doses to avoid a possible cumulative effect (Fig. 1C).

2.4.2. Sleep recordings

Following Cannabis administration, the animals were introduced to the recording set-up and the polysomnographic recordings began. Experimental sessions were conducted during 6 h of light (9 A.M. to 3 P.M) and dark periods (9 P.M to 3 A.M.), in a sound-attenuated chamber, which also acts as a Faraday box. The length of the recording sessions (6 h) was defined based on preliminary results of pilot experimental sessions, and according to previous studies of our laboratory (González et al., 2018; Schwarzkopf et al., 2018).

The recordings were performed through a rotating connector, to allow the rats to move freely within the recording box. Bioelectric signals were amplified (×1000), filtered (0.1–500 Hz), sampled (1024 Hz, 16 bits) and stored in a PC using the Spike 2 software (Cambridge Electronic Design).

2.5. Data analysis

The states of sleep and wakefulness (W) were determined in 10 s epochs (Fig. 2). W was defined as low voltage fast waves in frontal cortex, a mixed theta rhythm (5–9 Hz) in occipital cortex and relatively high EMG activity; light sleep (LS) as high voltage slow cortical waves interrupted by low voltage fast electroencephalographic activity. Slow wave sleep (SWS) was defined as continuous high amplitude slow (1–4 Hz) frontal, parietal and occipital waves and sleep spindles (10–15 Hz) combined with a reduced EMG activity, while REM sleep as low voltage fast frontal waves, a regular theta rhythm in the parieto-occipital cortex, and a silent electromyogram except for occasional myoclonic twitching. Total time spent in W, LS, SWS, Non-REM (NREM = LS + SWS) and REM sleep, as well as the duration and the number of episodes over the 6 h recording period, were determined. Operational, an episode was defined as consecutive epochs of the same sleep state without interruption. Sleep latencies (measured from the beginning of the recording) were also included in the analysis. The time spent in each state during the first recording hour was also analyzed.

EEG activity was studied in the same group of animals, but only for C_{200} and its control ($n = 6$). In order to analyze power spectrum (in each EEG channel) and coherence (between pairs of EEG channels) we used procedures similar to those utilized in our previous studies (Cavelli et al., 2017a, 2017b). The maximum number of non-transitional and artifact-free periods of 30 s was selected during each behavioral state along the 6 hs of recording, to determine the mean power and coherence for each rat. The power spectrum was estimated on Matlab using the *pwelch* function (hamming window, window size 5 s, with an overlap of 2.5 s, a frequency sample of 1024 Hz and a resolution of 0.5 Hz) The Magnitude Squared Coherence was analyzed between equidistant cortices. It was calculated by the *mscohere* Matlab function (window size: 10 s, overlap of 5 s, a frequency sample of 1024 Hz and a resolution of 0.5 Hz); for details about coherence definition see (Bullock and McClune, 1989). Once obtained, in order to normalize the data, values were Fisher-z transformed to get the Z′ coherence.

2.6. Statistical analysis

All values are presented as mean ± S.E.M. The experimental design for sleep analysis was a within-subject design, where statistical significance of the differences among groups (C_0, C_{40} and C_{80}) was evaluated utilizing one-way repeated measures ANOVA. *Post-hoc* comparisons were performed with the Bonferroni test when ANOVA indicated significance. For the analysis of the effect of C_{200}, a paired two-tailed Student *t*-test was performed. Statistical significance was set at $p < 0.05$.

We also compared the effects of C_{200} Vs. C_0 on the power and Z′ coherence means per rats, of different EEG bands, utilizing the paired two-tailed Student *t*-test. A Bonferroni correction for multiple comparisons was also applied. With this correction, $p < 0.0071$ was considered statistically significant. In all the cases, analysis during light and dark phases was done independently. The EEG bands that were analyzed were: delta, 1–4 Hz; theta, 5–9 Hz; sigma, 10–15 Hz; beta, 16–30 Hz; low gamma (LG), 31–48 Hz; high gamma (HG) 52–95 Hz; and HFO, 105–148 Hz (Cavelli et al., 2017b).

2.7. Determination of THC levels in plasma

In order to titrate the levels of THC in plasma, 12 animals were treated with 80 ($n = 6$) or 200 mg (n = 6) of vaporized Cannabis, during lights-on period (between 9 A.M. and 12 P.M.) employing the same methodology used in the previous experimental session. Subsequently, the rats were maintained in a quiet cage for 15 min and then euthanized by decapitation. Blood was collected in ice-chilled K2-EDTA coated collection tubes. Before two hours after the collection, blood was centrifuged 15 min at 1200*g* (Giuffrida et al., 2000; Takahashi et al., 2014). Plasma was collected in cryotubes and stored at −80 °C for later analysis. Plasma THC levels were determined by ELISA with high sensitivity for Δ9-THC and 11-OH-THC (Neogen Corporation, Lansing, USA). Δ9-THC used was T-005-1ML of (−)-Δ9-THC Certified Reference Material at 1.0 mg/mL in Methanol from Cerilliant. Each plasma sample was evaluated following the manufacturer's instructions. Briefly, a 25 µL aliquot of each plasma sample was transferred to individual wells of the ELISA plates together with 25 µL of optimization buffer and incubated for 60 min in the dark with gently shaking. Once this incubation stage was finished, 50 µL of drug-enzyme conjugate was added to each well and incubated in the dark at room temperature for 30 min, gently shaking the plate. Following the incubation, the liquid was dumped from the wells. After washing with buffer, 100 µL K-Blue® (TMB) substrates was added to each well and incubated for 30 min in the dark. The reaction was stopped with 100 µL H_2SO_4 (1 N) and the plate was read at 450 nm. The intensity of the color development was inversely proportional to the concentration of drug in the sample. A calibration curve was done for 1 ng/mL, 2 ng/mL and 5 ng/mL in blank plasma using Δ9-THC reference material. Sensitivity was determined by running a drug-free plasma sample and analyzing spiked at 0.5 ng/mL.

A negative control was also prepared and analyzed. Every 8 samples, a control plasma sample with 2 ng/mL was included; and, at the beginning and at the end of each batch, a blank sample was run.

3. Results

3.1. Determination of THC plasma levels

One of the samples of the C_{200} was hemolyzed and discarded. THC plasma concentrations 15 min after vaporization were 4.6 ng/mL ± 0.6 for C_{80}, and 6.7 ng/mL ± 0.04 for C_{200}.

3.2. Effects on sleep

Fig. 2 shows polysomnographic representative recordings following C_0 and C_{200} administration during the light period, whereas the hypnograms and spectrograms that were processed from the same recordings are shown in Fig. 3. In this animal, it is readily observed that C_{200} increases sleep time during the first recording hour.

When the whole population of animals was analyzed for the total recording time (6 h, Supplementary material; Tables 1 and 2), the administration of C_{40}, C_{80} or C_{200} during the dark phase did not affect W or sleep parameters. During the light phase, no effects were observed with C_{40} and C_{80} (Supplementary material; Table 3), while an effect in the number of W episodes was observed with C_{200} (C_{200}, 61.8 ± 4.9; C_0, 79.5 ± 4.5; $t(5) = -3.025$, $p = 0.029$) (Table 1).

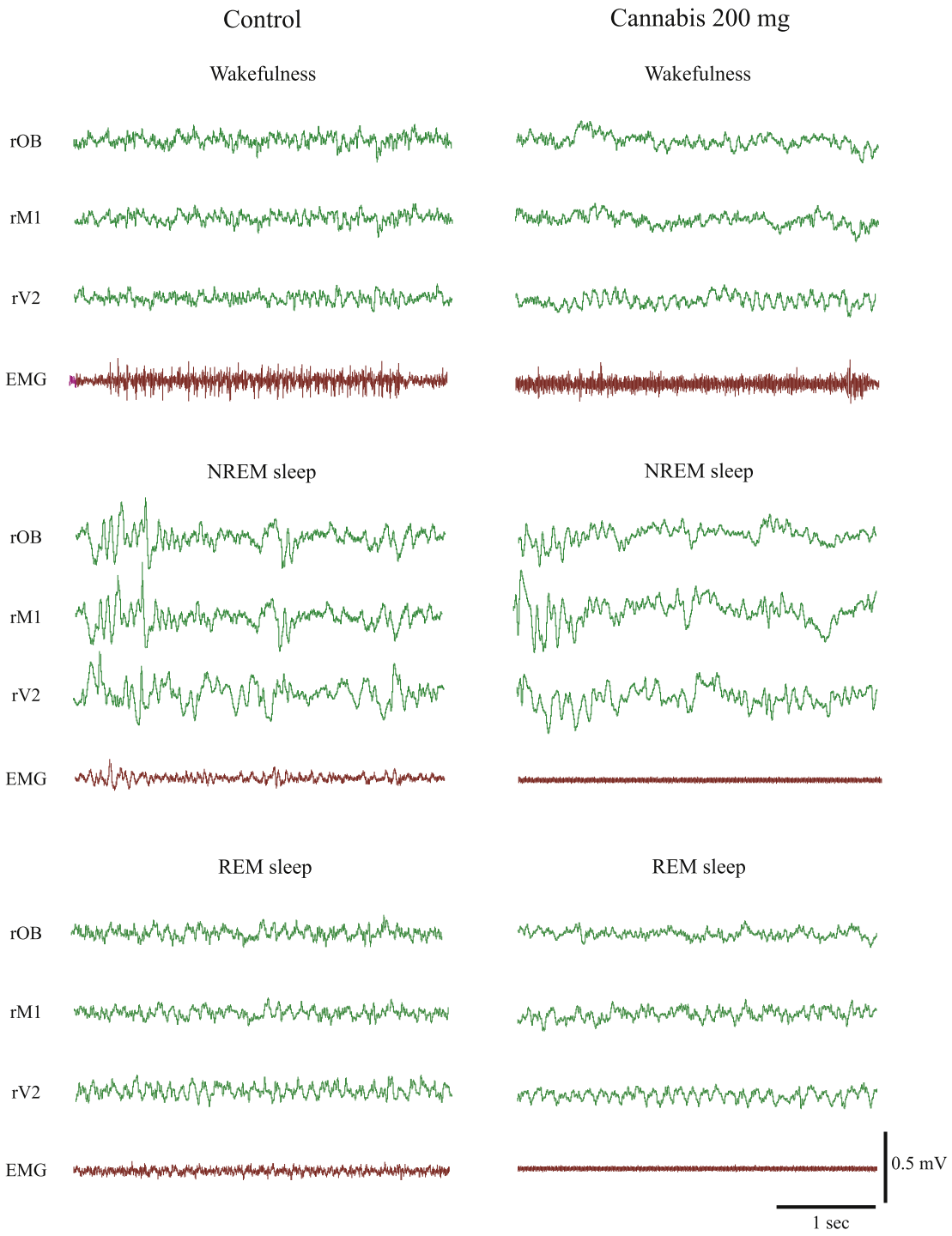

Fig. 2. Polysomnographic recordings of a representative animal after control (left) and Cannabis 200 mg (right) during the lights-on period. Recordings during wakefulness, NREM sleep and REM sleep for the right olfactory bulb (OB), right primary motor cortex (rM1) and right secondary visual cortex (rV2) are shown. EMG, Electromyogram.

When the first recording hour was analyzed C_{40} and C_{80} did not affect the time of W or sleep (Fig. 4A). However, following C_{200} there was a significant increase on NREM sleep (C_{200}: 185.8 ± 28.5; C_0: 115.0 ± 20.7; t(5) = −2.7 p = 0.04), but only during the light phase (Fig. 4B). This increment in NREM sleep time cannot be explained only by the increase of the number of episodes (C_{200}, 15.2 ± 1.2; C_0, 13.5 ± 2.8; t(5) = 0.58, p = 0.63), or by the duration of the episodes (C_{200}, 1.9 ± 0.4; C_0, 1.6 ± 0.4; t(5) = 0.74, p = 0.49). Hence, a combined effect of both factors is the cause of this increment.

3.3. Effects on spectral power

Because the effect on sleep was observed only for C_{200}, the quantitative analysis of the EEG was restricted to this dose. The effects of C_{200} on power spectrum are summarized in Fig. 5A.

During W in the light phase, C_{200} produced a reduction in power of theta (C_{200}, 88.5 ± 15.1; C_0, 110.7 ± 17.3; t(5) = 6.82, p = 0.001), sigma (C_{200}, 20.8 ± 2.6; C_0, 29.0 ± 2.7; t(5) = 8.64, p = 0.0003), beta (C_{200}, 10.4 ± 1.7; C_0, 14.0 ± 1.5; t(5) = 9.37, p = 0.0002) and LG (C_{200}, 5.6 ± 1.2; C_0, 8.1 ± 1.2; t(5) = 5.91, p = 0.002) in the OB.

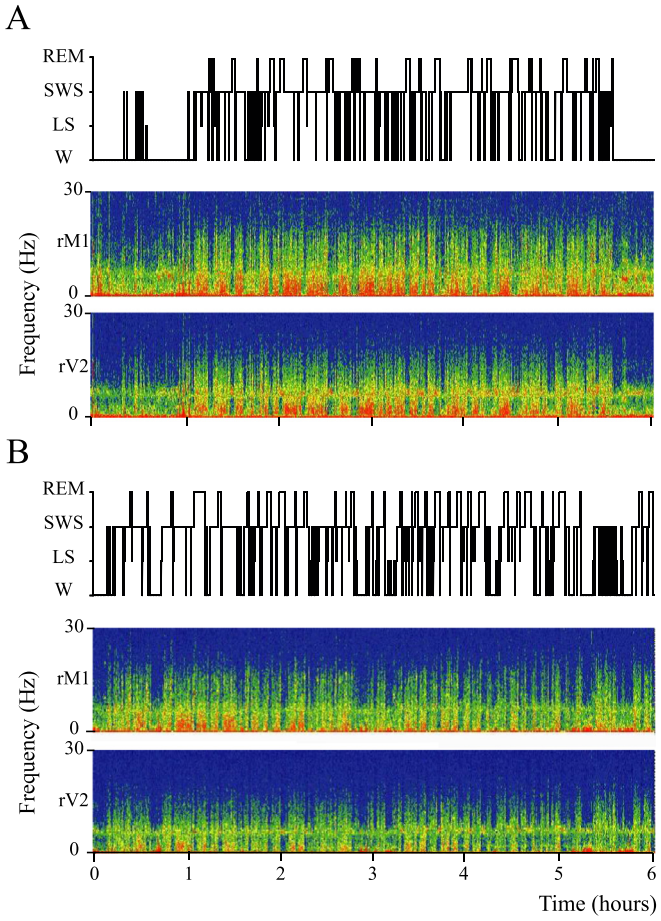

Fig. 3. Hypnograms and spectrograms (0.1–30 Hz) from the right primary motor (rM1) and right secondary visual (rV2) cortical recordings of a representative animal, are shown after control (**A**) and Cannabis 200 mg (**B**) administration during the lights-on period. During wakefulness (W) and REM sleep, theta activity (5–9 Hz) in the spectrograms can be readily observed. During slow waves sleep (SWS), delta activity (1–4 Hz) is more prominent and there are intermittent episodes of sigma activity (10–15 Hz), which correspond to the presence of sleep spindles. Color calibration of the spectrogram is not exhibited (larger power is exhibited in red). Cannabis increased sleep time during the first hour of recording. LS, light sleep.

LG power was also decreased in left M1 (lM1) (C_{200}, 0.4 ± 0.1; C_0, 0.6 ± 0.1; t(5) = 11.76, p = 0.0001). On the contrary, the EEG power was not affected during W in the dark phase.

No significant changes in the EEG power were detected with C_{200} administration during NREM sleep. During REM sleep in the light phase, HFO was reduced in lM1 (C_{200}, 0.3 ± 0.1; C_0, 0.4 ± 0.1; t(5) = 5.35, p = 0.0031), rM1 (C_{200}, 0.3 ± 0.1; C_0, 0.4 ± 0.1; t(5) = 6.91, p = 0.0023) and lS1 (C_{200}, 88.5 ± 15.1; C_0, 110.7 ± 17.3; t(5) = 5.65, p = 0.005). HG was also reduced in lM1 (C_{200}, 2.8 ± 0.7; C_0, 3.3 ± 0.7; t(5) = 6.47, p = 0.0013). No modifications were observed during the dark phase.

An example of the mean power spectrum of the OB during the light and dark phases is shown in Fig. 5B. The differences between dark (active) and light phases are readily observed.

3.4. Effects on spectral Z′ coherence

The effect of C_{200} on the spectral Z′ coherence was analyzed. The analyses were performed between right intrahemispheric cortices (OB-M1; M1-S1 and S1-V2), as well as between homologues interhemispheric cortices (M1, S1 and V2 of both hemispheres). These analyses were performed during W and sleep, either during the light and dark phases. In contrast to the effects on EEG power spectrum, no modification in Z′ coherence was observed during the light phase either in W or in sleep. During the dark phase, the only significant effect was observed during NREM sleep. A decrease in the intrahemispheric coherence of the sigma band (that correspond to the frequency of the sleep spindles) was observed between S1 and V2 cortices (C_{200}, 0.61 ± 0.04; C_0, 0.93 ± 0.009; t(5) = 5.23, p = 0.006); this result is shown in Fig. 6.

4. Discussion

To the best of our knowledge, this is the first report that describes the effects of vaporized Cannabis flowers on the sleep-wake cycle and EEG activity. In agreement with our hypotheses, we demonstrated in rats, that 200 mg of Cannabis produced a reduction in the number of episodes of W for the total time of recording and increased the time spent in NREM sleep during the first hour of recording, but only during the light (resting) phase. Furthermore, modifications in EEG power and coherence during W and sleep were also observed. Through the light phase, during W, 200 mg of Cannabis reduced the power spectrum from theta to LG bands in the OB, and the LG band in the left primary motor cortex. Additionally, during REM sleep, C_{200} reduced the power spectrum of HFO and HG bands in the motor cortex, as well as the LG band in the left primary somatosensory cortex. In regards to coherence, during dark phase, there was a reduction in interhemispheric coherence of the sigma band between S1 and V2 cortices. These data also show that the effects of Cannabis were highly dependent of the light/dark cycle.

4.1. Technical considerations

Vaporization was effective in delivering cannabinoids to the animals. The aim of our study was to deliver low doses of THC, and 6.7 ng/mL was the blood concentration of the maximal dose (C_{200}). This concentration is considered low, because there are studies that utilized THC blood concentration up to 150 ng/mL (Nguyen et al., 2016). In fact, higher levels of THC in blood can be obtained after similar procedures of vaporization (up to 301 ng/mL/kg), utilizing a larger time of administration and a larger amount of vegetal material (Manwell et al., 2014; Nguyen et al., 2016). However, THC levels of 2 to 4 ng/mL have been shown to produce objective and subjective effects (Brenneisen et al., 1996; Ohlsson et al., 1980). The sleep-promoting effect obtained with low doses of THC, encourage the use of medical cannabis avoiding side effects that may appear at higher doses, such as psychotic symptoms (Di Forti et al., 2009).

In order to get enough number of non-transitional and artifact-free periods for each behavioral state, the quantitative EEG analysis was made for the total recording time (6 h). However, because the effects of Cannabis on sleep were limited to the first hour of recording, the effect on the EEG is expected to be stronger in this time window.

4.2. NREM sleep promoting effect

The increment in NREM sleep time agrees with the findings of some clinical studies that showed that Cannabis (administered by others routes), may be useful to treat sleep disturbances in adults (Belendiuk et al., 2015; Bonn-Miller et al., 2014) and adolescents (Boys et al., 2001). However, this effect is not completely clear. Gates et al. (2014), after reviewing thirty nine publications regarding the influence of Cannabis administration on sleep, concluded that it is not beneficial, except among individuals who had pre-existing sleep interrupting symptoms such as pain (Gates et al., 2014). This inconsistency in the results might be due to different routes, and “chronic Vs. acute” type of administration, as well as the fact that several clinical researches had confounding factors such as pre-existing sleep problems, or participant gender and age.

Table 1
Effects of vaporization of 200 mg of Cannabis on sleep and wakefulness during the light phase.

	Control	Cannabis 200 mg
Wakefulness (W)		
Total duration (min)	113.3 ± 3.1	103.5 ± 7.0
Number of episodes	79.5 ± 4.5	**61.8 ± 4.9***
Episodes duration (min)	1.5 ± 0.1	1.7 ± 0.2
Light sleep (LS)		
Total duration (min)	19.5 ± 6.7	13.6 ± 4.6
Number of episodes	78.0 ± 22.4	53.5 ± 13.3
Episodes duration (min)	0.2 ± 0.0	0.2 ± 0.0
Slow wave sleep (SWS)		
Total duration (min)	180.0 ± 7.6	209.2 ± 10.9
Number of episodes	102.3 ± 12.8	82.0 ± 14.2
Episodes duration (min)	1.8 ± 0.2	2.4 ± 0.4
Latency (min)	15.3 ± 2.7	19.7 ± 5.8
NREM sleep		
Total duration (min)	199.5 ± 8.4	222.8 ± 7.5
REM sleep		
Total duration (min)	32.6 ± 5.0	33.6 ± 6.1
Number of episodes	22.5 ± 2.9	27.0 ± 8.8
Episodes duration (min)	1.5 ± 0.1	1.5 ± 0.3
Latency (min)	61.9 ± 10.2	78.9 ± 16.7

The analysis was done for the total recording time. Data is presented as mean ± standard error of six rats.

* Denotes significant difference compared to Sham values; two-tailed paired Student *t*-test ($p < 0.05$).

Concerning the effect of the isolated phytocannabinoids, most research concluded that THC had sedative properties, while CBD, according to the dose, could act as an activating compound (Nicholson et al., 2004). THC is a partial agonist of CB1 receptors (Paronis et al., 2012), and it is thought that its effect on sleep is caused by binding to these receptors (Murillo-Rodríguez, 2008; Santucci et al., 1996). In fact, the first report on the role of CB1 receptor on sleep modulation was done in 1996 by Santucci et al. (1996); the CB1 receptor antagonist "SR141716A" was injected intraperitoneal in rats, and induced a dose-dependent increase in W.

The plant material vaporized in the present study had high content of THC and undetectable levels of CBD. But, is just THC the responsible for the increase in NREM sleep? Although most research has focused on cannabinoids, the plant is composed by > 530 chemicals. It has been already determined that some of those compounds may have a synergic effect. In fact, Carlini et al. (1974), based on animal and human studies, determined that Cannabis extracts produced effects "two or four times greater than that expected from their THC content" (Carlini et al., 1974). This synergic effect was described as "entourage effect" (Mechoulam and Ben-Shabat, 1999). In fact, terpenes present in Cannabis have been shown to induce sedative effects (Booth et al., 2017; Do Vale et al., 2002). For example, terpenes such as αPinene (Yang et al., 2016), myrcene (Do Vale et al., 2002) and phytol (Costa et al., 2014), exert their effect acting on the benzodiazepine binding site of $GABA_A$ receptors, while others like limonene is thought to act on adenosine $A2_A$ receptor (Park et al., 2011); both sites of actions have been related to sleep regulation. Consequently, new studies are needed to elucidate the relative importance of THC and terpenes on the NREM sleep promoting effect.

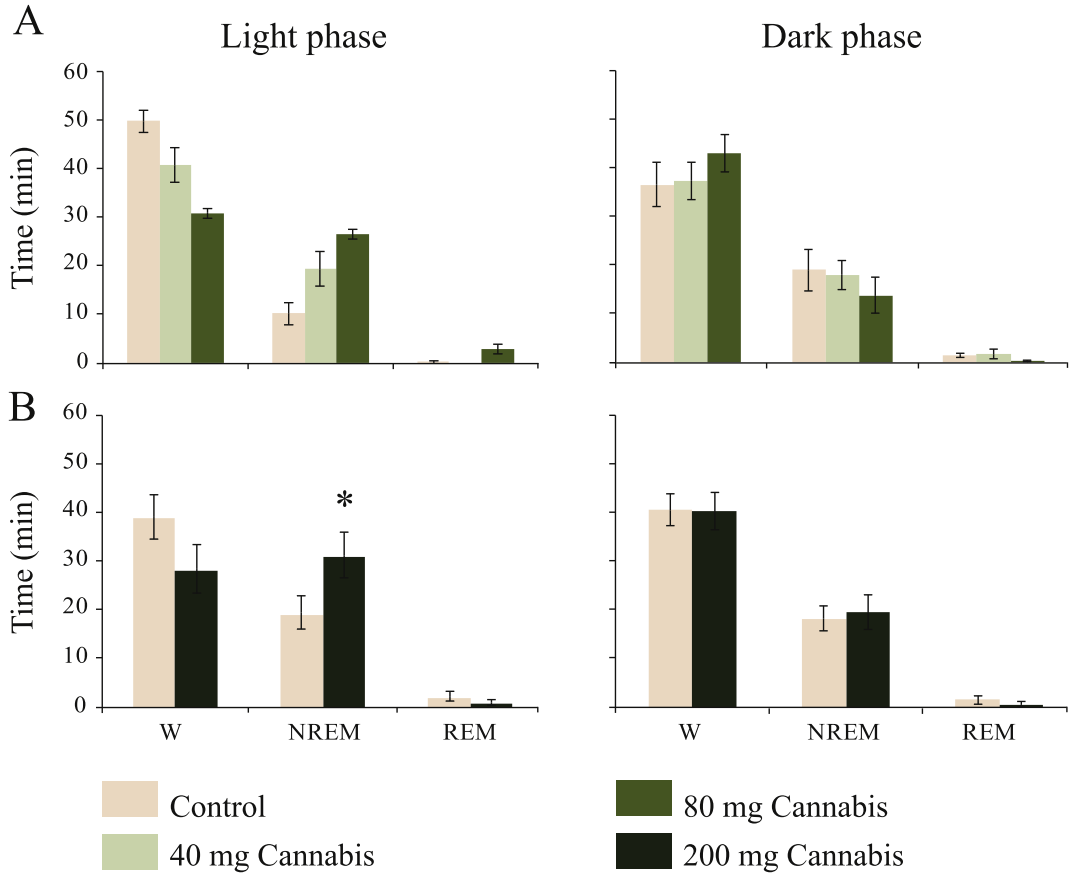

Fig. 4. Effects of Cannabis administration on sleep and W during the first recording hour ($n = 6$). Graphic chart shows the mean ± SEM time spent in wakefulness (W), NREM and REM sleep. The effects of the lower doses of Cannabis (40 and 80 mg) are shown in the top charts (**A**), while effect of 200 mg is shown in the bottom charts (**B**). Group mean differences among control, 40 and 80 mg were determined by one-way ANOVA repeated measures and Bonferroni tests, while mean differences between control and 200 mg were determined by the paired two-tailed Student *t*-test. The asterisk indicates significant differences ($p < 0.05$).

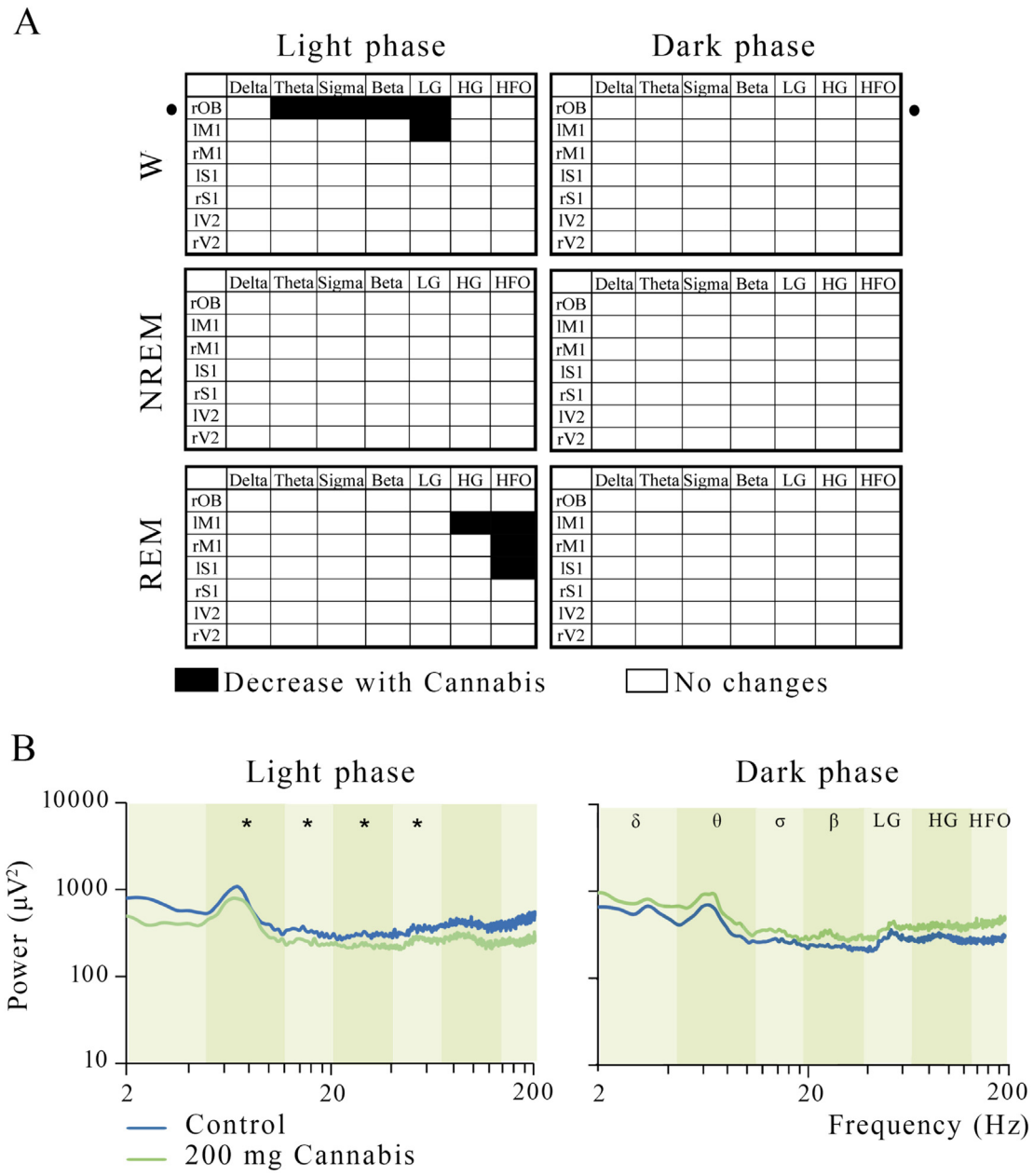

Fig. 5. Effects of Cannabis 200 mg administration on EEG power (n = 6). **A.** Summary of the effects according to the cortical site, frequency bands, behavioral states and light/dark phases. Significant decreases ($p < 0.0071$) are indicated in black. **B.** The effect of Cannabis 200 mg in the OB EEG power during W (indicated by a circle in A) is depicted as an example of all the analyzed cortices. The vaporization of Cannabis produced a statistically significant decrease in theta ($t(5) = 6.82$, $p = 0.0010$), sigma ($t(5) = 8.64$, $p = 0.0003$), beta ($t(5) = 9.37$) and LG ($t(5) = 5.91$, $p = 0.002$) bands power (indicated by asterisks) during the light phase. Pink noise was removed in this graphic by multiplying power values by their frequencies. Delta, 1–4 Hz; theta, 5–9 Hz; sigma, 10–15 Hz; beta, 16–30 Hz; LG, 31–48 Hz; HG, 52–95 Hz; HFO, 105–148 Hz. OB, Olfactory bulb; M1, primary motor cortex; S1, somatosensory cortex; V2, secondary visual cortex; r, right; l, left; LG; low gamma; HG, high gamma; HFO, high frequency oscillations; W, wakefulness; NREM, non-REM sleep; REM, rapid eye movement sleep.

4.3. Cannabis modified the EEG activity

At the OB, C_{200} decreased from theta to LG EEG bands power during W, but only during the light phase. CB1 receptors had been found in the OB (Moldrich and Wenger, 2000). Also, there is electrophysiological evidence that the endocannabinoid system plays a functional role in regulating neuronal activity and signaling in OB glomeruli (Wang et al., 2012). CB1 agonists and antagonists modulate the activity of the periglomerular and external tufted cells in the OB (Wang et al., 2012), and corticofugal feedback axons have CB1 receptors that could regulate the excitability of OB neurons (Pouille and Schoppa, 2018). Hence, C_{200} effect on OB EEG power may be an evidence that the endocannabinoid system modulates sensory processing within the OB.

C_{200} also reduced LG in M1 during W. This result is in accordance with other authors who demonstrated that THC administration reduces the power of EEG signal in various frequency bands in both hippocampus and neocortex (Willinsky et al., 1975); modifications either in the lower frequencies of the EEG (Böcker et al., 2010; Bounamici et al., 1982; Struve et al., 1999; Willinsky et al., 1975), as well as in low gamma oscillations have been observed (Cortes-Briones et al., 2015).

C_{200} also decreased HG and HFO power in motor and somatosensory cortices during REM sleep. Most dreams, a special form of cognitive activity, take place during REM sleep (Hobson, 2009). Both gamma and HFO oscillations are related to cognitive functions such as memory

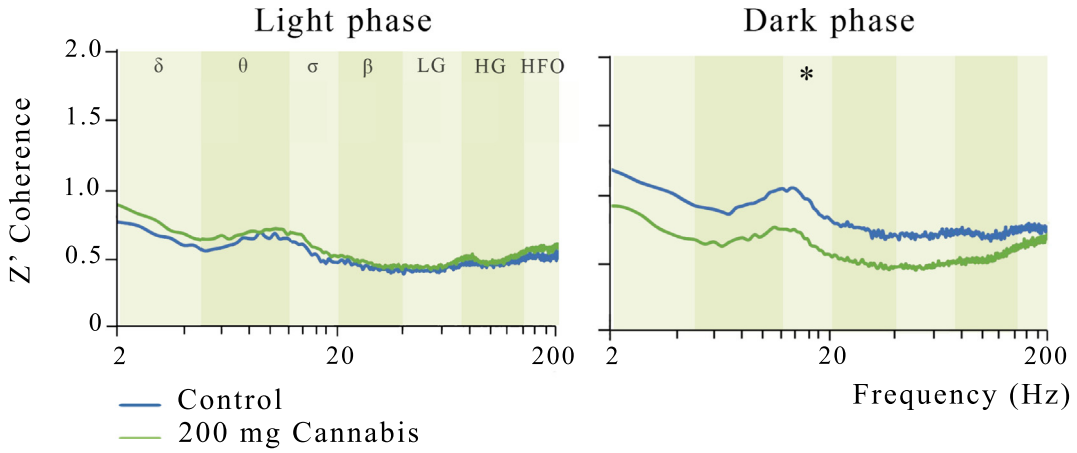

Fig. 6. Effects of Cannabis 200 mg administration on EEG Z′ coherence (n = 6). The figure shows the Z′ coherence during NREM sleep between the primary somatosensory and secondary visual area of the right hemisphere, following control and Cannabis administration. The vaporization of Cannabis produces a statistically significant decrease in the sigma band during NREM sleep (indicated by an asterisk, $t(5) = 5.23$, $p = 0.006$), but only during the dark phase.

processing (Bosman et al., 2014; Tort et al., 2013). It is known that acute and chronic administration of Cannabis induce cognitive alterations such as memory impairment (Crean et al., 2011; Shrivastava et al., 2011). Hence, we hypothesize that the reduction of HG and HFO power may be related to this effect.

The effect Cannabis vaporization on EEG oscillations during REM sleep also suggests that Cannabis may affect dreams. In this regards, it has been suggested that endocannabinoid system modulates the generation of dreams (Murillo-Rodriguez et al., 2017). In fact, post-traumatic stress disorder (PTSD) patients receiving Nabilone (a synthetic CB1 and CB2 receptor agonist) experienced either cessation or a significant reduction in nightmare intensity (Cameron et al., 2014; Fraser, 2009).

There was almost no effect on the EEG spectral coherence. However, a reduction in sigma intrahemispheric coherence between S1 and V2 was detected during NREM sleep; interestingly, only during the dark phase. This frequency band is associated with the sleep spindles. Those are brief (0.5–2 s) burst of 11–15 Hz synchronous activity generated by thalamocortical networks (Steriade et al., 1993). Sleep spindles play a role in consolidation of declarative memory (Fogel and Smith, 2011) and synchronize the occurrence of hippocampal ripples involved in memory replay (Latchoumane et al., 2017). Fewer synchronized spindle activity may affect memory consolidation by interfering with normal processes of coordinated memory reactivation and consolidation during sleep (Wamsley et al., 2012). In this regard, our results suggest that this reduction in the sigma coherence may be also related to the memory impairment produced by Cannabis (Fadda et al., 2004; Ranganathan and D'Souza, 2006).

4.4. Differential effects during the light and dark phases

We observed that C_{200} modified NREM sleep and the EEG power only during the light phase. On the contrary, C_{200} modified the EEG coherence just during the dark (active) period. Although these differential effects are difficult to interpret, it is important to highlight that most of the effects of C_{200} were during the light phase, where rats are mostly asleep.

How could those light/dark differences be explained? It has been demonstrated that the components of the endocannabinoid system show tissue-specific diurnal changes. The endocannabinoid concentrations on different anatomical regions, the CB1 receptor density, and the enzymes that control the synthesis and degradation of the endocannabinoids exhibit a circadian rhythm (Martínez-Vargas et al., 2003; Rueda-Orozco et al., 2008; Valenti et al., 2004). During the resting phase of the rats (lights-on period), CB1 receptor density is higher, while the anandamide concentrations are diminished (Martínez-Vargas et al., 2003; Valenti et al., 2004). As THC is a partial CB1 agonist (Paronis et al., 2012), we hypothesized that it would exert a bigger effect during the lights-on period when the levels of CB1 receptors is high, and there is less anandamide to compete with the receptors.

Our results suggest that the administration of medicinal Cannabis during the “resting” phase of the day may be optimal to treat sleep difficulties. Clinical studies should be carried out to confirm this hypothesis.

5. Conclusions and future directions

We demonstrate that the vaporization of Cannabis with high THC content, promotes sleep only during the “resting” phase. Furthermore, it induces interesting modification in the electrocortical activity, probably related to cognitive functions. These results encourage continuing the exploration of the effects on sleep of different doses, duration of the treatment, and Cannabis varieties.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.02.012.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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