Original Research

Paradoxical sleep deprivation potentiates epilepsy induced by homocysteine thiolactone in adult rats

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Abstract

There is an intriguing and still poorly understood relationship between sleep deprivation and epilepsy. It has recently been shown that paradoxical sleep deprivation decreases levels of homocysteine, an amino acid involved together with its thiolactone metabolite in epileptogenesis. The aim of the present study was to investigate the effects of paradoxical sleep deprivation on homocysteine thiolactone (H)-induced seizures in rats, a model of generalized seizures. Selective deprivation of paradoxical sleep in adult male Wistar rats was achieved by the platform method. Animals with implanted electrodes for electroencephalogram (EEG) registration were assigned to appropriate experimental conditions (dry cage for control, large platform for stress control and small platform for paradoxical sleep deprivation) and 72 h later were intraperitoneally treated with either H (5.5 mmol/kg) or saline (0.9% NaCl). This study showed that paradoxical sleep deprivation increased the incidence and number of H-induced seizure episodes, shortened latency time to seizures and led to significant rates of lethality after H administration, but without effect on the seizure severity. Paradoxical sleep deprivation increased the number and duration of spikes-and-wave discharges, while decreased latency to its appearance in EEG. Judging by the behavioral and EEG findings, it could be concluded that paradoxical sleep deprivation can provoke the expression of factors that can potentiate H-induced seizures in adult rats.

Keywords: sleep, seizures, homocysteine thiolactone, EEG, rats

Experimental Biology and Medicine 2013; **238**: 77–83. DOI: 10.1258/ebm.2012.012154

Introduction

Sleep is a cyclic and vital physiological process that constitutes one-third of human life.[1] Electrophysiological studies have shown that the rapid eye movement (REM) sleep is characterized by intensive brain activity, which is similar to the awake state, while the body is relaxed, what is the reason that REM sleep is termed as paradoxical sleep.[2] It is estimated that about 20% of the world's population still suffer from insufficient sleep time; lifestyle and sleep disorders being the major causes.[3,4] The cumulative effects of sleep deprivation, usually occurring during the final phases of paradoxical sleep,[5] as well as sleep disorders, are associated with an increased risk of metabolic, cardiovascular and neurological disorders.[3]

There are several experimental techniques of paradoxical sleep deprivation with different advantages and disadvantages.[6] Most are based on the original, so-called platform method by Jouvet *et al.*,[7] successfully modified by Šušić and Marković.[8]

Deprivation of sleep is associated with hyperexcitability and could provoke seizures in susceptible individuals.[6] On the other hand, epilepsy alters the sleep architecture and interferes with the patients' daily routine.[9] Complex interplay between sleep (most important physiological state) and epilepsy (frequent pathophysiological condition of the brain) has remained of special interest for neuroscientists since it is still only poorly understood.[10,11]

Homocysteine is a sulfur-containing amino acid generated during the metabolism of methionine.[12] In recent years, a growing body of evidence suggests that homocysteine together with its reactive thioester homocysteine thiolactone (H) are neurotoxins which can significantly increase the risk for the development of numerous neurological and psychiatric disorders such as Huntington's, Alzheimer's, Parkinson's disease, depression and cognitive decline,[13,14]

including epilepsy,[15,16] as well as sleep disorders.[17,18] Stanojlovic *et al.*[19] recently showed that acute administration of H to adult rats significantly alters neuronal circuits, leading to epileptogenic activity in the electroencephalogram (EEG) with characteristic spikes-and-wave discharges (SWDs) accompanied with absence-like behavior, as well as convulsive episodes in animal behavior. Therefore, they proposed it as a suitable model of generalized epilepsy with mixture of absence and convulsive behavior. Recent studies have shown that paradoxical sleep deprivation can lower plasma homocysteine levels in adult and juvenile rats.[20,21] These findings confirmed the problem of the relationship between paradoxical sleep deprivation and the effect of homocysteine and its thiolactone in initiation and development of epileptic activity.

Therefore, the aim of this study was to examine if the paradoxical sleep deprivation has effects on behavioral and EEG manifestations of H-induced seizures in adult rats.

Materials and methods

Animals and experimental conditions

Experimentally naive adult (2 months old) Wistar rat males were included in the study. The animals were obtained from a local supplier (The Military Medical Breeding Laboratories, Belgrade, Serbia) and kept under controlled environmental conditions (ambient temperature 21–23°C, 55–65% humidity and 12/12 light/dark cycle, with light on at 08:00) for one week before the experiments. They had free access to standard laboratory chow and tap water during all experimental phases.

All experimental procedures were in full compliance with Directive of the European Parliament and of the Council (2010/63/EU) and approved by The Ethical Committee of the University of Belgrade (Permission No 298/5–2).

Surgery and electrode implantation

Anesthetized animals (intraperitoneal pentobarbital sodium, 50 mg/kg) were placed in stereotaxic apparatus and three gold-plated electrodes were implanted to the level of the leptomeninges over frontal (2 mm rostrally to bregma and 2 mm from the median line), parietal (2 mm rostrally to lambda and 2 mm laterally to median line) and occipital (2 mm caudally to lambda) cortices for surface EEG recordings, as described in detail previously.[19] One week of recovery and habituation to EEG recording set-up were allowed to animals before the further experimental procedures described as follow.

Experimental groups and method of paradoxical sleep deprivation

Selective deprivation of paradoxical sleep was achieved by the platform method according to Jouvet and modified by Šušić and Marković.[8] Briefly, animals placed on small platform (6.0 cm in diameter, platform surface/rat weight ratio is 14 cm^2/100 g body weight [b.w.]) surrounded with water 1 cm beneath the platform top would awake when muscle atonia appear during paradoxical sleep. Therefore, small platform allows slow-wave sleep (SWS), but not paradoxical sleep (group S).

The corresponding stress control group included animals on the large platform (15.0 cm in diameter, platform surface/rat weight ratio 60 cm^2/100 g b.w.) also surrounded with water, but with a larger surface allowing both SWS and paradoxical sleep (group L). All other experimental conditions were the same for both groups. Water has been changed daily; during the short period of water change animals were maintained awake manually. Cage control (group C) comprised animals maintained in dry transparent plastic cages (50 cm × 35 cm × 30 cm) separately.

The outlined experimental conditions lasted for 72 h, after which animals received either (1) H (D,L-homocysteine thiolactone, Sigma Aldrich Co, St Louis, MO, USA) in a subconvulsive dose[19] (H = 5.5 mmol/kg, intraperitoneally) for the CH, LH and SH groups ($n = 8$ in each); or (2) a saline injection (0.9% NaCl, intraperitoneally) instead of H, for the Cc, Lc and Sc groups ($n = 6$ in each group).

H was freshly dissolved in saline and after adjusting the pH to 7.0 administered in a volume of 0.1 mL/100 g rat b.w.

Behavioral recordings

The rats were observed for 90 min after H (or saline) administration for signs of convulsive behavior in dry transparent plastic cages. Convulsive behavior was assessed by the incidence of motor seizures, the number of seizure episodes per rat and their severity. The severity of episodes of seizure was determined by a modified descriptive – rating scale as previously reported[19,22] with grades defined as follow: 1 – head nodding, lower jaw twitching; 2 – myoclonic body jerks (hot plate reaction), bilateral forelimb clonus with full rearing (Kangaroo position); 3 – progression to generalized clonic convulsions followed by tonic extension of fore and hind limbs and tail; 4 – prolonged severe tonic–clonic convulsions lasting over 20 s (status epilepticus) or repeated episodes of clonic convulsions for an extended period of time (over 5 min). Seizure latency, defined as the time to the first signs of motor seizure, was also recorded. In addition, lethality was recorded at 90 min (end of observational period) and at 24 h after the H injection.

EEG registration and analysis

EEG registration was done simultaneously with observation of convulsive behavior in freely moving rats starting immediately after drug injection. For EEG registration, an 8-channel apparatus (RIZ, Zagreb, Croatia) was used with digital signal acquisition using a SCB-68 data acquisition card (National Instruments Co, Austin, TX, USA) with a sampling frequency of 512 Hz/channel. The cut-off frequencies for the EEG recordings were set at 0.3 and 100 Hz for the high-pass and low-pass filters, respectively, while 50 Hz notch filter was used to eliminate ambient noise. Data acquisition and signal processing were performed using the LabVIEW platform software (NeuroSciLaBG, Belgrade, Serbia). All EEG recordings were stored on a

disk for further offline analysis. The spectral power density (obtained by the Fast Fourier transformation, Hanning window) of the 12 s epoch was plotted, and the integrated energy signals were expressed as $\mu V^2/Hz$.

SWDs, as EEG marker of non-convulsive type of H epilepsy, were determined by the following criteria defined earlier[19]: (1) spontaneous and generalized, rhythmic 5–7 Hz discharges, (2) with typical spike-wave complex lasting more than one second and (3) having its amplitude of at least twice the background EEG activity. The number and duration of SWD were calculated during a 90-min period after the H (or saline) administration, as well as latency time to the first SWD in EEG. All SWDs were detected visually.

Data analysis

Data on the incidence of seizures and lethality were evaluated by Fisher's exact probability test. The normal distribution of the data on seizure latency, the number and severity of seizure episodes, as well as the number and duration of SWD and SWD latency time have not been estimated by the Kolmogorov–Smirnov test. Therefore, the non-parametric analyses (Kruskal–Wallis analysis of variance and Mann–Whitney *U*-test) were used to evaluate these data ($^{*}P < 0.05$ and $^{**}P < 0.01$). The results were presented as medians with 25th and 75th percentiles.

Results

Behavioral effects

After the administration of a subconvulsive dose of H (5.5 mmol/kg intraperitoneally), animals from the cage control (CH) group responded with low seizure incidence (33.3%), long seizure latency (90 [48–90] min), small number of seizure episodes per rat (0 [0–2]), while the severity of those seizures was of grade 2 maximally (Figures 1 and 2a–c).

Seizure incidence ($P > 0.05$, Figure 1), seizure latency ($P > 0.05$, Figure 2a), number of seizure episodes per rat ($P > 0.05$, Figure 2b), as well as its severity ($P > 0.05$, Figure 2c) observed in the large platform group of animals (LH, stress control) were not statistically different from these parameters observed in the CH group.

A significantly higher incidence of H-induced seizures was observed in the paradoxical sleep deprivation group (SH), compared with the CH group ($P < 0.05$, Figure 1). Moreover, seizure incidence was higher in SH (90%) compared with the LH group (50%), but not statistically significant. Latency time to the first seizure was significantly shorter in the SH rats compared with the CH ($P < 0.05$, Figure 2a). The SH group has significantly higher numbers of seizure episodes per rat compared with either the CH or LH groups ($P < 0.05$, Figure 2b). Although maximal severity of seizure episodes in the SH group was of grade 4, and in the LH and CH groups of grade 2, differences regarding this parameter were not statistically significant ($P > 0.05$, Figure 2c).

Lethal outcome was observed in more than half of the animals from the SH group (62.5%) and the difference

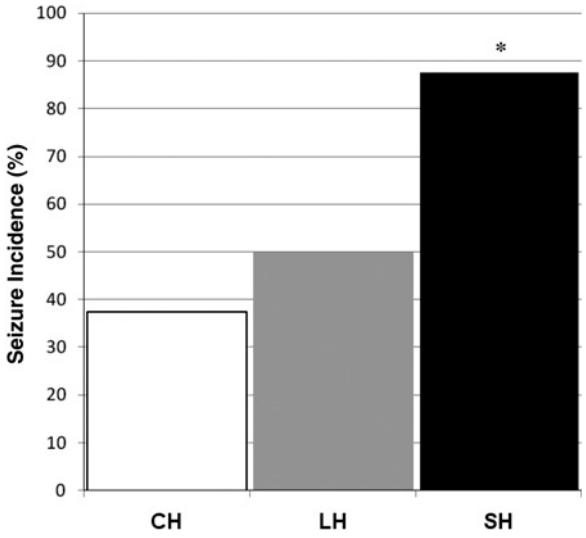

Figure 1 The influence of paradoxical sleep deprivation on the incidence of seizures induced by H in rats. Selective paradoxical sleep deprivation was achieved by platform methods. Adult male Wistar rats were assigned to appropriate experimental conditions (dry cage for control – C, large platform for stress control – L and small platform for paradoxical sleep deprivation – S) and 72 h later were intraperitoneally treated with either H (5.5 mmol/kg, CH, LH and SH groups, $n = 8$ in each) or saline (0.9% NaCl: Cc, Lc and Sc groups, $n = 6$ in each). Seizure incidence: percent of convulsing animals in group. The significance of the differences between the groups was estimated by Fisher's exact probability test ($P < 0.05$ versus CH)

observed 90 min and 24 h after the H administration was significant when compared with the CH (no lethality was recorded, $P < 0.05$) and LH groups (no lethality was recorded, $P < 0.05$) (Table 1).

Animals from the Cc, Lc and Sc groups, which received saline instead of H in otherwise equivalent conditions, showed no signs of convulsive behavior and no lethal outcome.

EEG analysis

Normal explorative behavior without EEG ictal activity was recorded in rats from the Cc, Lc and Sc groups after saline administration.

Ictal EEG activity in the form of SWD was recorded in rats from the CH, LH and SH groups after H administration (Figure 3a). The median number of SWD per rat was higher in the SH compared with the CH and LH groups ($P < 0.05$, Figure 3b). There were no differences in the median number of SWD per rat between the CH and LH. The same holds true for the duration of SWD (Figure 3c). On the other hand, the SWD latency time was decreased in the SH compared with the CH and LH group ($P < 0.05$, Figure 3d), while no differences existed between the CH and LH (Figure 3d).

DISCUSSION

This study showed that paradoxical sleep deprivation increased the incidence and number of H-induced seizure episodes per rat, as well as the number and duration of SWD in EEG. Paradoxical-sleep-deprived animals showed

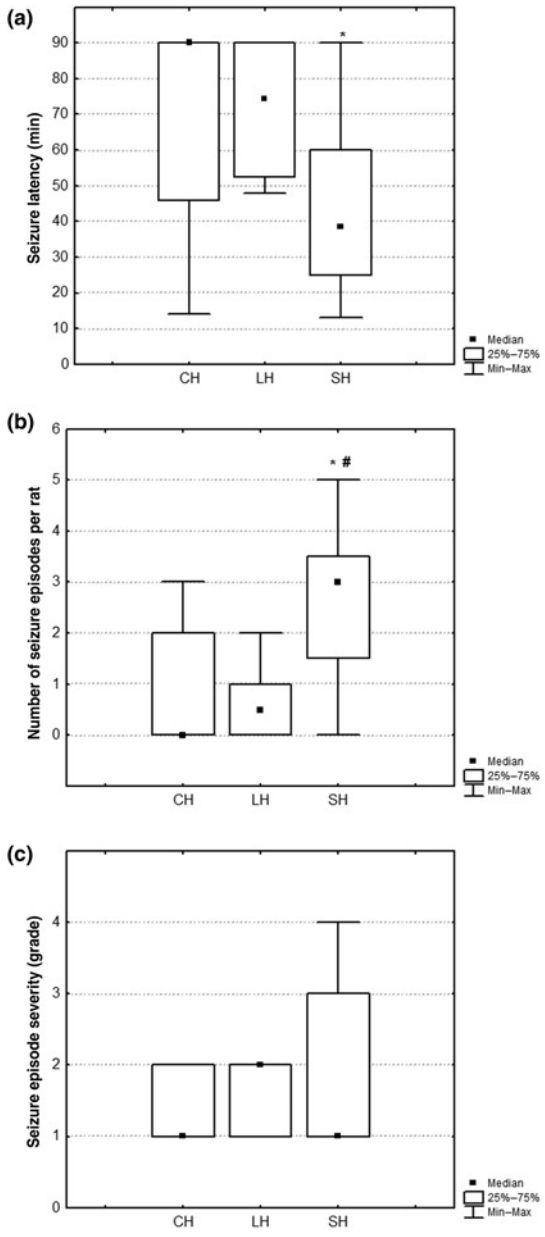

Figure 2 The effects of paradoxical sleep deprivation on median latency to the first seizure episode (a), a number of seizure episodes per rat (b) and their severity (c). Severity of seizure episodes was determined by a descriptive – rating scale with grades defined as follow: 1 – head nodding, lower jaw twitching; 2 – myoclonic body jerks (hot plate reaction), bilateral forelimb clonus with full rearing (Kangaroo position); 3 – progression to generalized clonic convulsions followed by tonic extension of fore and hind limbs and tail; 4 – prolonged severe tonic–clonic convulsions lasting over 20s (status epilepticus) or frequent repeated episodes of clonic convulsions for an extended period of time (over 5 min). The significance of the differences between the groups was estimated by Kruskal–Wallis analysis of variance and Mann-Whitney *U* test ($^{*}P < 0.05$ versus CH and $^{\#}P < 0.05$ versus LH)

shorter latency time to seizures in behavior and SWD appearance in EEG, as well as significant rate of lethality after H administration, while no significant effects were observed on the severity of seizures.

Sleep deprivation, especially paradoxical sleep deprivation, produces imbalance between excitatory and inhibitory neurotransmitters with consecutive hyperexcitabitlity.[6] On the other hand, subconvulsive dose of H do not produce major seizures and lethality.[19] The summation of these two independent processes that increase brain excitability, paradoxical sleep deprivation and H at a subconvulsive dose, resulted in the augmentation of all seizure parameters followed by lethal outcomes in this study.

Table 1 Lethality recorded in experimental groups 90 min and 24 h after H administration

	Experimental groups		
Lethality (%)	**CH**	**LH**	**SH**
After 90 min	0	0	$62.50^{*,\#}$
After 24 h	0	0	$75.00^{*,\#}$

Lethality rate of exited rats in groups expressed as percentage
Significance of the differences between the groups was estimated by Fisher's exact probability test ($^{*}P < 0.05$ versus CH and $^{\#}P < 0.05$ versus LH)
For details see caption to Figure 1

However, it was found in only two studies, that prolonged paradoxical sleep deprivation in rats led to a decrease in plasma homocysteine levels, which were not changed during the next 48 h of sleep recovery.[20,21] Sleep, as physiological process, contributes to the antioxidant defense,[23] while on the contrary, sleep deprivation causes the accumulation of free radicals. Therefore, Oliveira *et al.*[20] have suggested that this finding could be an autoregulatory response to the reduction of glutathione.

Mechanisms of the convulsive and neurotoxic effects of H are far from completely understood, but there is evidence that many excitatory effects are the result of excessive ionotropic and metabotropic glutamate receptors activation, oxidative stress, DNA damage and the induction of apoptosis,[19,24,25] as well as inhibition of Na^{+}/K^{+} ATPase activity.[26]

We investigated the functional involvement of the gaseous neurotransmitter NO in the convulsive activity of H in adult rats and found that systemic administration of L-arginine (NO precursor) reduced the incidence and number of convulsive rats in a dose-dependent manner.[27] NO achieves a neuro-protective and antioxidative role by building *S*-nitroso-L-glutathione,[28] contrary to H which increases the oxidative stress.[29] Oxidative stress increased after paradoxical sleep deprivation,[23,30] and further depleted antioxidative capacities and reduced the NO level and its anticonvulsive effects. It could be one of the possible explanations for the potentiating of H effects in the SH group of rats (paradoxical-sleep-deprived rats receiving H) observed in this study.

γ-Aminobutyric acid (GABA) system could also be a point of interaction, since there is evidence that sleep deprivation facilitates the induction of seizure by the modulation of GABA-ergic transmission.[31] Moreover, it was found that a high concentration of NO increased the GABA release.[32] In this regard, it is possible that reduction of the NO level after sleep deprivation could lead to a depression of the GABA-ergic system and consecutive hyperexcitability.

EEG is considered as a very useful tool to investigate cortical brain excitability in various conditions. Generalized SWD in EEG during absence seizures may result from aberrations of oscillatory rhythms that are normally generated during sleep by circuits connecting the cortex and thalamus. This oscillatory behavior involves an interaction between

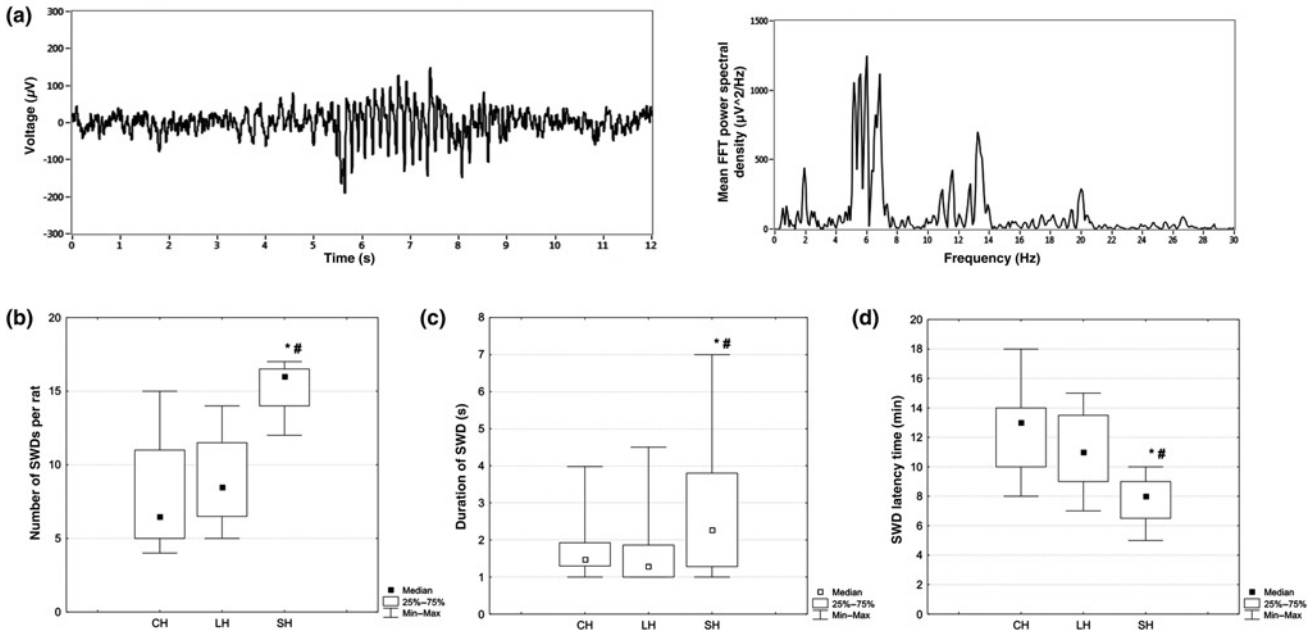

Figure 3 Representative electroencephalogram tracing (left panel) and corresponding power spectral density (right panel) recorded in rat from SH group 30 min after H administration showing spikes-and-wave discharge (SWD) as characteristic ictal pattern of H –induced absence –like seizures (a). The number (b), duration (c) and latency time to SWD (b) during 90 min after H administration in experimental groups. The significance of the differences between the groups was estimated by Kruskal–Wallis analysis of variance and Mann-Whitney *U* test (**P* < 0.05 versus CH and #*P* < 0.05 versus LH). For details see caption to Figure 1

$GABA_B$ receptors, Ca^{2+} channels and K^+ channels located within the thalamus.[33] These EEG graphoelements are clearly distinguishable from sleep spindles and are thought to be characteristic EEG manifestations of H proepileptic, absence-like activity.[19,33] In this study, we observed a significant increase in the number and duration of SWD and decrease in latency of its EEG appearance in the paradoxical-sleep-deprived rats by the administration of a subconvulsive dose of H (SH versus CH and LH). No SWD was registered in EEG of rats receiving saline instead of H in otherwise analog conditions (Cc, Lc and Sc group). These data indicate augmentation of EEG manifestations of H-induced seizures after paradoxical sleep deprivation.

It has been postulated that some of the epileptic activity of H could be attributed to the inhibition of Na^+/K^+ ATPase activity in different brain regions[26] including hippocampus. This enzyme is thought to be one of the major regulators of neuron excitability. Results of a recently reported study showed that paradoxical sleep deprivation also inhibits Na^+/K^+ ATPase activity in the brain cortex and predominantly the hippocampus through mechanisms involving oxidative stress.[30]

Behavioral alterations after paradoxical sleep deprivation are mostly a result of an imbalance in the neurotransmitter systems,[5] with key changes in: (1) generalized down-regulation of muscarinic (M2 type [34]) receptors which are necessary for initiation and coordination of paradoxical sleep[35] (paradoxical sleep-on neurons belong to the cholinergic system); (2) the dopamine neuronal circuits, inducing up-regulation of postsynaptic dopamine (D2 type) receptors[36] and (3) excitatory amino acid glutamate and aspartate levels with its elevation in the cortex and hippocampus.[37] On the other hand, it has been postulated that H acts on group I of metabotropic glutamate receptors, as well as on *N*-methyl-D-aspartate receptor complex.[15,16,24] Therefore, glutamatergic neurotransmission could be pointed out as a joint site of action for both paradoxical sleep deprivation and H, what could further explain the effects of its interaction obtained in the present study.

In this study, we observed a pronounced lethality rate in the SH (paradoxical-sleep-deprived rats receiving H) group compared with no lethality in the CH and LH groups. However, it should be pointed out that lethality was not recorded in the Sc group (paradoxical-sleep-deprived rats receiving saline instead of H) and that lethality was not recorded following the H injection alone in the same dose as in this study.[19] Paradoxical sleep deprivation is accompanied by a negative energy balance[38] induced by hypercatabolism; elevation of orexin levels, which could be a reason for hyperphagy, as well as increased sympathetic activity.[39] These factors, together with other alterations, could contribute to high lethality observed in the SH group.

Although most studies on bidirectional relationship between epilepsy and sleep disorders are based on clinical trials, experimental studies are of great significance for understanding this relationship.[40] Studies in human subjects are connected with limitations like use of antiepileptic drugs, stress, co-morbidities and habits, as well as some ethical questions. It has been shown that selective paradoxical sleep deprivation can potentiate metaphit (phencyclidine analog)-induced audiogenic seizures in rats[8] and provoke EEG abnormalities.[41] These observations are in agreement with our present study.

Different types of subthreshold excitatory stimuli occurring synchronously could induce brain hyperexcitability in

the process of temporal summation. In other words, paradoxical sleep deprivation induced some changes in neural activity which upon application of a subconvulsive H dose induced synchronization of activity in a critical population of neurons which triggered seizures. These individual components did not cause lethality, but together they led to a lethal outcome. Therefore, judging by the behavioral and EEG findings, it could be concluded that paradoxical sleep deprivation can provoke the expression of hyperexcitablity in neuronal circuits that can potentiate effects of subconvulsive H dose in adult rats.

Findings of this study revealed that paradoxical sleep deprivation augments process of epileptogenesis induced by H, which is risk factor for development of numerous disorders such as neurodegenerative disease, depression and cognitive decline.

Author contributions: All authors participated in the design of experiments and interpretation and analysis of the results. DH, AR-M and OS conducted the experiments. DH and OS wrote the manuscript.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education and Science of Serbia (grant no. 175032).

REFERENCES

1 Martins RC, Andersen ML, Tufik S. The reciprocal interaction between sleep and type 2 diabetes mellitus: facts and perspectives. *Braz J Med Biol Res* 2008;**41**:180–7
2 Jouvet M. Paradoxical sleep as a programming system. *J Sleep Res* 1998;**7**:1–5
3 Strine TW, Chapman DP. Associations of frequent sleep insufficiency with health-related quality of life and health behaviors. *Sleep Med* 2005;**6**:23–7
4 Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB. Physiological correlates of prolonged sleep deprivation in rats. *Science* 1983;**221**:182–4
5 Tufik S, Andersen ML, Bittencourt LR, Mello MT. Paradoxical sleep deprivation: neurochemical, hormonal and behavioral alterations. Evidence from 30 years of research. *An Acad Bras Cienc* 2009; **81**:521–38
6 Mallick BN, Singh A. REM sleep loss increases brain excitability: role of noradrenaline and its mechanism of action. *Sleep Med Rev* 2011;**15**:165–78
7 Jouvet D, Vilmont P, Delorme F, Jouvet M. Etude de la privation selective de la phase paradoxale de sommeil chez le chat. *C R Soc Biol Fil* 1964;**158**:756–9
8 Šušić V, Marković O. Potentiation of metaphite-induced audiogenic seizures bz REM sleep deprivation in rats. *Physiol Behav* 1993;**54**:331–8
9 Bazil CW, Walczak TS. Effects of sleep and sleep stage on epileptic and nonepileptic seizures. *Epilepsia* 1997;**38**:56–62
10 Malow B. Sleep and epilepsy. *Neurol Clin* 1996;**14**:765–89
11 Matos G, Tufik S, Scorza FA, Cavalheiro EA, Andersen ML. Sleep, epilepsy and translational research: what can we learn from the laboratory bench? *Prog Neurobiol* 2011;**95**:396–405
12 Hoffer LJ. Homocysteine remethylation and trans-sulfuration. *Metabolism* 2004;**53**:1480–3
13 Sachdev PS. Homocysteine and brain atrophy. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;**29**:1152–61
14 Kado DM, Karlamangla AS, Selhub J. Homocysteine versus the vitamins folate, B12 and B6 as predictors of cognitive function in older high functioning adults: Mac Arthur studies of successful aging. *Am J Med* 2005;**118**:161–7
15 Herrmann W, Obeid R. Homocysteine: a biomarker in neurodegenerative diseases. *Clin Chem Lab Med* 2011; **49**:435–41
16 Folbergrova J. Anticonvulsant action of both NMDA and non-NMDA receptor antagonists against seizure induced by homocysteine in immature rats. *Exper Neurol* 1997;**145**:442–50
17 Manni R, Terzaghi M, Zambrelli E. REM sleep behaviour disorder in elderly subjects with epilepsy: frequency and clinical aspects of the comorbidity. *Epilepsy Res* 2007;**77**:128–33
18 Kokturk O, Ciftci TU, Mollarecep E, Ciftci B. Serum homocysteine levels and cardiovascular morbidity in obstructive sleep apnea syndrome. *Respir Med* 2006;**100**:536–41
19 Stanojlovic O, Rasic A, Hrncic D, Susic V, Macut D, Radosavljevic T, Djuric D. Two types of seizures in homocysteine thiolactone-treated adult rats, behavioral and electroencephalographic study. *Cell Mol Neurobiol* 2009;**29**:329–39
20 Oliveira AC, D'Almeida V, Hipólide DC, Nobrega JN, Tufik S. Sleep deprivation reduces total plasma homocysteine levels in rats. *Can J Physiol Pharmacol* 2002;**80**:193–7
21 Andersen ML, Martins PJF, D'Almeida V, Santos RF, Bignotto M, Tufik S. Effects of paradoxical sleep deprivation on blood parameters associated with cardiovascular risk in aged rats. *Experimental Gerontol* 2004;**39**:817–24
22 Fischer W, Kittner H. Influence of ethanol on the pentylenetetrazole-induced kindling in rats. *J Neural Transm* 1998;**105**:1129–42
23 Reimund E. The free radical flux theory of sleep. *Med Hypotheses* 1994;**43**:231–3
24 Troen AM. The central nervous system in animal models of hyperhomocysteinemia. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;**29**:1140–51
25 Djuric D, Jakovljevic V, Rasic-Markovic A, Djuric A, Stanojlovic O. Homocysteine, folic acid and coronary artery disease: possible impact on prognosis and therapy. *Indian J Chest Dis Allied Sci* 2008; **50**:39–48
26 Rasic-Markovic A, Stanojlovic O, Hrncic D, Krstic D, Colovic M, Susic V, Radosavljevic T, Djuric D. The activity of erythrocyte and brain Na^+/K^+ and $Mg2^+$-ATPases in rats subjected to acute homocysteine and homocysteine thiolactone administration. *Mol Cell Biochem* 2009;**327**:39–45
27 Hrncic D, Rasic-Markovic A, Krstic D, Macut D, Djuric D, Stanojlovic O. The role of nitric oxide in homocysteine thiolactone-induced seizures in adult rats. *Cell Mol Neurobiol* 2010;**30**:219–31
28 Rauhala P, Lin AM, Chiueh CC. Neuroprotection by S nitrosoglutathione of brain dopamine neurons from oxidative stress. *FASEB J* 1998;**12**:165–73
29 Ramakrishnan S, Sulochana KN, Lakshmi S, Selvi R, Angayarkanni N. Biochemistry of homocysteine in health and diseases. *Indian J Biochem Biophys* 2006;**43**:275–83
30 Khadrawy YA, Nour NA, Aboul Ezz HS. Effect of oxidative stress induced by paradoxical sleep deprivation on the activities of Na+, K+-ATPase and acetylcholinesterase in the cortex and hippocampus of rat. *Transl Res* 2011;**157**:100–7
31 Jones BE. Glia, adenosine, and sleep. *Neuron* 2009;**61**:156–7
32 Getting SJ, Segieth J, Ahmad S, Biggs CS, Whitton PS. Biphasic modulation of GABA release by nitric oxide in the hippocampus of freely moving rats in vivo. *Brain Res* 1996; **717**:196–9
33 Sitnikova E, van Luijtelaar G. Electroencephalographic characterization of spike-wave discharges in cortex and thalamus in WAG/Rij rats. *Epilepsia* 2007;**48**:2296–311
34 Nunes JRGP, Tufik S, Nobrega JN. Decreased muscarinic receptor binding in rat brain after paradoxical sleep deprivation: an autoradiographic study. *Brain Res* 1994;**645**:247–52
35 McCarley RW. Neurobiology of REM and NREM sleep. *Sleep Med* 2007;**8**:302–30
36 Tufik S, Troncone LRP, Braz S. Does REM sleep deprivation induce subsensitivity of presynaptic dopamine or postsynaptic acetylcholine receptors in the rat brain? *Eur J Pharmacol* 1987; **140**:215–19

37 Mohamed SH, Ezz HAS, Khadrawy AY, Noor AN. Neurochemical and electrophysiological changes induced by paradoxical sleep deprivation in rats. *Behav Brain Res* 2011;**225**:39–46

38 Everson CA. Clinical manifestations of prolonged sleep deprivation. In: Schwartz WJ, ed. *Sleep Science: Integrating Basic Research and Clinical Practice*. Vol 15, Basel: Karger, 1997;34–59

39 Pedrazzoli M, D'Almeida V, Martins PJF. Increased hypocretin-1 levels in cerebrospinalfluid after REM sleep deprivation. *Brain Res* 2004;**995**:1–6

40 Matos G, Andersen ML, de Valle AC, Tufik S. The relationship between sleep and epilepsy: evidence from clinical trials and animal models. *J Neurol Sci* 2010;**295**:1–7

41 Méndez M, Radtke RA. Interactions between sleep and epilepsy. *J Clin Neurophysiol* 2001;**18**:106–27

(Received May 7, 2012, Accepted October 2, 2012)