High dose of ethanol decreases total spectral power density in seizures induced by D,L-homocysteine thiolactone in adult rats

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Abstract. The effects of ethanol on epilepsy are very complex. Ethanol can have depressant as well as excitatory effect on different animal models of epilepsy. Systemic administration of homocysteine can trigger seizures. The aim of the present study was to examine the changes of total spectral power density after ethanol alone and together with homocysteine thiolactone in adult rats. Adult male Wistar rats were divided into following groups: 1. saline-injected, (control) C; 2. D, L-homocysteine thiolactone, H (8 mmol/kg); 3. ethanol, E ($E_{0.5}$, 0.5 g/kg; E_1 , 1 g/kg; E_2 , 2 g/kg) and 4. E ($E_{0.5}$, E_1 , and E_2) 30 min prior to H, EH ($E_{0.5}$ H, E_1 H and E_2 H). For EEG recordings three gold-plated screws were implanted into the skull. Our results demonstrate that ethanol, when applied alone, increased total EEG spectral power density of adult rats with a marked spectrum shift toward low frequency waves. In EH groups, increasing doses of ethanol exhibited a dose-dependent effect upon spectral power density. Ethanol increased EEG spectral power density in $E_{0.5}$ H and E_1 H group, comparing to the H group (p > 0.05), the maximal increase was recorded with the lowest ethanol dose applied. The highest dose of ethanol (E_2 H) significantly decreased total power spectra density, comparing to the H group. We can conclude that high doses of ethanol depressed marked increase in EEG power spectrum induced by D,L-homocysteine thiolactone.

Key words: Epilepsy — EEG — Ethanol — D,L-homocysteine thiolactone

Introduction

Seizures and epilepsy have been documented since the earliest civilizations, before much was understood about the nervous system at all. Epilepsy, defined by a state of recurrent, spontaneous seizures (Scharfman 2007), is a major health problem that affects 1–2% of the population worldwide. Epileptogenic processes have been associated with an imbalance between excitatory and inhibitory control systems in selective regions of the brain (Brailowsky and Garcia 1999). Available drugs reduce seizures in the majority of

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patients, but only 40% are free of seizures despite optimal treatment (Loscher 2002). Animal models of epilepsy had a fundamental role in our understanding of the physiological and behavioral changes associated with human epilepsy, and have led to the discovery of antiepileptic drugs that are still in use. The physiological mechanisms underlying preictal, interictal, ictal and postictal states and transitions from one state to another are far from being elucidated, that is why animal models continue to be important for many aspects surrounding epilepsy (Sarkisian 2001).

Ethanol is used as a social drug and is the second psychoactive substance most widely used in the world after caffeine. The influence of ethanol on central nervous system (CNS) depends on the dose, drinking pattern, tolerance and other factors. The effects of ethanol on epilepsy are very complex. While chronic ethanol consumption is followed by series of

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seizures during the withdrawal period, acute ethanol intake exerts mainly inhibitory effects on the CNS and is usually associated with an increase of seizure threshold (Hillbom et al. 2003). Ethanol exerts important effects on membranes, voltage-gated ion channels, second messenger systems and a variety of different neurotransmitter systems such as adenosine (Concas et al. 1996), glycine (Aguayo and Pancetti 1996), acetylcholine (Coe et al. 1996), as well as monoamines and neuropeptides (Wang et al. 1996) systems.

Two major amino acid neurotransmitter systems, GABA and excitatory amino acids (glutamate and aspartate) are affected by ethanol. Numerous *in vivo* studies showed that ethanol inhibits calcium influx through N-methyl-D-aspartate (NMDA) glutamatergic receptors (Faingold et al. 1998) and enhances the inhibitory action of GABA (Davis and Jang-Yen 2001).

Homocysteine is a sulphur-containing amino acid normally present in human plasma and its concentration ranges from 1 to 15 µmol/l. Homocysteine is as one of the most potent excitatory agent of the CNS. Available data suggests that homocysteine can be harmful to human cells because of its metabolic conversion to a reactive thioester homocysteine thiolactone. Systemic administration of homocysteine or homocysteine thiolactone can trigger seizures in animals (Folbergova et al. 2000; Stanojlovic et al. 2000) and patients with homocystinuria suffer from epileptic seizures (Sachdev 2005). The various deleterious manifestations of hyperhomocysteinemia are due to overstimulation of NMDA and group I metabotropic glutamate receptors (mGluRs), oxidative stress, DNA damage and triggering of apoptosis. Normal human plasma levels of homocysteine thiolactone vary from 0 to 34.8 nmol/l and account for 0.002-0.3% total plasma homocysteine (Jakubowski 2006).

The aim of the present study was to examine the changes of total spectral power density after ethanol alone and together with homocysteine thiolactone in adult rats.

Materials and Methods

Animals

Adult (2-months-old) male Wistar rats, weighing 180–230 g, obtained from the Military Medical Academy Breeding Laboratories, Belgrade, were used in experiments. During the experiment, the animals were kept under controlled environmental conditions ($21 \pm 2^{\circ}$ C, 50% humidity and a 12/12 h light/dark cycle with light switched on at 9 a.m.) and housed individually with free access to standard laboratory animal chow and tap water. Animals were divided into following groups:

1. saline-injected, (control) C (n = 6)

2. D,L-homocysteine thiolactone, H (8 mmol/kg, n = 6)

- 3. ethanol, E: E_{0.5} (0.5 g/kg, n = 6), E₁ (1 g/kg, n = 6), E₂ (2 g/kg, n = 6)
- 4. E (E_{0.5}, E₁, and E₂) 30 min prior to H, EH: E_{0.5}H (*n* = 6), E₁H (*n* = 6), E₂H (*n* = 6).

All the substances were applied intraperitoneally (i.p.). Each rat was used only once. D, L-homocysteine thiolactone (Sigma-Aldrich Chemical Co., USA) was dissolved in saline and after adjusting the pH to 7.0 was administered in a volume of 1 ml/100 g body weight.

All experimental procedures were in full compliance with The European Council Directive (86/609/EEC) and approved by The Ethical Committee of the University of Belgrade (Permission No. 298/5-2).

EEG

The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), placed in a stereotaxic apparatus and three gold-plated recording electrodes were implanted over frontal, parietal and occipital cortices (for details see Stanojlović et al. 2000, 2007). Animals were allowed at least 7 days recovery from the surgery and then acclimated to the recording environment for at least 24 h. During that period, animals were supervised and no epileptiform phenomena were noticed. The animals were placed in separate transparent plastic cages ($55 \times 35 \times 15$ cm), and observed for 120 min for the occurrence of specific behavior and EEG.

An 8-channel EEG apparatus (RIZ, Zagreb, Croatia) was used. The signals were digitized using a SCB-68 data acquisition card (National Instruments Co., Austin, Texas, USA). A sampling frequency of 512 Hz/channel and 16-bit A/D conversion were used for the EEG signals. The cutoff frequencies for EEG recordings were set at 0.3 Hz and 80 Hz for the high-pass and low-pass filters, respectively. Ambient noise was eliminated using a 50 Hz notch filter. Data acquisition and signal processing were performed with LabVIEW software developed in the laboratory (NeuroSciLaBG).

All EEG recordings in freely moving rats were visually monitored and screened for seizure activity and stored on disk for subsequent off-line analysis. EEG epochs containing artifacts, were rejected prior to further analysis. The observational period (120 min) was divided into eight 15 min intervals. From each interval, a 5 min period of EEG was chosen (near to the midpoint of the 15 min interval) during typical and characteristic vigilance state. After that, ten consecutive 12 s epochs of EEG were extracted and the mean total power spectrum density was calculated. Energies in different frequency bands were calculated integrating the power spectrum in these frequency windows. The fast Fourier transform method (linear detrending, Hanning window, 0.083 Hz resolution) was applied to obtain estimates of total spectral power densities ($\mu V^2/Hz$) and spectral power densities in δ (0.5–4 Hz); θ (4–7 Hz); α (7–15 Hz) and β (15–30 Hz) frequency band. Relative powers were calculated by dividing the absolute amplitude within a given frequency range by corresponding measures of total amplitude.

Data analyses

Differences in total power spectral density between the groups were compared with one-way ANOVA. For data not normally distributed, Kruskal-Wallis one-way ANOVA on Ranks and a Dunns post hoc analysis (SigmaStat, SPSS Inc., Chicago, USA) was applied.

Results

The power spectra analysis showed (Fig. 1A) that there were significant differences in the mean total power spectra densities between C and E groups. Ethanol increased mean total spectral power density 15 min and 30 min after administration, in all E groups $E_{0.5}$ (p < 0.05, p < 0.01), E_1 (p < 0.01, p < 0.01), E_2 (p < 0.01, p < 0.01), respectively, compared to the C group. The highest total power density in both time points was recorded in E_1 group and it was statistically significant (for 15 min p < 0.01, p < 0.05; and E_2 group, respectively.

There were no differences in total power spectra densities between E and C groups, 45 and 60 min after ethanol administration. However, 75 min after ethanol administration spectral power density in all E groups were significantly higher (p < 0.05) compared to the C group. The highest spectral power density was recorded in E₁ group in consecutive 90, 105 and 120 time points, and it was significantly different in comparison with C group.

EEG recording 15 min after ethanol injection displayed synchronization, decreased frequency and increased amplitude of EEG and spectral power in the δ frequency range, together with an increase in the mean voltage. Additionally, a significant decrease in the highest frequency ranges was recorded 30, 45, and 60 min (Fig. 2A) after ethanol administration. The analysis of EEG frequency bands, revealed dose-dependent increase in proportion on δ rhythm recording with a decrease in proportion of β and of α rhythm.

In another series of experiments, D, L-homocysteine thiolactone in 45 min time point caused initial decrease in total spectral power density which was significantly lower (p < 0.05) comparing to the C group (Fig. 1B). In consecutive periods 60, 75, 90, 105 and 120 min, spectral power densities in H group were significantly higher comparing to the C group (p < 0.05, p < 0.05, p < 0.05, p < 0.01, p < 0.05, respectively). The increased power density was due to epileptiform activity induced by D, L-homocysteine thiolac-



Figure 1. Time-course of EEG total spectral power densities in ethanol and control (A), D, L-homocysteine thiolactone and control (B), and ethanol 30 min prior to D, L-homocysteine thiolactone group (C). C, control group; E, ethanol groups (E_{0.5}, 0.5 g/kg; E₁, 1 g/kg; E₂, 2 g/kg); EH (E_{0.5}H ; E₁H and E₂H), E_{0.5}, E₁, and E₂ 30 min prior to H. All the substances were applied intraperitoneally (i.p.). Statistical significance was estimated by one-way ANOVA. For data not normally distributed, Kruskal-Wallis one-way ANOVA. * p < 0.05; ** p < 0.01 vs. C; * p < 0.05; ** p < 0.01 vs. E₁(A). * p < 0.05; ** p < 0.01 vs. E₁(A). * p < 0.05; ** p < 0.01 vs. E₁(A). * p < 0.05; ** p < 0.01 vs. E₁(A). * p < 0.05; ** p < 0.01 vs. E_{0.5}H; + p < 0.05; ** p < 0.01 vs. E₁(A).



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Figure 2. Representative EEG recordings and spectral power densities in the 60 min time point for ethanol and control groups (A) and for ethanol 30 min prior to D, L-homocysteine thiolactone groups (B) (see the caption for Fig. 1).

tone, with characteristic high voltage spikes and spike-wave complexes (Fig. 2B (H)).

Application of D, L-homocysteine thiolactone after ethanol, in 45 min time point, caused initial decrease in total spectral power densities in all experimental groups, although statistical significance was not attained (Fig. 1C). In the 60 min time point, lower doses of ethanol prior to homocysteine ($E_{0.5}H$, E1H groups) increased power densities, but without statistical significance. In consecutive time points 75 and 90 min, spectral power density in E_{0.5}H group decreased gradually but did not return to the basal values. The same holds true with E1H group, in 105 and 120 min, in comparison to the H group $(p < 0.01, p < 0.01), E_{0.5}H$ group (p < 0.01, p < 0.01) and E_1H group (p < 0.01, p > 0.05), respectively. On the other hand, in E₂H group we observed significantly reduced total power density in comparison with H (p < 0.01, p < 0.01), E_{0.5}H (p <0.01, p < 0.01) and $E_1H(p < 0.01, p < 0.01)$ group in 60 and 75 time point, respectively. This reduction of total spectral power density persisted until the end of the observational period; although a transient peak was detected in 105-time point, which was significantly lower in comparison with H group (p < 0.05). At the end of the observational period, spectral power density in E₂H group returned to its basal value and it was significantly lower in comparison with the H group (p < 0.01). The highest dose of ethanol (E2H group) almost completely abolished spiking activity in all time points and restored baseline-like activity (Fig. 2B (E₂H group)), while lower doses of ethanol increased the amplitude of high voltage spikes (Fig. 2B ($E_{0.5}H$ group) and Fig. 2B (E₁H group)).

Discussion

In the present study, we investigated the changes of total spectral power density after ethanol alone and together with homocysteine thiolactone in adult rats. Recording electrical activity of the brain represents a measure of both brain functions and dysfunctions. The changes in the brain spontaneous activity produced by alcohol have been widely investigated by EEG.

Our results presented here demonstrate that ethanol, when applied alone, induced changes in the total EEG spectral power density of adult rats. Characteristic changes in EEG of ethanol-treated rats were accompanied by hypnotic changes. The increase in power density was most obvious 15 and 30 min after ethanol administration, and at the very end of the observational period, e.g. 105 and 120 min after ethanol administration. Interestingly, between 45 and 60 min period there were no differences in EEG power density between E and C groups, because the hypnotic effect of ethanol in E groups overlapped with sleeping period in control rats (Fig. 1A).

The first two doses employed (0.5 and 1 g/kg), showed a dose-dependent increase, while the highest dose applied

decreased total power spectra. It is known that ethanol produces various changes in the EEG in both humans and animals, depending upon dose. High doses of ethanol induced a shift of the power spectrum to slower frequencies, whereas the effect of smaller doses depends on individual reaction. Increases in both slow and fast frequencies may be observed (Sauerland and Harper 1970). Our results presented here revealed a marked spectrum shift toward low frequency waves (Fig. 2A).

Together with the data of the others (Young et al. 1982; Ilian and Gevins 2001; Pietrzak and Czarnecka 2005, 2006), we have clearly demonstrated that EEG changes are associated with increased proportion of a low-frequency waves with a high amplitude, corresponding to δ and θ rhythm and accompanied by decreased proportion of α and β rhythm recording.

Ethanol and epilepsy are complexly interrelated and have been linked since Hippocrates. Alcohol-related changes in the CNS are thought to be mediated through the unbalance of excitation–inhibition with GABAergic activity being predominantly affected by alcohol. An important action of ethanol involves enhancement of the activation of the GABA_A receptor, which results in hyperpolarization of the neuronal membrane by opening of the Cl⁻ channel and influx of Cl⁻ ions and the resultant inhibition of the neuron, mechanism opposing epilepsy (Faingold et al. 1998).

Acute administration of ethanol inhibits NMDA-induced ion currents *in vitro* (Lovinger et al. 1989) and *in vivo* (Simson et al. 1991), Ca²⁺ influx, cyclic GMP production (Hoffman et al. 1989), neurotransmitter release and reduces NMDA-evoked neurotoxicity (Chandler et al. 1993). In contrast to NMDA receptors, ethanol potentiates the action of serotonin (5-HT) at central 5-HT3 receptors.

Animal models have played a key role in the discovery and characterization of all significant antiepileptic drugs. It is highly likely that no single model system could be useful for all types of epilepsy. Ethanol administered by different routes and doses modified convulsive activity in bicuculine (Zhuk et al. 2001), pentylenetetrazol (Fischer and Kittner 1998), maximal electroshock (Fischer 2005) and amygdalekindled (Kim 1995) models of epilepsy. Ethanol also raised the threshold to elicit an electrographic seizure (Cohen et al. 1993a,b). Some studies proved that ethanol induced susceptibility to audiogenic seizures in rats during the withdrawal syndrome (Faingold et al. 1998). This question seems to be matter of special interest, since ethanol is considered to be a high risk factor for epileptic patients.

D,L-homocysteine thiolactone induced significant increase in total EEG spectral power density 30 min after its administration (60 min time point, Fig. 1B) due to synchronized EEG activity which developed into high-amplitude spikes and waves, especially during the tonic phase of a maximal behavioral response. This is in agreement with previous results which have shown that median latency to the first seizure episode in H group was 28 (21–39) min (Stanojlovic et al. 2009). The total power density remained increased, comparing to the C, till the end of the observational period, due to convulsive and epileptic activity of D, L-homocysteine thiolactone.

Homocysteine and its oxidized forms could provoke seizures and act on NMDA ionotropic glutamate receptors together with group I mGluRs, major gates for Ca²⁺ influx and intraneuronal calcium mobilization in the presence of glycine (Zieminska et al. 2003; Sachdev 2005). Increased cytosolic Ca²⁺ concentrations affect enzyme activities and synthesis of nitric oxide, a retrograde messenger that overstimulates excitatory amino acid receptors including glutamate, to convulsive threshold (Meldrum 1994). It seems that homocysteine exerts a direct excitatory effect comparable to the action of glutamate (Wuerthele et al. 1982). Homocysteine was shown to enhance either the release or uptake of other endogenous excitatory amino acids (Folbergrova 1997). Furthermore, there is some data that homocysteine sequesters adenosine, an endogenous anti-convulsant and thereby reduces the seizure threshold (Marangos et al. 1990). Homocysteine relates to an additional important issue in the management of patients with epilepsy. Most anticonvulsants (phenytoin, carbamazepine and valproic acid) lower plasma folate levels and as a result, increases homocysteine levels significantly. These data suggest that increased homocysteine levels may be related to epileptogenesis and suboptimal control of seizures in the patients with epilepsy (Sener et al. 2006).

Interestingly, in EH groups, increasing doses of ethanol exhibited a dose-dependent effect upon spectral power density. When applied in doses 0.5 and 1 g/kg, ethanol increased total power spectra in $E_{0.5}H$ and E_1H group, comparing to the H group (p > 0.05), the maximal increase was recorded with the lowest ethanol dose applied. The highest dose of ethanol (E_2H) significantly decreased total power spectra density, comparing to the H, and all EH groups, in all time points. At the end of the observational period, spectral power densities in E_1H and E_2H group returned to its basal value, which was not the case with the $E_{0.5}H$ group (Fig. 1C).

Action of ethanol on electrographic pattern was found to be biphasic, with potentiation of epileptiform activity in one dose range and depression in another one. Low ethanol doses causing euphoria and behavioral arousal are associated with desynchronization of the EEG, decrease in the mean amplitude, and increase in the theta and alpha activity (Lucas et al. 1986; Cohen et al. 1993a; Little 1999).

Literature data (Little 1999; Cohen et al. 1993a,b), similarly to our results, demonstrated that higher ethanol doses, led to decreased frequency and increased amplitude in the EEG. All aforementioned data on EEG monitoring concerning the ethanol influence on homocysteine-induced epilepsy support the idea that acute ethanol treatment could represent one of the factors of the exogenous stabilization of brain excitability and that high doses of ethanol have depressed EEG power spectra effect.

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References

- Aguayo L. G., Pancetti F. C. (1994): Ethanol modulation of the GABA_A- and glycine-activated Cl⁻ current in cultured mouse neurons. J. Pharmac. Exp. Ther. **270**, 61–69
- Brailowsky S., Garcia O. (1990): Ethanol, GABA and epilepsy. Arch. Med. Res. **30**, 3–9
- Chandler L. J., Newsom H., Sumners C., Crews F. (1993): Chronic ethanol exposure potentiates NMDA excitotoxicity in cerebral cortical neurons. J. Neurochem. **60**, 1578–1581
- Coe I. R., Yao L. N., Diamond I., Gordon A. S. (1996): The role of protein kinase C in cellular tolerance to ethanol. J. Biol. Chem. 271, 29468–29472
- Cohen H. L., Porjesz B., Begleiter H. (1993b): Ethanol-induced alterations in electroencephalographic activity. Electroencephalogr. Clin. Neurophysiol. **86**, 368–376
- Cohen S. M., Martin D., Morrisett R. A., Wilson W. A., Swartzwelder H. S. (1993a): Proconvulsant and anticonvulsant properties of ethanol: studies of electrographic seizures *in vitro*. Brain Res. **601**, 80–87
- Concas A., Mascia M. P., Cuccheddu T., Floris S., Mostallino M.
 C., Perra C., Satta S., Biggio G. (1996): Chronic ethanol intoxication enhances [³H]CCPA binding and does not reduce A₁ adenosine receptor function in rat cerebellum. Pharmac. Biochem. Behav. 53, 249–255
- Davis K., Jang-Yen W. (2001): Role of glutamatergic and GABAergic systems in alcoholism. J. Biomed. Sci. **8**, 7–19
- Faingold C. L., Gouemo P., Riaz A. (1998): Ethanol and neurotransmitter interactions – from molecular to integrative effects. Prog. Neurobiol. 55, 509–535
- Fischer W. (2005): Influence of ethanol on the threshold for electroshock-induced seizures and electrically-evoked hippocampal afterdischarges. J. Neural. Transm. **112**, 1149–1163
- Fischer W., Kittner H. (1998): Influence of ethanol on the pentylenetetrazole-induced kindling in rats. J. Neural. Transm. **105**, 1129–1142
- Folbergová J., Haugvicová R., Mareš P. (2000): Behavioral and metabolic changes in immature rats during seizures induced by homocysteic acid: the protectie effect of NMDA and non-NMDA receptor antagonists. Exp. Neurol. **161**, 336–345
- Folbergrova J. (1997): Anticonvulsant action of both NMDA and non-NMDA receptor antagonists against seizures induced by homocysteine in immature rats. Exp. Neurol. 145, 442–450

- Hillbom M., Pieninkeroinen I., Leone M. (2003): Seizures in alcohol-dependent patients. CNS Drugs 17, 1013–1030
- Hoffman P. L., Rabe C. S., Moses F., Tabakoff B. (1989): N-methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production. J. Neurochem. 52, 1937–1940
- Ilian A. B., Gevins A. (2001): Prolonged neurophsiological effects of cumulative wine drinking. Alcohol **25**, 137–152
- Jakubowski H. (2006): Pathophysiological consequences of homocysteine excess. J. Nutr. **136**, S1741–1749
- Kim C. K., Kalynchuk L. E., Pinel J. P., Kippin T. E. (1995): Tolerance to the anticonvulsant and ataxic effects of pentobarbital: effect of an ascending-dose regimen. Pharmacol. Biochem. Behav. 52, 825–829
- Little H. J. (1999): The contribution of electrophysiology to knowledge of the acute and chronic effects of ethanol. Pharmacol. Ther. **84**, 333–353
- Loscher W. (2002): Current status and future directions in the pharmacotherapy of epilepsy. Trends Pharmacol. Sci. 23, 113–118
- Lovinger D. M., White G., Weight F. F. (1989): Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science **243**, 1721–1724
- Lucas S. E., Mendelson J. H., Benedikt R. A., Jones B. (1986): EEG alpha activity increases during transient episodes of ethanol-induced euphoria. Pharmacol. Biochem. Behav. 25, 889–895
- Marangos P. J., Lofturs T., Weisner J., Lowe T., Rossi E., Browne C. E., Gruber H. E. (1990): Adenosine modulation of homocysteine-induced seizures in mice. Epilepsia 31, 239–246
- Meldrum B. S. (1994): The role of glutamate in epilepsy and other CNS disorders. Neurology **44**, 4–23
- Pietrzak B., Czarnecka E. (2005): Effect of the combined administration of ethanol and acamprosate on rabbit EEG. Pharmacol. Rep. **57**, 61–69
- Pietrzak B., Czarnecka E. (2006): The effect of combined administration of ethanol and gabapentin on rabbit electroencephalographic activity. Basic Clin. Pharmacol. Toxicol. 99, 383–390
- Sachdev P. S. (2005): Homocysteine and brain atrophy. Prog. Neuropharmacol. Biol. Psychiatry **29**, 1152–1161
- Sarkisian M. R. (2001): Overview of the current animal models for human seizure and epileptic disorders. Epilepsy Behav. 2, 201–216

- Sauerland E. K., Harper R. M. (1970): Effects of ethanol on EEG spectra of the intact brain and isolated forebrain. Exp. Neurol. 27, 490–496
- Scharfman H. E. (2007): The neurobiology of epilepsy. Curr. Neurol. Neurosci. Rep. **4**, 348–354
- Sener U., Zorlu Y., Karaguzel O., Ozdamar O., Coker I., Topbas M. (2006): Effects of common anti-epileptic drug monotherapy on serum levels of homocysteine, vitamin B12, folic acid and vitamin B6. Seizure 15, 79–85
- Simson P. E., Criswell H. E., Johnson K. B., Hicks R. E., Breese G. R. (1991): Ethanol inhibits NMDA-evoked electrophysiological activity *in vivo*. J. Pharmacol. Exp. Ther. 257, 225–231
- Stanojlović O., Živanović D., Šušić V. (2000): N-methyl-D-aspartic acid and metaphit-induced audiogenic seizures in rat model of seizure. Pharmacol. Res. 42, 247–253
- Stanojlović O., Hrnčić D., Rašić A., Lončar-Stevanović H., Djuric D., Šušić V. (2007) Interaction of delta sleep-inducing peptide and valproate on metaphit audiogenic seizure model in rats. Cell Mol. Neurobiol. 27, 923–932
- Stanojlović O., Rašić-Marković A., Hrnčić D., Sušić V., Macut D., Radosavljević T., Djuric D. (2009): Two types of seizures in homocysteine thiolactone-treated adult rats, behavioral and electroencephalographic study. Cell. Mol. Neurobiol. 29, 329–339
- Wang Y., Jeng C. H., Lin J. C., Wang J. Y. (1996): Serotonin modulates ethanol-induced depression in cerebellar Purkinje neurons. Alcohol Clin. Exp. Res. 20, 1229–1236
- Wuerthele S. E., Yasuda R. P., Freed W. J., Hoffer B. J. (1982): The effect of local application of homocysteine on neuronal activity in the central nervous system of the rat. Life Sci. 31, 2683–2691
- Young G. A., Wolf D. L., Khazan N. (1982): Relationships between blood ethanol levels and ethanol-induced changes in cortical EEG power spectra in the rat. Neuropharmacology **21**, 721–723
- Zhuk O. V., Zinkovsky V. G., Golovenko N. Y. (2001): The pharmacodynamics of anticonvulsant and subconvulsant effects of ethanol in CBA and C57BL/6 mice. Alcohol 23, 23–58
- Zieminska E., Stafiej A., Lazarewicz J. (2003): Role of group I metabotropic glutamate receptors and NMDA receptors in homocysteine-evoked acute neurodegeneration of cultured cerebellar granule neurons. Neurochem. Int. **43**, 481–492