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Research report

Neurochemical and electrophysiological changes induced by paradoxical sleep deprivation in rats☆

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ABSTRACT

The present study aims to investigate the effects of paradoxical sleep deprivation (PSD) on the waking EEG and amino acid neurotransmitters in the hippocampus and cortex of rats. Animals were deprived of paradoxical sleep for 72 h by using the multiple platform method. The EEG power spectral analysis was carried out to assess the brain's electrophysiological changes due to sleep deprivation. The concentrations of amino acid neurotransmitters were assessed in the hippocampus and cortex using HPLC. Control data showed slight differences from normal animals in the delta, theta and alpha waves while an increase in the beta wave was obtained. After 24 h of PSD, delta relative power increased and the rest of EEG wave's power decreased with respect to control. After 48 h and 72 h the spectral power analysis showed non-significant changes to control. The amino acid neurotransmitter analysis revealed a significant increase in cortical glutamate, glycine and taurine levels while in the hippocampus, glutamate, aspartate, glutamine and glycine levels increased significantly. Both the waking EEG and neurotransmitter analyses suggest that PSD induced neurochemical and electrophysiological changes that may affect brain proper functionality.

1. Introduction

Sleep and wakefulness are controlled by a network of brain nuclei that interact in a complex fashion, integrating homeostatic and circadian regulations [1]. Sleep in mammals is composed of two major stages, rapid eye movement sleep (REMS) and non-rapid eye movement sleep. Sleep deprivation has long been used as a tool in sleep research to interfere into normal sleep/wake cycles in rodents [2] and human [3] to elucidate the sleep function.

REMS loss affects neuronal responsiveness [4] and brain excitability [5]. Similar alterations are observed with several paradigms of either total or PSD (paradoxical sleep deprivation) in rats [6,7]. The hippocampus is a limbic structure involved in learning and memory and it has been focus of intensive research aimed at understanding the function of REMS as it relates to memory function [8].

Neuropharmacological studies have shown that the changes in brain levels of specific neurotransmitters could trigger or suppress specific stages of wake and sleep [9]. Studies that measured the state-dependent neurotransmitter levels of the brain have suggested that the levels of different neurotransmitters in the brain are lightly regulated by behavioral states of wake and sleep [10]. The transition between wakefulness, NREM and REM sleep is accompanied by neuromodulatory changes. Acetylcholine, norepinephrine, serotonin, histamine and hypocretin levels are high in wakefulness and low in NREM sleep and noradrenergic, histaminergic and serotonergic become silent during REM sleep [11].

After neuromodulation, the EEG and the underlying patterns of neuronal activity also changes, especially in the cerebral cortex. Some reports measured tissue glutamate content in whole brain after total or REM sleep deprivation with mixed results, no changes [12] or an increase in glutamate after sleep loss [13]. REM sleep deprivation was also shown to increase glutamine turnover [14] and protein levels of glutamine synthetase [15]. Transcript levels of genes coding for enzymes involved in glutamate synthesis (glutamine synthase, glutaminase), glutamate receptor subunits (GLUR2, GluR3) [16] and intracellular proteins implicated in glutamate receptor clustering (Homer/Vesl, Narp) are also upregulated during waking [17]. In addition, the number of GluR1-containing AMPA receptors increases after waking relative to sleep in cortex and hippocampus [18].

Recently, Naylor et al. [19] found rising L-glutamate levels during extended (>15 min) waking periods, whereas an overall decline

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in brain ECF L-glutamate concentration was demonstrated after long periods of sleep (>15 min of combined SW and REM sleep).

Several studies aimed to investigate the nature of brain functioning underlying the extended wakefulness [3,20,21]. Recording of the brain electrical signals (EEG) is considered as a non-invasive sensitive tool used frequently to investigate changes in cortical brain excitability under different conditions [22].

The distinct variations in the theta and alpha band may represent electrophysiological correlates of different aspects of a circadian change in arousal [23]. Furthermore, the combined increase in theta activity and decrease in alpha activity seems to correspond to a slowing of encephalographic rhythms, that is, a shift of spectral power toward lower frequencies. A prevalence of slow-wave activity might thus provide an index of sleep propensity and/or cortical deactivation [24].

Rhythmical slow activity in the hippocampus, described as theta (3–12 Hz), can be recorded during wakefulness, and represents one of the tonic markers of rapid eye movement (REM) sleep [25].

Theta oscillations or phase-locked neuronal discharges were observed in hippocampal afferent and efferent structures, i.e., the entorhinal cortex, the perirhinal cortex, and the amygdala [26,27], although these structures are not capable of independently generating theta activity.

Task-related theta responses in both the scalp EEG and hippocampal recordings were suggested to reflect temporal cooperation between the hippocampus and its immediate efferent and afferent structures [28,29].

It was therefore hypothesized that scalp recorded theta oscillations may serve as a window to hippocampal activity [28], which is not directly volume-conducted to the scalp, per se [30].

Most of the animal studies that pursued the changes in EEG due to sleep deprivation aimed to characterize those effects on the subsequent sleep structure and sleep rebound [31,32].

Few studies have focused on the study of waking EEG changes during or after sleep deprivation [33]. EEG recordings of the brain yield a wealth of information regarding neuronal activity, but are limited in that these measurements only report gross neuronal firing patterns [19]. The authors emphasized the importance of understanding the underlying physiology driving these large-scale neuronal events through techniques that can accurately monitor neurochemical release and uptake.

The present study aims to investigate the effect of paradoxical sleep deprivation on the concentrations of amino acid neurotransmitters in the cortex and hippocampus of adult rats. In addition, waking EEG changes and its functional significance after paradoxical sleep deprivation in rat were also studied.

2. Material and methods

2.1. Experimental animals

The experimental animals used in the present study were adult male Wistar albino rats weighing 200–250 g. The animals were obtained from the animal house of the National Research Center, Egypt. They were maintained on stock diet and kept under fixed conditions of housing and handling. They were under controlled light-dark cycle (light on at 7 a.m. and off at 7 p.m.) and temperature conditions (25 ± 2 °C). All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center, Egypt which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Design of experiment

Paradoxical sleep deprivation was induced according to the method described by Zager et al. [34]. Sleep deprivation procedures were carried out using the multiple platform method to reduce the immobilization stress. The animals were placed in glass cages (50 cm × 50 cm × 50 cm) equipped with platforms and filled with water to 1 cm below the platform. Two different sizes platforms were used; one with large diameter (15 cm) for control animals and the other with small diameter (3 cm) suitable for sleep-deprived animals. Two rats, to avoid social isolation stress, were placed in each cage containing two circular platforms (3 cm) with water 1 cm below the upper surface of the platform. When the animal enters the paradoxical phase of sleep, it falls into the water due to muscle atonia and wakes up. Food and water were available through a grid on the top of the cage containing water.

At the beginning of the experiment the number of animals was 24. They were divided into three groups ($n = 8$). The first group was subjected to paradoxical sleep deprivation for 72 h. The second group that acts as a control group was subjected to the same conditions of the first group but the platform diameter was 15 cm which permitted the animals to sleep on it. Each animal of sleep deprived rats and the control group was weighed at the beginning and at the end of the 72 h of paradoxical sleep deprivation (before decapitation). The weight gain was expressed as the difference between the initial weights of the animals and their final weights after 72 h in the sleep deprived and control groups.

After 72 h both the sleep-deprived rats and the control group were sacrificed. The cortex and hippocampus of each rat were dissected out, weighed and kept frozen until analysis

The third group of animals ($n = 8$) was used for EEG recording. The animals served first as control and then were subjected to the sleep deprivation procedure to reduce the number of animals used and to secure the comparison of the EEG data.

2.3. Determination of amino acids concentration

The quantitative determination of the amino acids (glutamate, aspartate, glutamine, GABA, glycine and taurine) was carried out by using the high performance liquid chromatography (HPLC) method employed by Márquez et al. [35].

The HPLC system consisted of a Wellchrom Mini-star K-501 pump (Knauer, Germany), a column thermostat 5–85 °C with injector equipped with a 20 μL loop (Knauer, Germany), a luna 5u C-18 reversed phase column (5 μm particle size, 150 × 4.6 mm I.D.) from phenomenex, USA, a Wellchrom spectrophotometer K-2600 with variable wavelength (Knauer, Germany) and a chromatography workstation (Eurochrom 2000).

The mobile phase consisted of 50/50 (v/v), methanol/water containing 0.6% glacial acetic acid and 0.008% triethylamine. The concentrations of the amino acids studied in the different brain areas were expressed as μmol/g fresh tissue.

2.4. Electrode implantation

Under deep pentobarbital anesthesia (40 mg/kg, i.p.) the rats were positioned in the stereotaxic device (David Kopf instruments, Tujunga, California, USA) and implanted with stainless steel miniature screws (1 mm diameter) that served as EEG electrodes. The two cortical electrodes were implanted above the hippocampus region at 2.7 mm lateral to the midline, 3.5 mm posterior to the bregma in both hemispheres. Both electrodes were referenced to the third electrode above the cerebellum implanted 2 mm posterior to the lambda, on midline [36]. The electrodes were fixed in their place and isolated by a layer of dental cement (zinc polycarboxylate, Spofa-Dental-Praha, Czech Republic). After surgery, the animals were housed individually in separate glass cages for 7–10 days to recover from surgery. During this period the animals were habituated to the EEG recording setup.

2.5. EEG recording and analysis

EEG recording sessions were started at the light phase and the animals were kept awake during this period via gentle handling. All recordings were done at the same time of the day (between 9 and 11 a.m.) to avoid the circadian variation of the EEG signals. Firstly, a baseline recording was obtained from normal wake/sleep cycles animals (cage housed animals), then the recording was done for the same animals kept for one day on a large platform (control animals). The same procedures were carried out for the sleep-deprived animals after 24 h, 48 h and 72 h of PSD except they were put on the smaller platform.

During EEG recordings, rats were kept in a sound attenuated and electrically shielded cage (25 cm × 25 cm × 30 cm). Rats were left 30 min prior recording for acclimatization. EEG recording electrodes connected to the amplifier, which in turn was connected to the analog-to-digital conversion (A/D) card (National instruments, USA). Biobench software (National instruments, USA) was used to acquire EEG signals. EEG recordings were FFT analyzed under the following parameters: hamming window type, the frame size used was 1024 points, the resolution was 0.195 Hz and the sampling rate was 200 samples/sec. The EEG recording session continued for 1 h. The obtained averaged power spectra were segmented into five frequency bands; delta (0.1–4 Hz); theta (4.1–8 Hz); alpha (8.1–13 Hz); beta-1 (13.1–18 Hz) and beta-2 (18.1–30 Hz). The power of each frequency band was relative (percentage) to the total power (0.1–30 Hz) of the bands for comparison purpose.

2.6. Statistical analysis

Significant differences between normal, control and PSD EEG relative band powers were obtained by using *t*-test. The differences between the initial weights of the rats and their weights after 72 h were expressed as mean ± S.E.M. The amino acid concentrations were expressed as means ± S.E.M. Data were analyzed by Student's *t*-test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software in a PC-compatible computer. The difference between means was

Table 1
The levels of amino acid neurotransmitters in the cortex in control and PSD3 (after 72 h of PSD).

	Control	PSD3
Glutamate	5.047 ± 0.091	5.602 ± 0.212*
Aspartate	3.433 ± 0.206	3.358 ± 0.263
Glutamine	2.526 ± 0.038	2.602 ± 0.029
Glycine	0.624 ± 0.019	0.771 ± 0.028**
GABA	1.039 ± 0.020	1.069 ± 0.022
Taurine	3.454 ± 0.112	4.218 ± 0.273*

Mean ± S.E.M.
PSD (paradoxical sleep deprivation).
* *P*-value < 0.05.
** *P*-value < 0.01.

significant at $p < 0.05$. Percentage difference = [(mean of sleep deprived rat − mean of control)/mean of control] × 100.

3. Results

3.1. Effect of PSD on weight gain of rats

Paradoxical sleep deprivation for 72 h resulted in a significant decrease in the weight gain of the sleep-deprived rats (4.125 ± 0.666) as compared to that of control rats (10.374 ± 0.73), recording a percentage difference of −60.237% below the control.

3.2. Effect of PSD on amino acid neurotransmitter concentrations

Table 1 shows the effect of paradoxical sleep deprivation on the levels of amino acid neurotransmitters in the cortex. After 72 h of paradoxical sleep deprivation, a significant increase in cortical glutamate, glycine and taurine levels was obtained, recording +10.997%, +23.558% and +22.119% above the control levels, respectively.

In the hippocampus of rats deprived from paradoxical sleep for 72 h, a significant increase in the levels of the excitatory amino acids glutamate and aspartate, was recorded, being +22.813% and +27.466% above the control values, respectively (Table 2). This was accompanied by a significant increase in glutamine and glycine, recording a percentage difference of +19.916% and +19.048%, respectively.

3.3. Effect of PSD on the EEG frequency bands

The EEG power spectral analysis is shown in Table 3 and depicted in Fig. 1 and the actual power spectra were shown in Fig. 2. There are slight differences recorded between normal and control data in delta, theta and alpha waves. However, there was a non-significant increase in both beta-1 and beta-2 waves. A peak difference was obtained after 24 h of PSD. There was a significant increase in delta band (66%) with respect to control. However, a significant decrease was obtained in theta (−62.1%), alpha (−63.6%), beta-1 (−66.7%) and beta-2 (−75%) with respect to control. After the second and third days of PSD, there was a non-significant difference with respect to control data. The delta band recorded a non-significant increase in the second and third days of PSD with respect to control, while theta, alpha, beta-1 and beta-2 recorded a non-significant decrease.

Table 2
The levels of amino acid neurotransmitters in the hippocampus in control and PSD3 (after 72 h of PSD).

	Control	PSD3
Glutamate	9.065 ± 0.318	11.133 ± 0.356***
Aspartate	2.403 ± 0.071	3.063 ± 0.112 ***
Glutamine	2.606 ± 0.085	3.125 ± 0.092**
Glycine	0.609 ± 0.017	0.725 ± 0.027*
GABA	1.141 ± 0.025	1.206 ± 0.027
Taurine	3.671 ± 0.153	3.677 ± 0.147

Mean ± S.E.M.
PSD (paradoxical sleep deprivation).
* *P*-value < 0.05.
** *P*-value < 0.01.
*** *P*-value < 0.001.

4. Discussion and conclusions

It has been hypothesized that sleep/waking cycle may be metabolic in origin. Thus, it has been It has been hypothesized that sleep/waking cycle may be metabolic in origin. Thus, it has been suggested that the prolonged waking induces accumulation of brain metabolites responsible for excessive sleepiness and participating sleep rebound [13].

In the present study, paradoxical sleep deprivation for 72 h resulted in a significant increase in hippocampal glutamate, aspartate, glutamine and glycine. This was accompanied by a significant increase in cortical glutamate, glycine and taurine. The present results are consistent with the findings of Bettendorff et al. [13] who observed an increase in glutamate content in rat cerebral cortex due to paradoxical sleep deprivation. More recently, the study of Cortese et al. [37] revealed that paradoxical sleep deprivation induced a significant increase in glutamate levels in the hippocampus and thalamus.

Large evidence has shown that prolonged PSD leads to a reduction in body mass, elevated energy metabolism, changes in circulating hormones, loss of immune integrity and other disorders [7]. Moreover, it has been observed that paradoxical sleep deprivation produced a fall in glucose, glucose-6-phosphate and pyruvate in the cerebral frontal lobe indicating high metabolism [38]. Sleep deprivation produced weight loss, increased energy expenditure, increased plasma catecholamine and hyperthyroidism [39]. All these features are indicative of an elevated level of brain metabolism. These features in turn may explain the significant decrease in the weight gain of sleep-deprived rats recorded in the present study after 72 h.

In waking and REM sleep overall brain metabolism is high in both animals and humans [40,41]. During deep NREM sleep, instead, cerebral glucose metabolism is 30–40% lower than during waking [41,42], and so are cerebral blood flow and oxygen metabolism (18% and 25%, respectively) [40]. Moreover, most of the brain energy budget is required to sustain synaptic activity at glutamatergic synapses [43]

Thus, the increase in the rate of cerebral metabolism due to PSD will result in an increase in the rate of cerebral glucose utilization that represents the main substrate for the brain [44]. Since glucose is metabolized in the neurons into amino acid neurotransmitters [45] and most of the brain energy budget is required to sustain synaptic activity at glutamatergic synapses [15], it could be concluded that the significant increase in the excitatory amino acid neurotransmitters (glutamate and aspartate) in the hippocampus and cortex after 72 h of paradoxical sleep deprivation may be at the expense of glucose as a consequence of the elevated rate of metabolism. Sallanon-Moulin et al. [15] showed that the expression of glutamine synthetase increases after paradoxical sleep deprivation. This may give an explanation for the net increase in hippocampal glutamine content recorded in the present study.

Earlier data suggested that PSD increases ammonia content in rat brain, as the brain ammonia content increases during prolonged intense cerebral activity [46]. Furthermore, it has been reported that the production of glutamate by rat brain mitochondria increased by ammonia [47]. Consequently, this mechanism may underlie the significant increase in glutamate induced by sleep deprivation in the hippocampus and cortex.

Table 3
The relative band power of EEG frequency bands in normal, control, and PSD1 (after 24 h of PSD), PSD2 (after 48 h of PSD) and PSD3 (after 72 h of PSD).

	Delta	Theta	Alpha	Beta-1	Beta-2
Normal	0.56 ± 0.13	0.27 ± 0.09	0.09 ± 0.02	0.04 ± 0.02	0.03 ± 0.02
Control	0.50 ± 0.21	0.29 ± 0.13	0.11 ± 0.06	0.06 ± 0.03	0.04 ± 0.02
PSD1	0.83 ± 0.07*	0.11 ± 0.06*	0.04 ± 0.01**	0.02 ± 0.01**	0.01 ± 0.003*
PSD2	0.70 ± 0.11	0.18 ± 0.07	0.07 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
PSD3	0.65 ± 0.04	0.23 ± 0.03	0.08 ± 0.01	0.03 ± 0.003	0.02 ± 0.002*

Mean ± standard deviation.
PSD (paradoxical sleep deprivation)
* *P*-value < 0.05.
** *P*-value < 0.01.

The present data revealed that the hippocampus was more severely affected by sleep deprivation than the cortex. In the hippocampus, the percentage of change in glutamate (22.813%) was not only higher than in the cortex (10.997%) but was also accompanied by a significant increase in glutamine and aspartate. It has been reported that sleep deprivation increases neuronal excitability and decreases the threshold for seizures in epileptic model [48]. In animals, sleep deprivation resulted in a lowering of the thresholds to electric shock convulsions [49] and for kindling to occur [50]. Accordingly, this increase in glutamine could be an adaptive mechanism to alleviate the state of excitation mediated by glutamate. The present recorded increase in glycine content in the hippocampus and cortex may enhance the state of excitation as glycine potentiates NMDA receptors [51].

In our previous work [52], we observed that 72 h of paradoxical sleep deprivation induced oxidative stress as indicated by the significant increase in lipid peroxidation, and nitric oxide and decreased reduced glutathione level. These effects were more prominent in the hippocampus than the cortex which may reflect the great vulnerability of this brain area. There are a large number of animal studies that suggest a relationship between paradoxical sleep and memory [53]. PSD reduces spatial memory acquisition [54] and memory consolidation [55]. Although the mechanisms whereby sleep loss produces these effects are unknown, recent theories have proposed that the neural activity associated with waking if sustained for long periods may damage brain cells and eventually lead to cell death [56]. Cell death cascades in the brain emphasize the role of excess glutamate which can damage several cellular components by increasing the intracellular calcium and free radicals damaging cells [57].

Supporting our present findings, Dash et al. [58] observed that glutamate levels were low in an awake rat if it had been asleep for several hours and high in a sleeping rat if it had been mostly awake for the previous hours. Thus, the previous sleep–wake history contributes to the absolute levels of glutamate at any given time, whereas the direction of these changes is determined by the current behavioral state.

Spontaneous EEG is an objective non-invasive measure of the dynamical activity of the brain that provides not only a local but also a global spatiotemporal description of the collective neuronal activity [59]. Quantitative EEG (QEEG; spectral analysis) can bring a new and fundamental approach to describing the electrophysiological changes in animal models. Spectral EEG analysis describes the voltage/power distribution in frequency bands under each electrode [60].

In the present study, the recording of the waking EEG following PSD aimed to investigate the reflection of the physiological changes occurring in the brain under this condition on the EEG frequency bands. While extensive studies have been done in animals to investigate the effects of sleep deprivation on the subsequent sleep recovery and rebound in animals, only few studies have addressed the changes in brain physiology accompanying sleep deprivation in waking state.

The slight non-significant variations in EEG frequency bands in control data with respect to data of the home-cage animals indicated the absence of PSD consequences in these animals, as they

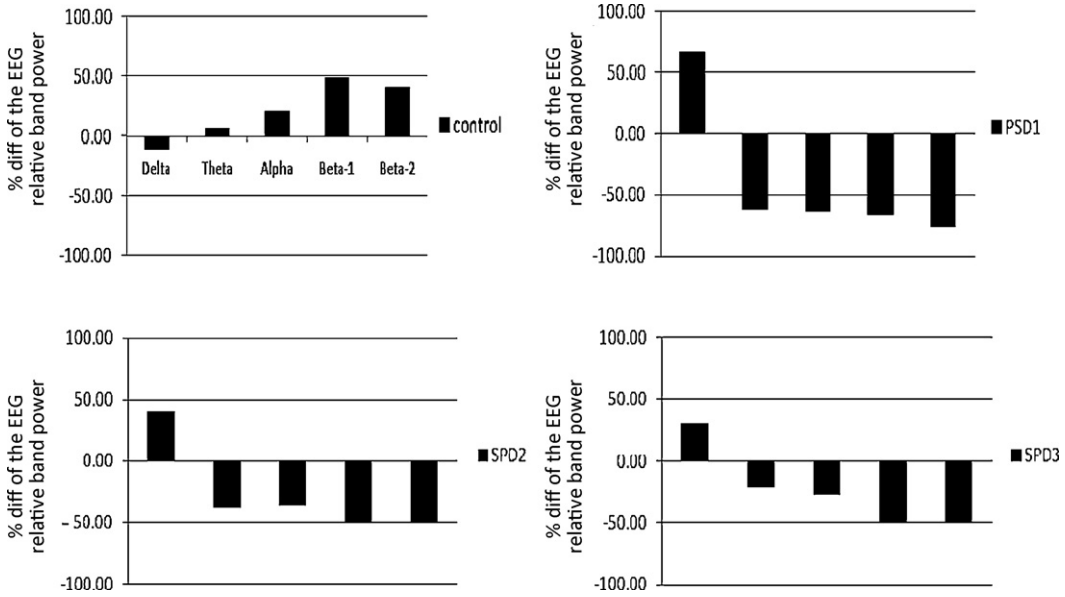

Fig. 1. The percentage difference from normal data in the EEG relative band power of control, PSD1 (after 24 h of PSD), PSD2 (after 48 h of PSD) and PSD3 (after 72 h of PSD). The bars represent the EEG frequency bands as indicated in the first chart from the left PSD (paradoxical sleep deprivation).

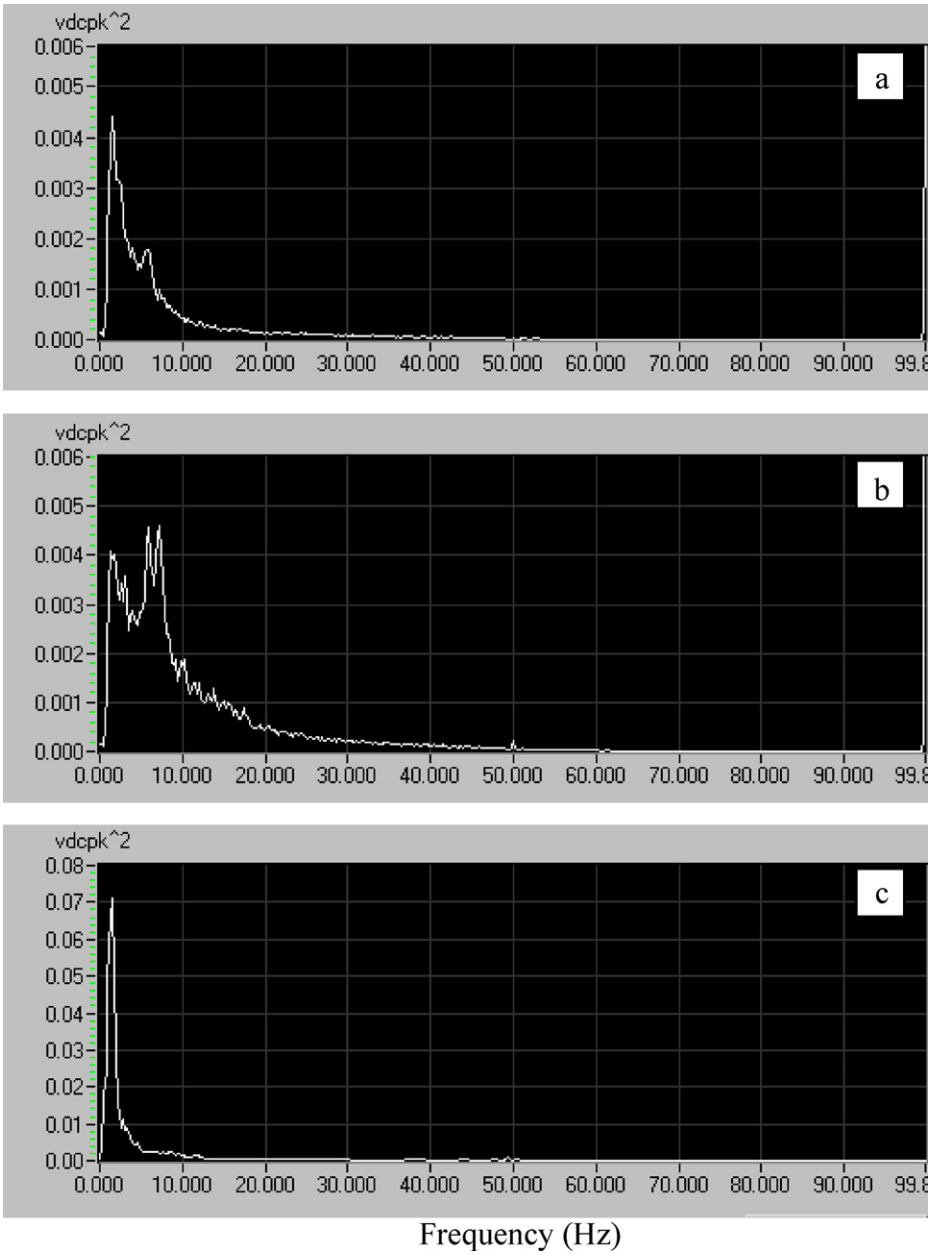

Fig. 2. The average power spectra of the normal (a), control (b) and after 24 h of sleep deprivation (c) of the same animal.

slept over a large platform. However, the increase in beta-1 and beta-2 waves suggests a kind of increase in alertness of these animals [61].

There was an increase in the power of the slow frequency delta band in the waking EEG of the PSD data after 24 h compared to control in the present study. It is well known that extended waking enhances sleep propensity, while extended sleep raises the likelihood of waking. The physiological processes underlying this sleep regulation are reflected by EEG parameters. The high sleep pressure during or after sleep deprivation may give rise to EEG synchronization in the awake animal [62] and also impressively demonstrates that a slow wave EEG pattern is compatible with the awake behavior [63]. It has been proposed that the increase of delta activity in the waking EEG during sleep deprivation reflects sleep propensity in mice [2]. Also, in humans, EEG delta power increased during a prolonged waking episode [64]. Brunner et al. [65] reported increased delta wave after four nights of sleep restriction. These waking EEG data showed a pattern of brainwave synchronization similar to what was observed in the nocturnal sleep episode.

In the present study, the simultaneous enhancement in the slow frequencies and suppression in the fast frequencies of EEG signals after 24 h could be considered as a decrease in the level of arousal and drive-related behaviors resulting from PSD. Recently, Christopher et al. [66] reported attention impairment on a choice reaction time task in rats after sleep deprivation. The effect of sleep deprivation on attention has been well documented in humans. Sleep loss in a single night leads to impairments in sustained attention tasks that require selective attention to an auditory channel [67] and psychomotor vigilance to unpredictable visual stimuli [68].

It is clear from the EEG data that the highest increase in the delta wave was recorded after 24 h of paradoxical sleep deprivation and was accompanied by a significant decrease in theta, alpha, beta-1 and beta-2 indicating decreased arousal and increased sleep pressure. This significant increase in delta frequency started to

decline gradually reaching a non-significant increase after 48 and 72 h of paradoxical sleep deprivation. Simultaneously, the recorded decrease in the other frequencies was attenuated gradually showing non-significant changes after 48 and 72 h of paradoxical sleep deprivation. In the present study, the EEG data were analyzed after 24, 48 and 72 h of paradoxical sleep deprivation, however, the neurochemical results were obtained after 72 h only.

It has been reported that paradoxical sleep is increased after learning [69], and sleep deprivation interferes with learning and memory [70]. Several studies have reported the effects of PSD on learning and memory in both human [71] and rat [72]. The investigation of the central nervous system demonstrated the significant role of the rhythmic slow brain electrical activity in the theta wave range in information processing [73]. Theta oscillations are essential for the physiological operation of the hippocampus, and abolishing those results in severe behavioral deficits [74]. The phasic patterning of neuronal activity provides spatiotemporal coding of information in the hippocampus [75]. Furthermore, theta oscillations support the compression of representations carried by neuronal spiking from the time scale of behavior into short time scales (referred to as "temporal compression") [76], which may be important for spike timing dependent plasticity [77] and for the proper temporal packaging and transfer of neuronal information [78]. The suppression of theta wave seen in the present results after 24 h of PSD may suggest a decrease in the firing activity of the theta wave generator and consequently expect a memory-deficit behavior of these animals. It has been shown that restoring theta-range rhythmicity restores hippocampal function [79]. Theta-range activity is not exclusive to the hippocampus. Many cortical [80–85] and subcortical [86–90] areas also exhibit theta-range activities. The occurrence of theta-range activity and its synchronization between the hippocampus and other parts of the brain appears to be essential for the correct expression of learned behaviors [85,88,91]. Cortico-hippocampal interactions have long been postulated to support memory formation, consolidation, and retrieval—and to involve theta-range oscillations [92].

Field potentials recorded from the rat midline cortices confirmed the existence of hippocampal theta-like activities of modest amplitude throughout the rostro-caudal axis [93]

Interestingly, there is strong evidence that the glutamate system is involved in mediating hippocampal theta activity. For instance, the removal or isolation of glutamate-containing pathways eliminates hippocampal theta activity [29]. Gallinat et al. [94] found a robust relationship between hippocampal glutamate and frontal theta activity during stimulus processing and their results suggested a functional coupling between the frontal cortex and hippocampal region during stimulus processing and supported the idea of the hippocampus as a neural rhythm generator driven by glutamatergic neurotransmission. Evidence of the influence of glutamatergic neurotransmission on theta activity was also shown in scalp-recorded human EEG, specifically in a decrease in theta activity after administration of caroverine, an agent with glutamate-antagonistic effects [95]. This follows the theory that glutamate is the major excitatory neurotransmitter in the hippocampus, mediating excitatory postsynaptic potentials [96] that are essential for EEG oscillations and evoked potentials [97].

Because the hippocampus has widespread anatomical and functional connections to all parts of the cortex [98] and possesses intrinsic oscillatory properties [29], this structure is in a key position to modulate large-scale network oscillations in the theta frequency range. Gallinet et al. [94] emphasized the role of the hippocampus as a rhythm generator acting on current generators in cortical areas, which in turn may be related to other neurotransmitter mechanisms, e.g., GABA [99].

As evident from EEG recordings and neurochemical data obtained after 72 h of sleep deprivation, it may be concluded that the increase in glutamate in both the hippocampus and cortex may lead to the initiation of the hippocampal theta rhythm which can be measured from both the cortical and subcortical structures together with the other frequency bands and consequently lead to a state of forced arousal.

In conclusion, there is electrophysiological and neurochemical evidence that sleep deprivation impairs brain function. The decreased arousal and increased sleep pressure after 24 h as indicated by the increase in delta waves may lead to inconsistent behavior, being mirrored by brief intrusions of sleepy features into the waking EEG. After 72 h of PSD, the neurochemical data of the present study showed a state of hyperexcitability mediated by the increase in the excitatory amino acid neurotransmitters and was supported by the EEG data being changed toward a decrease in slow wave activity. These changes may be responsible for the impairment of many brain functions especially those related to emotional (fear), memory and learning behaviors induced by PSD.

Conflict of interest

There is neither conflict of interest nor financial support to any of the authors of this research.

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