Camphor alters occipital electrocorticographic patterns during sleep deprivation in Wistar rats

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Introduction: Sleep disorders are common in the general population, necessitating the search for new strategies to address this public health challenge. The study aims to describe the electrocorticographic and behavioral changes in sleep deprived Wistar rats exposed to varying doses of camphor, to assess its effects on sleep and its potential as a sleep-inducing drug.

Materials and Methods: For the electrocorticographic evaluation, seventy-two rats were randomly assigned to distinct groups: a control group, a sleep-deprived group, three sleep-deprived groups receiving 10, 20, and 30 mg/kg i.p. of camphor respectively, and three groups that received these doses without sleep deprivation. For the behavioral analysis, twenty-seven rats were divided into three groups, each receiving the same doses as the previous test.

Results and Discussion: Our results showed that there was a decrease in the frequency of brain oscillatory patterns when camphor was administered at 10 mg/kg i.p. whereas there was a dose-dependent increase in the spectral power and distribution following the administration of 20 and 30 mg/kg i.p., with the emergence of Delta, Theta, Alpha, and Beta waves. As for the behavioral analysis, it was demonstrated that testicular relaxation, decreased motility, and light sleep induction also occurred in a dose-dependent manner. Thus, we conclude that camphor administration intensifies occipital electrocorticographic patterns in sleep-deprived rats, and its electrocorticographic and behavioral analysis could indicate a potential as a supporting agent in the insomnia treatment.

KEYWORDS

camphor, electrocorticography, sleep deprivation, insomnia, neuropharmacology, sleep disorders

1 Introduction

Sleep disorders are highly prevalent in the general population (Gale et al., 2024; Qian et al., 2023; Rössler et al., 2017). It is well known the critical role of sleep quality in overall wellbeing, as it is associated with cortisol levels (Henry et al., 2021) and immunity (Ballestar-Tarín et al., 2023). Due to its significance, sleep restriction and circadian cycle disruption can impair systemic functioning of the organism and are correlated with the onset of numerous diseases (Touitou et al., 2017), including cancer (Straif et al., 2007; Stevens et al., 2011; Schernhammer et al., 2006; Papantoniou et al., 2015) and cardiovascular problems (Karlsson et al., 2001). Most importantly, these disturbances affect proper brain function, leading to cognitive decline (Marquié et al., 2015; Vallières et al., 2014; Fonken et al., 2012) and mood disorders (Lee et al., 2016; Bara and Arber, 2009; Kalmbach et al., 2015; Fonken et al., 2012).

Recent guidelines establish that cognitive-behavioral therapy (CBT) should be the first-line treatment for insomnia. When CBT proves insufficient, pharmacological interventions may be considered. For short-term insomnia, recommended medications include benzodiazepines (BZ), benzodiazepine receptor agonists (BZRAs), low-dose sedating antidepressants, and daridorexant (Riemann et al., 2023). Although BZ and BZRAs show positive outcomes, they should be prescribed with caution due to their association with tolerance, nocturnal confusion, falls, and cognitive impairment (Treves et al., 2018; Barker et al., 2004; Stranks and Crowe, 2014). Low-dose sedating antidepressants and daridorexant have proven useful for treating insomnia, but evidence supporting their effectiveness remains limited (Everitt et al., 2018). For chronic insomnia in adults, the latest American Academy of Sleep Medicine guidelines suggest that current pharmacological treatments — such as BZ, BZRAs, orexin receptor agonists, and melatonin agonists — have a weak strength of recommendation according to the GRADE methodology, which assesses the certainty of evidence in healthcare (Sateia et al., 2017; Morgenthaler et al., 2016). This does not imply that these drugs are ineffective, but rather that there is a lack of comprehensive understanding of their efficacy and potential risks. This underscores the ongoing need for research into these medications and the development of innovative approaches to address this public health issue.

Several essential oils have been described in the literature as influencing sleep induction (Dougnon and Ito, 2021; Lillehei and Halcon, 2014). Xiao et al. (2022) reported that borneol essential oil, a by-product of Cinnamomum camphora steam distillation, reduced locomotor activity, shortened sleep latency, and prolonged sleep duration in rats. Camphor is an aromatic terpene compound with a polyvalent structure that exhibits diverse biological properties, including antimicrobial, anti-inflammatory, analgesic, and antioxidative effects (Chen et al., 2013; Lee et al., 2022). However, its neurological actions remain poorly understood, with few studies examining camphor's role in the induction and maintenance of sleep. Further research could potentially establish camphor as an alternative treatment for sleep disorders.

With this considerations in mind, the upcoming investigation aims to analyze and describe the electrocorticographic and behavioral changes observed in Wistar rats submitted to different doses of camphor with or without sleep deprivation, in order to verify the electrocorticographic changes generated in the medial visual cortex of these animals during sleep and assess the potential of camphor as a sleep inducer.

2 Materials and methods

2.1 Ethical considerations

The research was conducted in accordance with the rules of the National Legislation for the Use and Breeding of Experimental Animals, as well as in accordance with the ethical principles of the National Council for the Control of Experimental Animals (CONCEA), the ARRIVE 2.0 guidelines and the NIH Guide for the Care and Use of Laboratory Animals. The research project was submitted to and approved by the UFPA Research Ethics Committee file number 1380040822 [ID 002046].

2.2 Animals

The study used a total of ninety-nine adult male Wistar rats weighing 180–200 g. The animals were obtained from the Central Animal Facility of the Federal University of Pará (UFPA) and allocated individually to the Experimental Animal Facility of the Laboratory of Pharmacology and Toxicology of Natural Products. The animals received water and food *ad libitum* and were kept in a temperature-regulated environment (25°–28°C) with a 12-h light-dark cycle.

2.3 Chemical substances

The following chemical substances were used to carry out the research: ketamine hydrochloride anesthetic obtained from the Köing Laboratory (Santana de Parnaíba, SP, Brazil); xylazine hydrochloride obtained from the Vallée laboratory (Montes Claros, MG, Brazil); lidocaine obtained from the Hipolabor laboratory (Sabará, MG, Brazil) for electrode implantation; and camphor obtained from the Êxodo Científica laboratory (Sumaré, São Paulo, Brazil).

2.4 Experimental design

Seventy-two rats were randomly divided into the following groups: a) Control group (Control group without sleep deprivation); b) Control SLD (Control group with 24-h sleep deprivation); c) CPH 10 (Camphor-treated group 10 mg/kg i.p. without sleep deprivation); d) SLD- CPH 10 (Camphor-treated group 10 mg/kg i.p. with sleep deprivation); e) CPH 20 (Camphor-treated group 20 mg/kg i.p. without sleep deprivation); f) SLD- CPH 20 (Camphor-treated group 20 mg/kg i.p. with sleep deprivation); g) CPH 30 (Camphor-treated group 30 mg/kg i.p. without sleep deprivation); h) SLD- CPH 30 (Camphor-treated group 30 mg/kg i.p. with sleep deprivation). All ECoG recordings lasted 3 min. As for the behavioral analysis, twenty-seven rats were randomly separated into three groups: 10 mg/kg i.p. camphor-treated group; 20 mg/kg i.p. camphor-treated group; 30 mg/kg i.p. camphor-treated group. All groups were consisted of nine animals.

2.5 Electrocorticography (ECoG)

The entire experiment was conducted in a Faraday cage. The electrodes for ECoG acquisition were implanted in the bregma

coordinate (−5.04 and 2 mm to the side of each hemisphere) represented by the medial visual cortex (Paxinos and Watson, 2013). On the fifth postoperative day, the electrodes were connected to a data acquisition system consisting of a high-impedance amplifier (Grass Technologies, P511), monitored by an oscilloscope (Protek, 6510). The data was continuously digitized at a rate of 1 KHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX). The data was stored on a hard disk and processed using specialized software (LabVIEW express). The recording electrode was implanted in the right cerebral hemisphere, while the reference electrode was implanted in the left hemisphere. The electrophysiological recordings were analyzed up to 40 Hz. Brain oscillations were analyzed according to (Jalilifar and Yadollahpour, 2017; Hamoy et al., 2018), which correspond to: Delta (1–4 Hz), Theta (4–8 Hz), Alpha (8–12 Hz), Beta (12–28) and Gamma (28–40 Hz).

2.6 Behavioral analysis

All the tests were conducted between 8 and 10 a.m. The animals were observed for 30 min after the administration of camphor to non-sleep-deprived animals. The behavioral parameters observed were according to the following sequence: a) testicular relaxation (maintenance of the testicle in the scrotum due to the relaxation of the Cremaster muscle); b) decrease in the animals' motility; c) induction of superficial sleep (where the animal could still be awakened by stimulation).

2.7 Electrophysiological data analysis

For the analysis of acquired signals, a program was built using Python 2.7. The Numpy and Scipy libraries were employed for mathematical processing, while the Matlab library was utilized for graphical representation. The graphical interface was developed using the PyQt4 library. Amplitude graphs were employed to demonstrate the potential difference between the reference and recording electrodes. A sampling rate of 1,000 samples per second was observed for signal analysis. Spectrograms were computed using a Hamming window with 256 points (256/ 1,000 s). Each frame was generated with an overlap of 128 points per window. For each frame, the Spectral Power Density (SPD) was calculated using the Welch's average periodogram method. The frequency histogram was generated by first computing the SPD of the signal using the 256-point Hamming window without overlap, resulting in a histogram constructed with 1 Hz bins.

To analyze the differences between experiments, a graph was constructed with the mean and standard deviation (SD) of the SPD from multiple experiments. Each wave in the graph was generated from a test set, where the SPD was generated, and the mean and SD calculated for each group of 256 points without overlap were used for the Hamming window PSD calculation.

2.8 Statistical analysis

The results are presented as the mean value ± SD. The normality of the variances was verified by the Kolmogorov-Smirnov test and the homogeneity by the Levene test. The groups were compared using a one-way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* test, whenever appropriate. All the analyses were run in GraphPad® Prism 6 and considered a p < 0.05 significance level.

3 Results

The ECoG recordings of the Control group showed amplitude below 0.05 mV (Figure 1A, left), as shown in the 1-s amplification with the background frequency in Alpha (Figure 1A, center) with spectrogram showing greater energy intensity below 10 Hz (Figure 1A, right). For the Control SLD group, the ECoG showed an increase in amplitude to 0.06 mV (Figure 1B, left), demonstrated in the 1-s amplification, with a preponderance of the background frequency in Alpha (Figure 1B, center). The frequency spectrogram showed a greater distribution of energy between the frequencies up to 40 Hz (Figure 1B, right).

The electrocorticographic recording of the CPH 10 group showed amplitude with a variation of up to 0.05 mV (Figure 1C, left) as shown in the amplification of the recording (Figure 1C, center). The spectrogram shows energy intensity below 5 Hz with a lower distribution of energy in the frequencies up to 40 Hz (Figure 1C, right). The SLD-CPH 10 group showed an increase in the amplitude of the ECoG tracing by 0.07 mV with a preponderance of low frequency rhythms (Figure 1D, left), the amplification of the recording shows background Alpha oscillations (8–12 Hz) (Figure 1D, center), and the spectrogram shows a power distribution below 15 Hz (Figure 1D, right).

The CPH 20 group displayed an amplitude of 0.05 mV (Figure 1E, left). This group showed a preponderance of low-frequency oscillations (Figure 1E, center), as the spectrogram showed power distribution up to 10 Hz (Figure 1E, right). The SLD-CPH 20 group displayed trace slowdown with a preponderance of frequencies between Delta, Theta, and Alpha. There was also a considerable gain in the power at the frequencies from 1 to 12 Hz (Figure 1F, left), which is a characteristic of electroencephalogram (EEG) and ECoG slowing (Figure 1F, center). The spectrogram demonstrated an energy intensity above 15 Hz (Figure 1F, right), but greater intensity up to 40 Hz.

The electrocorticographic recordings for the CPH 30 group displayed an amplitude up to 0.05 mV (Figure 1G, right) with a decrease in frequency that can be observed in the amplification of the recording (Figure 1G, center). The spectrogram showed energy intensity below 15 Hz (Figure 1G, right). For the SLD-CPH 30 group, the animals' electrocorticographic tracings showed a preponderance of low frequencies such as Delta, Theta, Alpha, and Beta (below 30 Hz). The amplification of the recordings demonstrates a trace slowdown (Figure 1H, center). The spectrogram shows a greater distribution of power up to 40 Hz than the other groups (Figure 1H, right).

The mean power in the Control group (0.4588 ± 0.034 mV^2/ Hz × 10^{-3}) was lower than the Control SLD group (0.587 ± 0.0668 mV^2/Hz × 10^{-3}) but was similar to the groups: CPH 10 (0.536 ± 0.0668 mV^2/Hz × 10^{-3}) (p= 0.1720), SLD-CPH 10 (0.546 ± 0.0699 mV^2/Hz × 10^{-3}) (p= 0.0804), CPH 20 (0.482 ± 0.0861 mV^2/ Hz × 10^{-3}) (p= 0.993), and CPH 30 (0.505 ± 0.0670 mV^2/Hz × 10^{-3}) (p= 0.769). The Control group had lower potency than the SLD-
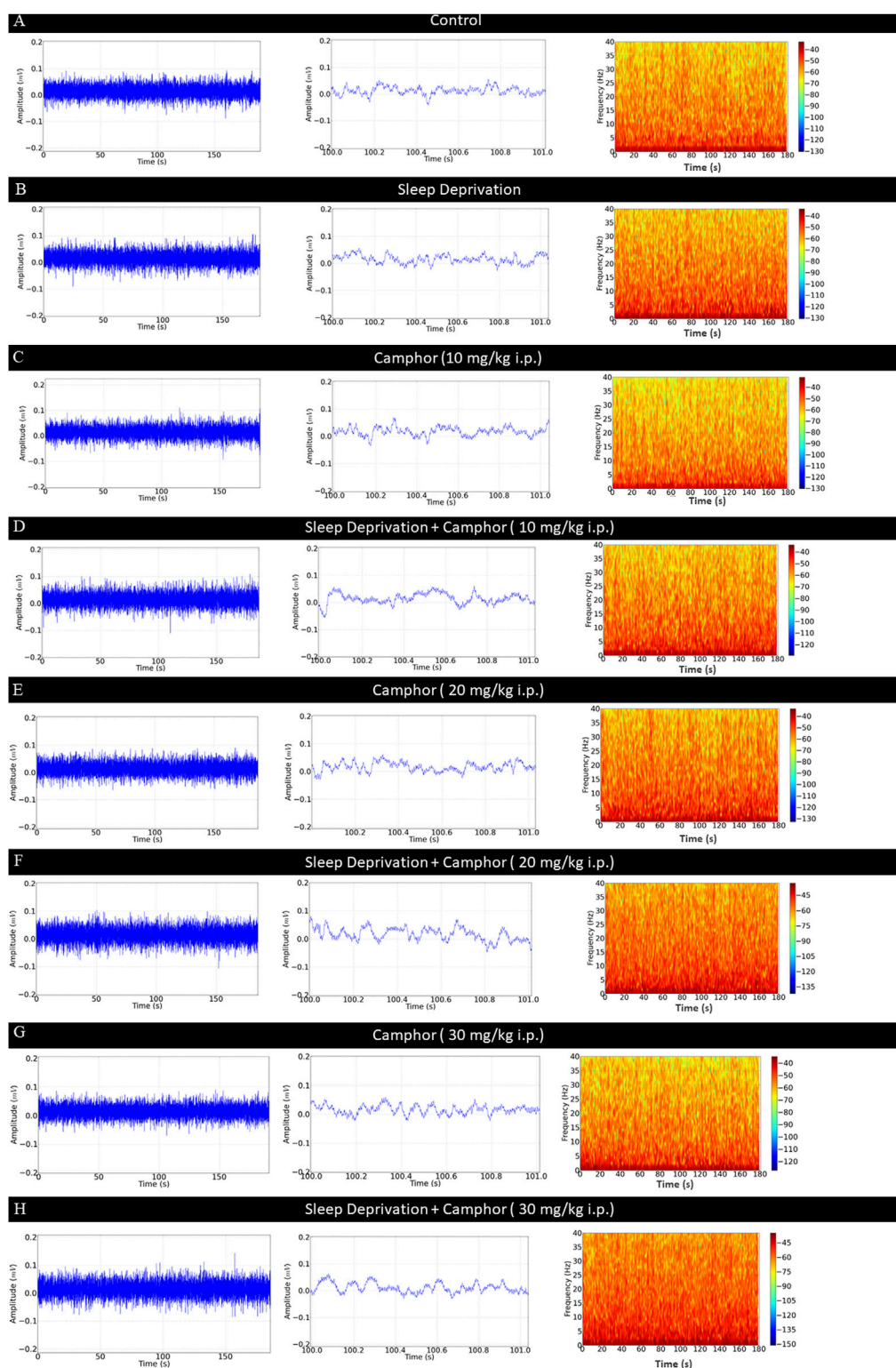
FIGURE 1
Demonstrations of 3-min ECoG recordings. ECoG tracing of the Control group (left), 1-s amplification of the tracing (center) and power distribution spectrogram (right) **(A)**; ECoG tracing of the Control SLD group (left), 1-s amplification of the recording (center) and respective spectrogram with power distribution in frequencies up to 40 Hz (right) **(B)**; ECoG tracing of the CPH 10 group (left), amplification of the recording in 1 s (center) and spectrogram (right) **(C)**; ECoG tracing of the SLD- CPH 10 group (left), amplification of the recording in 1 s (center) and spectrogram (right) **(D)**; ECoG tracing of the CPH 20 group (left), 1-s amplification (center) and spectrogram of energy distribution in frequencies up to 40 Hz (right) **(E)**; ECoG tracing of the SLD - CPH 20 group (left), 1-s amplification (center) and spectrogram of energy distribution in frequencies up to 40 Hz (right) **(F)**; ECoG recording of the CPH 30 group (left), amplification (center) and spectrogram (right) **(G)**; ECoG recording of the SLD - CPH 30 group (left), amplification (center) and spectrogram (right) **(H)**.
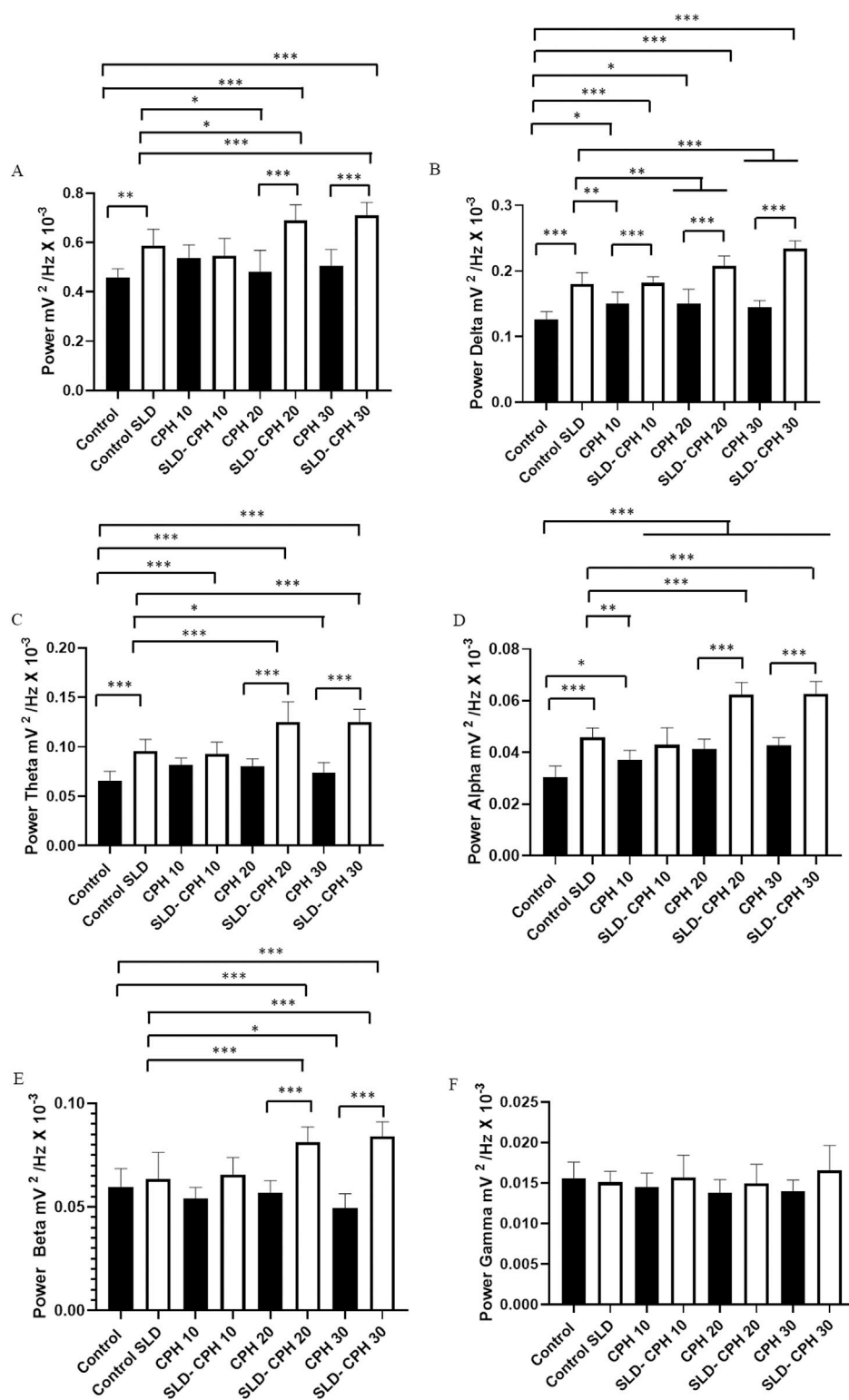
FIGURE 2
Graph of linear power distribution in the frequencies up to 40 Hz in the ECoG recordings between the groups with different camphor treatments **(A)**;
Average linear power between the groups with frequency in delta oscillations (1−4 Hz) **(B)**; average power distribution in theta frequency (4−8 Hz) for the
groups **(C)**; Graph of linear power distribution in the recordings between the groups in the oscillations in Alpha (8−12 Hz) **(D)**; Average power distribution
of the groups in the frequency in beta (12−28 Hz) **(E)**; Graph of linear power distribution between the groups in the frequency in gamma (28−40 Hz)
(F). (After ANOVA followed by Tukey, *P< 0.05 **P< 0.01 ***P< 0.001, n = 9).

CPH 20 (0.689 ± 0.0639 mV^2/Hz × 10^{-3}) and SLD-CPH 30 (0.710 ± 0.0516 mV^2/Hz × 10^{-3}) groups. The Control SLD group had a mean power similar to the CPH 10 (p= 0.685), SLD-CPH 10 (p= 0.8686) and the CPH 30 groups (p= 0.1275). The mean linear potency was higher than that of the CPH 20 group, but it was lower than the mean potency of the SLD-CPH 20 and SLD-CPH 30 groups. The mean potency of the CPH 10 group was similar to the SLD-CPH 10 group (p = 0.999). The mean potency of the CPH 20 and CPH 30 groups was lower when compared to the SLD-CPH 20 and SLD-CPH 30 groups (Figure 2A).

For Delta oscillations (1–4 Hz), the Control group (0.1258 ± 0.0122 mV^2/Hz × 10^{-3}) had lower mean Delta power than the groups: Control SLD (0.180 ± 0.0173 mV^2/Hz × 10^{-3}), CPH 10 (0.150 ± 0.0173 mV^2/Hz × 10^{-3}), SLD- CPH 10 (0.182 ± 0.009 mV^2/Hz × 10^{-3}), CPH 20 (0.150 ± 0.022 mV^2/Hz × 10^{-3}), SLD-CPH 20 (0.207 ± 0.0153 mV^2/Hz × 10^{-3}) and SLD-CPH 30 (0.234 ± 0.0116 mV^2/Hz × 10^{-3}), but was similar to the CPH 30 group (0.144 ± 0.0105 mV^2/Hz × 10^{-3}) (p = 0.160). The Control SLD group had a higher value than the groups treated with camphor alone, however, it was lower than the SLD-CPH 20 and SLD-CPH 30 groups. All the camphor-treated groups showed lower Delta power when compared to the SLD-CPH 10, SLD-CPH 20, and SLD-CPH 30 groups (Figure 2B).

The mean Theta power in the Control group (0.0654 ± 0.0099 mV^2/Hz × 10^{-3}) was lower than the Control SLD (0.0955 ± 0.0120 mV^2/Hz × 10^{-3}), SLD-CPH 10 (0.0928 ± 0.0119 mV^2/Hz × 10^{-3}), SLD-CPH 20 (0.125 ± 0.0202 mV^2/Hz × 10^{-3}) and SLD-CPH 30 groups (0.124 ± 0.0130 mV^2/Hz × 10^{-3}), but it was similar to the CPH 10 (0.0821 ± 0.0066 mV^2/Hz × 10^{-3}) (p = 0.0829), CPH 20 (0.080 ± 0.0078 mV^2/Hz × 10^{-3}) (p = 0187) and CPH 30 groups (0.073 ± 0.010 mV^2/Hz × 10^{-3}) (p= 0.8023). The Control SLD group had lower mean Theta power than the SLD-CPH 20 and SLD-CPH 30 groups and was higher than the CPH 30 group. The Control SLD group was similar to the following groups: CPH 10 (p = 0.2818), SLD-CPH 10 (p = 0.999) and CPH 20 (p= 0.1355). The CPH 10 group was similar to the SLD-CPH 10 group (p = 0.5563). The power of the CPH 20 and CPH 30 groups was weaker than the SLD-CPH 10, SLD-CPH 20 and SLD-CPH 30 groups (Figure 2C).

The mean power of Alpha oscillations for the Control group (0.0304 ± 0.0043 mV^2/Hz × 10^{-3}) was lower than the following groups: Control SLD (0.0458 ± 0.0035 mV^2/Hz × 10^{-3}), CPH 10 (0.0371 ± 0.0036 mV^2/Hz × 10^{-3}), SLD-CPH 10 (0.042 ± 0.0065 mV^2/Hz × 10^{-3}), CPH 20 (0.041 ± 0.0037 mV^2/Hz × 10^{-3}), SLD-CPH 20 (0.062 ± 0.0046 mV^2/Hz × 10^{-3}), CPH 30 (0.042 ± 0.0029 mV^2/Hz × 10^{-3}) and SLD-CPH 30 (0.062 ± 0.0046 mV^2/Hz × 10^{-3}). The Control SLD group had greater power than the CPH 10 group and lower than the SLD- CPH 20 and SLD- CPH 30 groups but was similar to the following groups: SLD-CPH 10 (p = 0.859), CPH 20 (p = 0.403) and CPH 30 (p= 0.818). The SLD-CPH 20 and SLD-CPH 30 groups had higher power means than the groups without sleep deprivation (Figure 2D).

The mean power in Beta oscillations for the Control group (0.059 ± 0.0086 mV^2/Hz × 10^{-3}) was similar to the Control SLD (0.063 ± 0.012 mV^2/Hz × 10^{-3}) (p= 0.977), CPH 10 (0.054 ± 0.0054 mV^2/Hz × 10^{-3}) (p = 0.790), SLD- CPH 10 (0.0657 ± 0.0060 mV^2/Hz × 10^{-3}) (p= 0.773), CPH 20 (0.0566 ± 0.0060 mV^2/Hz × 10^{-3}) (p= 0.999), and CPH 30 groups (0.049 ±
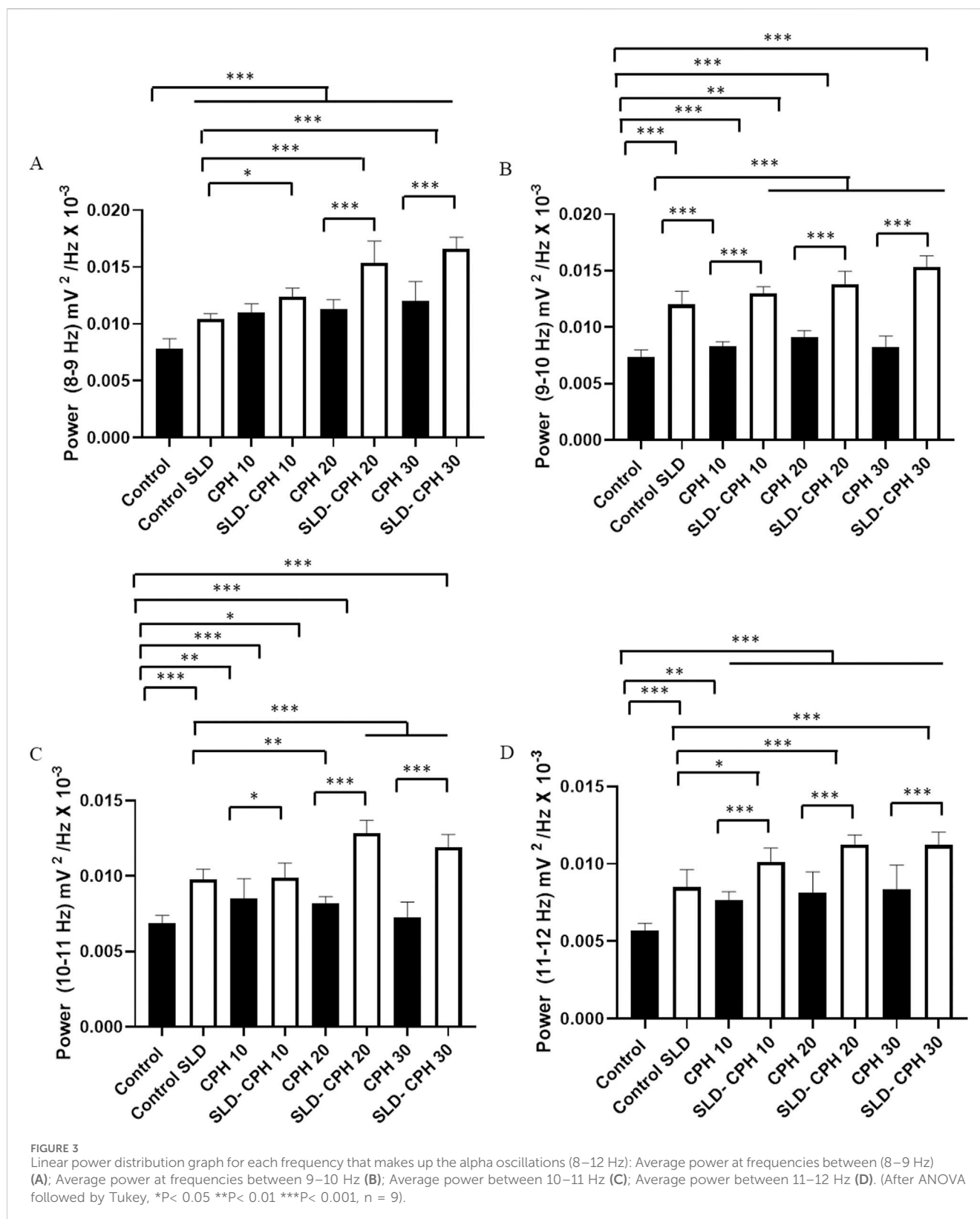
0.0067 mV^2/Hz × 10^{-3}) (p= 0.141). The Control group Beta power was weaker than the SLD-CPH 20 (0.0813 ± 0.0072 mV^2/Hz × 10^{-3}) and SLD-CPH 30 groups (0.084 ± 0.0068 mV^2/Hz × 10^{-3}). The groups of animals deprived of sleep showed similar power in Beta oscillations to the CPH 10 (p= 0.216), SLD-CPH 10 (p= 0.998) and SLD-CPH 20 groups (p= 0.626). However, the Control SLD group had a power mean power than the SLD- CPH 20 and SLD-CPH 30 groups (Figure 2E).

The average power in Gamma oscillations for the Control group (0.0155 ± 0.0020 mV^2/Hz × 10^{-3}) was similar to the following groups: Control SLD (0.0150 ± 0.0013 mV^2/Hz × 10^{-3}) (p = 0.999), CPH 10 (0.0145 ± 0.0017 mV^2/Hz × 10^{-3}) (p= 0.969), SLD-CPH 10 (0.0157 ± 0.0027 mV^2/Hz × 10^{-3}) (p= 0.999), CPH 20 (0.0137 ± 0.0016 mV^2/Hz × 10^{-3}) (p= 0.643), SLD-CPH 20 (0.0149 ± 0.0023 mV^2/Hz × 10^{-3}), CPH 30 (0.0140 ± 0.0135 mV^2/Hz × 10^{-3}) (p= 0.786), and SLD-CPH 30 (0.016 ± 0.0030 mV^2/Hz × 10^{-3}) (p= 0.961) (Figure 2F).

By individualizing the frequencies in the Alpha spectrum, we can assess camphor's contribution to the increase of Alpha waves. This way, the mean power evaluated between 8 and 9 Hz for the Control group (0.00786 ± 0.00083 mV^2/Hz × 10^{-3}) was lower than the following groups: Control SLD, (0.010 ± 0.00043 mV^2/Hz × 10^{-3}), CPH 10 (0.011 ± 0.00075 mV^2/Hz × 10^{-3}), SLD- CPH 10 (0.012 ± 0.00077 mV^2/Hz × 10^{-3}), CPH 20 (0.0112 ± 0.00083 mV^2/Hz × 10^{-3}), SLD-CPH 20 (0.0153 ± 0.00190 mV^2/Hz × 10^{-3}), CPH 30 (0.0120 ± 0.0016 mV^2/Hz × 10^{-3}), and SLD-CPH 30 (0.0166 ± 0.0010 mV^2/Hz × 10^{-3}). The Control SLD group power was lower than the SLD-CPH 10, SLD-CPH 20, SLD-CPH 30 groups. And it was similar to the CPH 10 (p= 0.964), CPH 20 (p= 0.7716), CPH 30 (p = 0.077) groups. The CPH 10 group was similar to the SLD-CPH 10 group (p= 0.1907). The SLD-CPH 20, SLD-CPH 30 groups had higher powers than the groups treated with camphor alone (Figure 3A).

The mean power evaluated between 9 and 10 Hz for the Control group (0.00737 ± 0.00061 mV^2/Hz × 10^{-3}) was lower than all the sleep deprived groups: Control SLD (0.0120 ± 0.00116 mV^2/Hz × 10^{-3}), SLD-CPH 10 (0.0129 ± 0.00059 mV^2/Hz × 10^{-3}), SLD-CPH 20 (0.0138 ± 0.0011 mV^2/Hz × 10^{-3}), SLD-CPH 30 (0.0152 ± 0.00102 mV^2/Hz × 10^{-3}). The camphor-treated groups were similar to the Control group: CPH 10 (0.0083 ± 0.00039 mV^2/Hz × 10^{-3}) (p= 0.3080), and the CPH 30 (0.0082 ± 0.00103 mV^2/Hz × 10^{-3}) (p= 0.470). The CPH 20 group (0.00911 ± 0.00058 mV^2/Hz × 10^{-3}) was lower than the Control group. The sleep-deprived group showed higher mean power than the camphor-treated groups. However, it was lower than the SLD-CPH 20 and SLD-CPH 30 groups (Figure 3B).

The mean power evaluated between 10 and 11 Hz for the Control group (0.00689 ± 0.00049 mV^2/Hz × 10^{-3}) was lower than the groups: Control SLD (0.0097 ± 0.00067 mV^2/Hz × 10^{-3}), CPH 10 (0.00852 ± 0.001304 mV^2/Hz × 10^{-3}), SLD-CPH 10 (0.0098 ± 0.00098 mV^2/Hz × 10^{-3}), CPH 20 (0.0081 ± 0.00044 mV^2/Hz × 10^{-3}), SLD-CPH 20 (0.0128 ± 0.00086 mV^2/Hz × 10^{-3}), and SLD- CPH 30 (0.0119 ± 0.00084 mV^2/Hz × 10^{-3}). However, it was similar to the CPH 30 group (0.0072 ± 0.000998 mV^2/Hz × 10^{-3}) (p= 0.9821). The Control SLD group had a lower mean power than the SLD-CPH 20 and SLD-CPH 30 groups. The CPH 20 and CPH 30 groups had lower mean power. The CPH 10 group (p= 0.060) and the SLD-CPH 10 group (p=

FIGURE 3
Linear power distribution graph for each frequency that makes up the alpha oscillations (8−12 Hz): Average power at frequencies between (8−9 Hz) **(A)**; Average power at frequencies between 9−10 Hz **(B)**; Average power between 10−11 Hz **(C)**; Average power between 11−12 Hz **(D)**. (After ANOVA followed by Tukey, *P< 0.05 **P< 0.01 ***P< 0.001, n = 9).

0.999) were similar. The SLD-CPH 10, SLD-CPH 20, SLD-CPH 30 groups had higher means than the groups treated with camphor alone (Figure 3C).

The mean power evaluated between 11 and 12 Hz for the Control group (0.0056 ± 0.00049 mV^2/Hz $\times 10^{-3}$) was lower than the following groups: Control SLD (0.0084 ± 0.0011 mV^2/Hz $\times 10^{-3}$), CPH 10 (0.0076 ± 0.00052 mV^2/Hz $\times 10^{-3}$), SLD-CPH 10 (0.010 ± 0.00090 mV^2/Hz $\times 10^{-3}$), CPH 20 (0.0081 ± 0.0013 mV^2/Hz $\times 10^{-3}$), SLD CPH 20 (0.011 ± 0.00061 mV^2/Hz $\times 10^{-3}$), CPH 30 (0.0083 ± 0.0015 mV^2/Hz $\times 10^{-3}$) and SLD-CPH 30 ($0.011 \pm$

TABLE 1 Demonstrates the average latency(s) for the appearance of the analyzed behaviors (n = 9).

Dose of camphor	Testicular relaxation	Decreased motility	Light sleep induction
10 mg/kg i.p.	477.8 ± 46.91 s	508.4 ± 44.27 s	—
20 mg/kg i.p.	394.4 ± 42.28 s	449.7 ± 45.90 s	—
30 mg/kg i.p.	318.3 ± 46.90 s	327.0 ± 47.43 s	553.8 ± 46.33 s
	$F_{(2, 24)}$ = 27.75 $P < 0.001$	$F_{(2, 24)}$ = 36.63 $P < 0.001$	

0.00084 mV²/Hz × 10⁻³). The Control SLD group had a lower mean than the SLD-CPH 10, SLD-CPH 20 and SLD-CPH 30 groups. And it was similar to the CPH 10 (p= 0.6614), CPH 20 (p= 0.996) and CPH 30 (p = 0.999). The CPH 10, CPH 20, and CPH 30 groups had lower values than the SLD-CPH 10, SLD-CPH 20, and SLD-CPH 30 groups (Figure 3D).

As for the behavioral analyses, the first observed behavior in the animals was the total relaxation of the testicles while they remained in the scrotum, and the latency time for this behavior was shorter with higher doses of camphor. Shortly afterward, the animals showed decreased motility, remaining still for longer periods, although stimuli could provoke movement. This pattern exhibited similar characteristics in terms of latency of occurrence and administered dose to the previous test. Regarding the induction of light sleep, the only dose that induced sleep in the animals was 30 mg/kg i.p., with a latency of 553.8 ± 46.33 s. The induced sleep was superficial, and the animals could be awakened by sound and tactile stimuli (Table 1).

4 Discussion

Camphor, a crystalline white substance with a strong odor, is one of the byproducts extracted from this plant and has various known pharmacological applications (Hamidpour et al., 2013). Not much is known about the mechanism of action of camphor on the central nervous system. Vatanparast and Andalib-Lari., 2017 proposed, through experiments on snail neurons, that camphor can inhibit K+ channels, resulting in increased Ca2+ influx into neurons. This inhibition reduces the neuron's hyperpolarization time and facilitates the generation of action potentials. At higher doses of the substance, this may lead to epileptiform patterns on the electroencephalogram, marked by waves with multiple peaks (polyspike waves) indicative of ictal activity (Ferreira et al., 2020). The hyperpolarization phase typically serves as a negative feedback mechanism to regulate neuronal excitability (Janahmadi et al., 2008; Vatanparast and Andalib-Lari., 2017 proposed mechanism of action justifies well how camphor is capable of inducing seizures, but it is still unclear how low doses of camphor induce light sleep as observed in our results.

Studies have shown that zolpidem, a widely used hypnotic drug that acts through interaction with γ-aminobutyric acid receptor subunit alpha-1, tends to intensify Delta waves and attenuate the power of waves in the 4–12 Hz range, particularly Theta waves, while also intensifying Beta waves in the 25–30 Hz range. Consequently, this drug is effective in prolonging total sleep time and slow-wave

sleep (SWS). In comparison, camphor also increases the power of Delta and Beta waves; however, it additionally tends to potentiate Theta and Alpha waves (Crestani et al., 2000; Landolt et al., 2000; Kopp et al., 2004; Bettica et al., 2012; Fox et al., 2013).

Baclofen, in the other hand, is a γ-aminobutyric acid type B (GABAb) receptor agonist that has similar effects to camphor in the CNS. It induces a hypersynchronous slow wave EEG pattern characterized by high amplitude Delta frequencies, enhancing the power density in non-rapid eye movement (NREM) sleep significantly in the 2.25–5.5 and 10.75–14.75 Hz, which are correspondent to Delta, Theta, and Alpha frequencies. It also generates behavioral alterations consistent with relaxation that depended on other variables such as light exposure, and was able to increase sleep intensity (Pressman, 2004; Hodor et al., 2015).

Gamma-hydroxybutyrate, another GABAb receptor agonist, induces a hypersynchronous EEG pattern similar to that of Baclofen. It increases EEG power density in NREM sleep within the frequency range associated with slow-wave activity. However, a significant effect was observed only under low light exposure (Hodor et al., 2015). Based on these electroencephalographic similarities, we hypothesize that low doses of camphor may have agonistic effects on GABAb receptors, which could explain its effects on the CNS.

Despite the current limited understanding of the neural mechanisms underlying cognitive impairments and electrocortical alterations caused by sleep restriction, Vyazovskiy et al. (2013) suggest that prolonged periods of wakefulness affect the cortex's responsiveness to stimuli by altering cortical bistability, in other words, the ability of neurons to stabilize themselves in a state of both depolarization and hyperpolarization (Wilson, 2008). According to the author, heightened excitability, functional connectivity, and bistability increase the likelihood of bursts of activity, as cortical responsiveness is enhanced, with stimuli often coming from sources far from the neuronal network. This finding could explain why rats exposed to both sleep deprivation and camphor show a significant increase in Delta, Theta, Alpha, and Beta wave power compared to those not subjected to sleep deprivation. The altered responsiveness of the neurons may synergize with camphor's action, thereby enhancing brain wave power, especially at lower frequencies.

Furthermore, it is known that sleep deprivation can promote the detachment of pericytes from the endothelium of cerebral vessels by decreasing the expression of proteins important for maintaining the integrity of the blood-brain barrier (Medina-Flores et al., 2020), thus increasing the permeability of the blood-brain barrier by multiple factors and possibly allowing greater passage of camphor into brain tissue. Therefore, it is possible that sleep deprivation allows camphor to reach neurons more easily, increasing its concentration in the

tissue and potentiating its effects, especially considering camphor's highly lipophilic structure, which would have a high affinity for brain tissue, rich in lipids (Vatanparast and Andalib-Lari, 2017).

Our results also showed that there was a decrease in the predominant frequency of brain oscillatory patterns in the group exposed to 10 mg/kg i.p. of camphor, shifting the oscillatory patterns toward the Alpha frequency range. When administered 20 and 30 mg/kg i.p., there was a preponderance of low-frequency Delta, Theta-Theta, and Alpha bands, indicating an oscillatory pattern with deceleration characteristics, with a considerable gain in power in the 1–12 Hz range. When observed at a dose of 30 mg/kg i.p., despite the pattern of trace slowdown, there was also an intensification in the high frequency bands, especially Beta, which must be correlated with the excitatory effect of camphor on neurons. It was observed that the power of the Delta, Theta, Alpha, and Beta waves changed in a dose-dependent manner and the Alpha waves showed an increase in distribution between the 8–10 Hz and 11–12 Hz frequency bands, also in a dose-dependent manner. All the changes described were potentiated by sleep deprivation.

These findings indicate camphor exhibits properties suggestive of a sleep-inducing agent when administered within therapeutic concentrations. Specifically, camphor induces electrocorticographic patterns of trace slowdown, which is characterized by a preponderance of low-frequency oscillations akin to those observed during physiological sleep. Moreover, our behavioral evaluation demonstrated light sleep induction within non-sleep-deprived rats that received 30 mg/kg i.p. of camphor, preceded by decreased mobility and testicular relaxation, further supporting this hypothesis.

According to the most common classification, sleep is divided into 5 phases: Wake, characterized by low-amplitude Beta waves in alert states, and by a predominance of Alpha waves in states of relaxation, similar to those obtained in our study among groups that received doses of 10 mg/kg i.p. of camphor; N1 - Light Sleep, characterized by low voltage Theta waves; N2 - Heavy Sleep, characterized by sleep spindles and K complexes; N3 - SWS, characterized by higher amplitude Delta waves; and rapid eye movement (REM) sleep, characterized by the presence of Beta waves similar to the alert state. Our findings show similarities in the tracings of the sleep deprived groups who received doses of 20 and 30 mg/kg i.p. with brainwave oscillatory sleep patterns. The sleep deprived group that received 20 mg/kg showed patterns that were similar to SWS due to the trace slowdown and potentiation of Delta waves, while the 30 mg/kg group resembled REM sleep patterns, due to the trace slowdown plus the appearance of Beta waves. These findings could indicate that camphor can facilitate sleep induction through changes in neuronal activity, which is potentiated by sleep deprivation. However, due to the neuro-excitatory nature of camphor, higher doses could induce the appearance of increasingly higher frequencies, up to the appearance of potentially harmful epileptiform patterns.

It is important to note that camphor can induce toxicity when administered in high doses. Gastrointestinal symptoms frequently arise due to direct irritation of the mucous membranes, oxidative stress, and cardiotoxicity (Eldridge et al., 2007; Love et al., 2004; Du et al., 2021). Additionally, camphor toxicity can precipitate neurological effects, including agitation, anxiety, spasms, convulsions, hallucinations, hyperreflexia, and, in severe cases, coma and respiratory arrest, with these severe outcomes more commonly observed in pediatric patients (Rahimi et al., 2017; Eldridge et al., 2007; Love et al., 2004; Phelan, 1976; Bahr et al., 2019). The International Programme on Chemical Safety (1989) estimates that the probable lethal oral dose of pure camphor is approximately 4 g for adults, while in children, the lethal dose is estimated to be between 0.5 and 1 g (70 mg/kg). In rats, the intraperitoneal lethal dose is reported to be 900 mg/kg.

In conclusion, applying camphor to sleep-deprived Wistar rats alters occipital electrocorticographic patterns by increasing the total mean power of spikes, especially in the frequency bands of Delta, Theta, Alpha, and Beta, and inducing sleep-like behavior in Wistar rats. This activity is likely due to multiple factors, including the synergistic effect of sleep deprivation and camphor and the increased blood-brain barrier permeability caused by sleep deprivation. While camphor may be potentially harmful at high doses due to its toxicity, it could be a useful adjunctive medication in the treatment of insomnia. However, further studies are needed to confirm its efficacy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the animal study was approved by the Animal Ethics Committee (CEUA—UFPA) number 1380040822 [002046]. The study was conducted in accordance with the local legislation and institutional requirements. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ALGA: Methodology, Investigation, Writing–original draft, Writing–review and editing. GBB: Methodology, Investigation, Writing–original draft, Writing–review and editing. MFS: Writing–review and editing. LVS: Writing–review and editing. RGS: Methodology, Investigation, Writing–original draft. TMC: Methodology, Writing–review and editing. TSR: Writing–review and editing. PFPH: Writing–review and editing. LEQ: Writing–review and editing. CAP: Writing–review and editing. DBA: Writing–review and editing. DCFL: Writing–review and editing. MTS: Writing–review and editing. MH: Data curation, Methodology, Supervision, Conceptualization, Formal analysis, Project administration, Validation, Investigation, Funding acquisition, Resources, Visualization, Software, Writing–original draft, Writing–review and editing.

Funding

Acknowledgments

Conflict of interest

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

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Nomenclature

CBT	Cognitive-behavioral therapy
BZ	Benzodiazepines
BZRA	Benzodiazepine receptor agonist
CNS	Central nervous system
SD	Standard deviation
SLD	Sleep deprivation
CPH	Camphor
SPD	Spectral power density
ECoG	Electrocorticography
EEG	Electroencephalogram
REM	Rapid eye movement
NREM	Non-rapid eye movement
SWS	Slow-wave sleep