Indian Journal of Experimental Biology
Vol. 44, December 2006, pp. 949-954

Influence of transcranial magnetic stimulation on spike-wave discharges in a genetic model of absence epilepsy

Leonid S Godlevsky[a], Evgeniy V Kobolev[a], Egidius L J M van Luijtelaar[b], Antony M L Coenen[b], Konstantin I Stepanenko[a] & Igor V Smirnov[a]

[a]Department of Biophysics, Informatics and Medical Devices, Medical University, Odessa, Valehovsky Lane 2, Ukraine

[b]NICI, Department of Biological Psychology, Radboud Nijmegen University, BOX 9104 6500 HE Nijmegen, The Netherlands

Received 23 September 2005; revised 12 September 2006

Transcranial magnetic stimulation (TMS) impulses, (0.5 Hz, 3 impulses) were presented at threshold intensity to male WAG/Rij rats. One group received stimuli, which involved motor responses of hindlimbs, rats of the second group received sham stimulation. Electrocorticograms (ECoG) were recorded before and up to 2 hr from the moment of transcranial magnetic stimulation. It was established that such stimulation engendered a reduction of spike-wave discharge (SWD) bursts duration. This effect was most pronounced in 30 min from the moment of cessation of stimulation, when a decrease of 31.4% was noted in comparison with sham-stimulated control group. The number of bursts of spike-wave discharges was reduced, but did not reach significant difference when compared both with pre-stimulative base-line level and with sham-stimulated control rats. Bursts of spike- wave discharges restored up to pre-stimulative level in 90-150 minutes from the moment of cessation of transcranial stimulation. It can be concluded that transcranical magnetic stimulation possessed an ability to engender short- time suppression of bursts of spike-wave discharges in WAG/Rij rats.

Keywords: Absence epilepsy, Spike-wave discharges, Transcranial magnetic stimulation.

Transcranial magnetic stimulation (TMS) is a procedure in which electric activity of the brain is influenced by pulsed magnetic field. Such an influence is performed via small electric field, which in turn is produced by passing a large current through an insulated copper-wire coil placed above the scalp. Circling current induced by magnetic impulses is able to activate superficial neuronal layers and is proper for the examination of conductance in the central and peripheral nervous system [1].

Later on the meta-analysis of data on the effects of central application of TMS in depressed patients revealed its therapeutic efficacy in patients suffering from pharmacologically resistant endogenous depression[2]. The functional state of cortical inhibition may be assessed in paired TMS tests when measurements of silence period (period of inhibition), induced in the cortex by the first impulse of TMS are determined[1,3]. That is why the investigation of the intracortical inhibitory and excitatory processes became the next field of TMS clinical application. Also, such a technology permits to evaluate the net effects of different antiepileptic drugs upon excitative and inhibitory mechanisms in the brain cortex[4].

It is of worth to assume that functional involvement of inhibitory interneurons into full-time spike-wave (SW) development may be interfered with increase of TMS frequency and prevalence of excitation of cortical neurons may be expected at some "critical" value of frequency of rTMS. Such an assumption was proposed by Chen *et al.*[5] who investigated the safety of TMS. They stressed that 10 rTMS trains at 20 Hz for 1.6 sec and stimulus intensity of 110% of motor threshold were found to be safe at the inter train interval of 5 sec. However, inter-train intervals of 1 sec or less were unsafe for trains of 20 Hz for 1.6 sec and stimulus intensities higher than 100% of MT with regard to induction of seizures.

Antiepileptic parameters of TMS were identified by Tergau[6], who revealed that low-frequency repeated TMS (rTMS) (0.33 Hz, 500 impulses at 100% of the threshold of motor response) delivered for 5 consecutive days in 9 patients with drug-resistant form of focal epilepsies (two temporal, 7 extra-temporal) was followed by pronounced reduction of initially very frequent partial and secondary generalized seizures.

Phone: +48-717-89-16
E-mail: godlevsky@odmu.od.ua

In another work slow-frequency TMS (0.5 Hz, 100 impulses at 5% below of motor threshold) were given biweekly for four consecutive weeks to patients with medically refractory partial seizures due to focal cortical dysplasia[7]. The EEG was recorded for 30 min before and after the first 100 stimuli and the number of seizures during the month of stimulation was compared with that of the month before stimulation. Authors revealed that stimulation caused 70% reduction in the frequency of seizures and a 77% reduction in the frequency of interictal spikes. Hence, authors came to conclusion that slow- frequency TMS induced cortical inhibition and thus reduced seizure manifestations.

In accordance with Theodore *et al.*[8] weekly seizure frequency in 24 patients suffering from localization-related epilepsy compared for 8 weeks before and for the similar period after 1 week of 1 Hz TMS (15 min twice daily, 120% of motor threshold) was reduced by 0.045 ± 0.13 in experimental and by 0.004 ± 0.20 in sham- stimulated group. Comparison of data collected for 2 weeks after the moment of cessation of TMS revealed the corresponding reduction by 0.16 ± 0.18 in experimental and 0.01 ± 0.24 in sham- stimulated patients. Hence, authors concluded that TMS caused mild and short lived effect upon seizures.

Hence, the data are in favor for the dependence of the final effect of TMS in patients with epilepsy upon clinician manifestations on parameters of TMS (intensity with regard to motor threshold, their frequency, and general number of stimuli). But, it should be stressed as in a case of electrical stimulations (ES) of the brain[9] the type of response in epileptogenic zone, in the course of TMS may depend upon form of epilepsy as well. Namely, two experimental models—generalized absence and seizure one may be explained by opposite functional state of GABA-ergic inhibition. Thus, while absence manifestations are resulted from hyperexcitable intracortical inhibition, generalized seizures may be regarded as an outcome of breaking down of GABAergic neuronal control[10-12]. That is why ant-absence medication may be followed by strengthening of seizures precipitation and vice versa[13].

There are few experimental works on investigations of TMS effects. Thus, experimental data of Ebert and Ziemann[14] pointed on the elevation of the threshold of kindled afterdischarge induced via ES of amygdala in rats—by 55% when compared with the corresponded data in sham-stimulated kindled rats. Antiseizure effect was observed in two weeks after a single high-frequency rTMS train (20 Hz for 3 sec). Authors used 12.5 cm diameter coil, which was located tangentially to the parietal bones, and stimulus intensity were adjusted to 120 A/μs, which was above the threshold for evoking motor responses in hindlimb muscles. Godlevsky *et al.*[15] also confirmed decreasing of ES-amygdalarly induced kindled seizures after trial of TMS (0.1 Tesla, 3 Hz, 20 impulses) in comparison with the base-line seizures in the same rats.

Nevertheless, till nowdays effects of TMS upon absence SWD rhythm and corresponded behavior were not the focus of investigations. That is why the aim of the present work is to investigate effects of TMS of low frequency (0.5 Hz) upon slow-wave discharges registered in cortex of WAG/Rij rats and connected minor behavioral manifestations, regarded as and inherited form of absence epilepsy.

Materials and Methods

Animals and surgery—Male 6-7 months old WAG/Rij rats (14), were used. Animals were kept at standard conditions (23°C, 60% RH, 12 hr dark/light cycles, with standard diet and tap water given *ad libitum*). Procedures involving animals and their care conform to the university guidelines that are in compliance with international laws and policies [European Community Council Directive 86/609, OJ L 358, I, December 12, 1987; National Institute of Health *Guide for Care and Use of Laboratory Animals*, US National Research Council, 1996].

Animals were anaesthetized with Nembutal (Pentobarbital[@]) ("Ceva", France, 35 mg/kg, ip) and implanted with monopolar cortical electrodes (nichrome wires isolated till tips, 0.12 mm diam.) stereotaxically into frontal (AP=1.5; L=1.8), temporal (AP= -5.0; L=6.0) and occipital (AP= -6.0; L=2.5) zones of cortex of both hemispheres in accordance to coordinate of stereotaxic atlas[16]. Indifferent electrodes were fixed in nasal bones. Electrodes were fixed to the skull by dental cement. After surgery, all animals received gentamicin (5 mg/kg, ip) to prevent sepsis. All animals survived in postoperative period. Starting one week after surgery, the rats were daily handled and adapted to the experimental setup. In the course of such adoption 30 sec immobilization of animal head in special plastic tube was used, and TMS-characteristic clicking sounds were noted.

EEG registration and TM stimulations—The EEG signals were sampled at 256 samples/sec, and were

stored for off-line analysis. Signals were filtered with band pass set at 0.5-40 Hz. Continuous ECoG registrations were divided in 30 min length epochs, SWD were identified and number of SWD bursts and mean duration of SWD were calculated and used for statistical analysis (Fig. 1). Altogether 7 epochs (referent points) were used for comparative statistical procedures (Fig. 1). For within group comparisons, the number of SWD per 30 min was multiplied by 2.

The generated magnetic impulses ("AVIMP", FSU) were biphasic with 3.0 ms duration and with the inductivity of 1.0 Tesla at the height of impulse. Intensity of TMS, which produced motor reaction of hind limbs was identified and used for further stimulations[6]. The foremost part of a 10 cm diameter circular coil was hold tangentially to the parietal bones. Lines of magnetic field were directed in parallel to the electrodes in such a position, and all wires were disconnected from the ECoG plugs during TM stimulation, which permitted to minimize the effect of induction of currents in electrodes during TMS.

While positive correlation between slow-wave sleep and SWD is well known[17,18], and active behavior induced in the course of TMS-induced clicks, sham stimulated control group (7 animals) was affected by those characteristic clicks as well. Each rat of this group was timely immobilized for 1 min in a plastic tube with an oval opening near the parietal bones. The coil was tilted 90° away from the rat's skull and held at about 50 cm distance from the skull. Thus, effective stimulation of the brain was excluded, while characteristic sound clicks were produced[6]. Each rat received TMS (experimental group) or sham-TMS (control group) only once.

Statistical analysis—Values were expressed as mean ± SE. Comparison between indices before and after TMS in the same group was investigated with Wilcoxon Matched-pairs Singlet ranks test. The Kruskal–Wallis test was appropriate for the estimation of differences between control and experimental groups. $P<0.05$ was considered significant.

Results

Electrographic data—All rats showed SWD during the base-line period. The significant reduction of the mean duration of SWD in comparison with the pre-TMS value was seen in the first three epochs after TMS, and maximal reduction (37.5%, $P<0.05$) was registered in 30 min from the moment of cessation of TMS (Table 1). An example of SWD bursts registered in WAG/Rij rat before and in 30 min from the moment of cessation of TMS both with characteristic frequency of dischargers at 8/sec is presented in Fig. 2. The duration of SWD bursts remained reduced for 1.5 hr, a restoration of investigated index was seen at 90th and 150th min, along with a significant reduction still present at 120th min (14.0%, $P<0.05$). The number of SWD bursts was also reduced by 29.9% ($P<0.05$) at 30th min post-TMS in comparison with the pre-TMS period (Table 1). The data revealed the reduction of the average duration of SWD bursts in sham-stimulated rats in the first 15 min after TM-

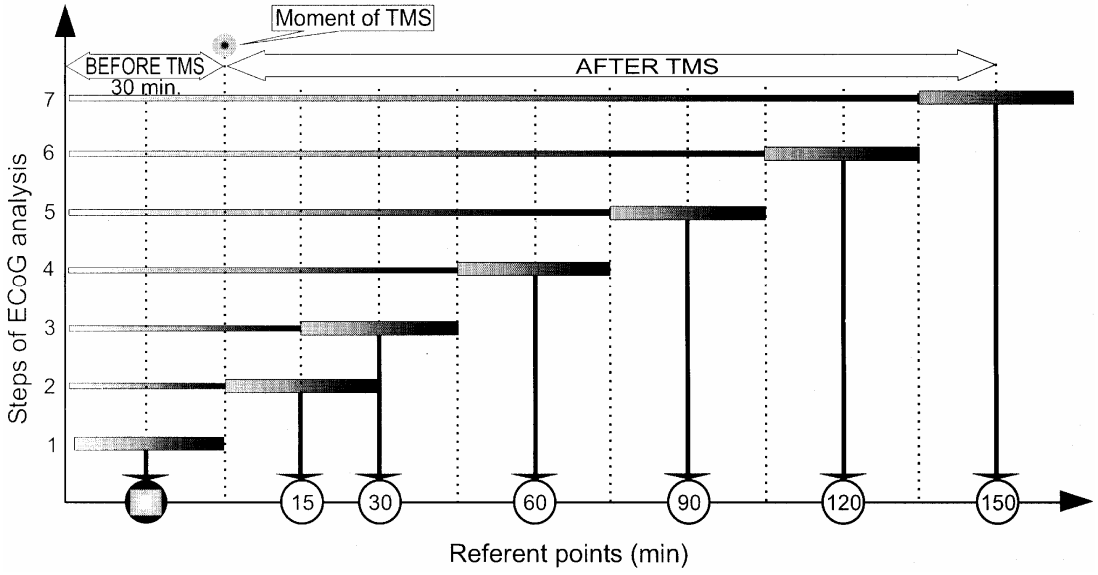

Fig. 1—Paradigm of the ECoG data collection. The moment of TMS is marked with dark circle on the abscissa.

Table 1—Effect of TMS upon duration and number of SWD bursts in WAG/Rij rats
[Values are mean ± SE from 7 rats in each group]

		Period of time (min)						
		Before TMS	15	30	60	90	120	150
Duration of SWD	Control	6.1 ± 0.6	4.8 ± 0.5*	5.2 ± 0.5	5.9 ± 0.5	6.7 ± 0.7	6.5 ± 0.6	5.9 ± 0.5
	Exp.	5.7 ± 0.4	3.7 ± 0.5*	3.6 ± 0.3*	4.1 ± 0.4*	5.6 ± 0.4	4.9 ± 0.3*	5.8 ± 0.4
Number of SWD bursts	Control	14.9 ± 1.6	14.6 ± 1.2	13.9 ± 1.5	14.9 ± 1.16	15.3 ± 1.3	16.1 ± 1.5	16.0 ± 1.5
	Exp.	16.7 ± 1.4	15.3 ± 1.5	11.7 ± 1.3*	15.0 ± 1.07	14.1 ± 1.1	16.0 ± 1.2	17.1 ± 1.1

*P<0.05 (Wilcoxon Matched-pairs Singlet ranks test), when compared with the corresponded background indices (before TMS)

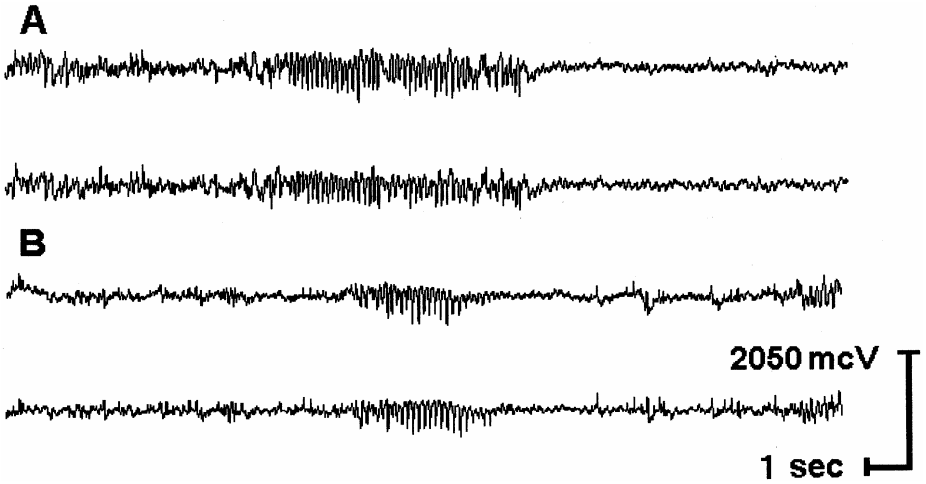

Fig. 2—Shortening of SWD bursts observed on the 30th min of post—TMS period. [A- before, and B- after TMS (3 impulses, 0.5 Hz). Upper reading-left fronto-occipital, and lower reading-right fronto-occipital leads].

sham stimulation in comparison with the base-line period. The investigated index was reduced by 20.6% (P<0.05).

The comparison between the control and experimental groups revealed the TMS-induced reduction of the duration of SWD by 31.4% (H=1.0; P=0.025) at 30th min from the moment of cessation of TMS (Table 1).

Behavioral peculiarities—Subtle motor components of hind limbs from each single impulse were observed during TMS. Sham stimulation did not result in such reactions, rats of this group showed a characteristic startle reflex, as a response to the sound clicks. All mentioned measurements were performed during active or passive wakefulness.

Minor clinical signs, characteristic for absence epilepsy manifestations in rodents were present during shortened SWD in the post-TMS period, these include cessation of locomotor activity, tremor of vibrissae, head tiltings, slight short-time breathing acceleration. All mentioned reactions coincided with electrographically registered bursts of SWD. Therefore, behavioral peculiarities pointed on the resemblance of post-TMS behavioral structures to those ones registered during base-line period.

Discussion

The present data are in favour of induction of short-term decreasing of duration of SWD bursts in WAG/Rij rats—genetic model of absence epilepsy, as a result of TMS. That effect was pronounced, when compared with sham control animals. Analogous comparison with the background absence-like epileptic activity in the same rats revealed more prolonged period of reduction of SWDs duration as well as decreasing of the number of SWD bursts. Behavioral manifestations of absence SWDs manifestations were mainly confined to characteristic "disruption" of on-going locomotions, which have been identified as "freezings"[19-21]. During such short time period animals displayed subtle tremor of vibrissae, head tiltings, and slight short-time breathing

acceleration. Total duration of freezing corresponded to the duration of SWDs. Hence, it could be concluded that TMS-caused shortening of SWDs did not abolish minor behavioral absence manifestations.

All effects were noted when rats demonstrated similar level of wakefulness thus, vigilance did not contribute to observed suppression of SWD[17,18]. Nevertheless, the role of click-activation of brain may be suspected as a mechanism of short-time decreasing of SWD duration in sham-stimulated group, when index of SWD duration was compared with that one registered in the same animals before TMS-clicks.

The duration of SWD bursts was affected more remarkably than number of SWD bursts, suggesting that mechanisms of precipitation of SWD were not influenced by TMS, while those which were in charge for the maintenance of SWD rhythm, suppressed. It is interesting to note that initiation of SWD bursts was more probable to start from cortex as far as application of penicillin to the feline cortex, but not the thalamus, was sufficient to cause typical 3–4 Hz seizures[12].

The most rodent models differ from higher mammals in having spike-wave discharges at a frequency of 6–8 Hz. This frequency more closely overlaps with the frequency range of normal thalamo-cortical oscillations in rodents, such as sleep spindles (8–11 Hz), or theta oscillations[20-23]. Nevertheless, sensitivity of SWD bursts to anti-absence medication, such as ethosuximide and valproic acid, short-time impairment of cognitive processes, decrease of responsiveness in behavioral task, and specific localization of zones of SWD bursts generation point on their "absence" seizures nature[18,19].

Keeping above in view, affection of thalamo-cortical synchronization, as a target of TMS is most probable. Estimation of the depth of excitation produced by TMS revealed that TMS-evoked response in projection area of the hind limb muscles as a depth of stimulation of more than 1 cm[24]. But, usage of TMS at some distance from the skull in such a way that only "threshold" motor responses were observed may be in favour of affecting by TMS those layers of neuronal tissue, which are most closely located to the source of impulses, namely-surface of hemisphere. Cortical motor neurons are therefore most intensively affected by circular currents, induced by impulses of magnetic field.

Therefore, TMS may affect SWD in WAG/Rij rats via functional destabilization of regular oscillations in thalamo-cortical chains at the level of cortical structures, which are most probably affected by TMS. In this case, due to TMS-induced cortical excitation, and consequent interference of induced excitation and inhibition, the cortex is less well able to compose SWD bursts, as a response to afferent inputs from thalamic nuclei. Meanwhile, it is difficult to regard the induced hyperexcitable state of cortical structures by TMS as an "antiabsence" mechanism of TMS, because of excitation of cortical neurons clearly contributes to SWD generation[10,21]. On the other hand, processes contributing to *eliciting* of SWD may be different from those involved in the control of *duration* of a SWD. It may be assumed that spreading of local excitation from cortical structures to other brain structures with consequent backward effects, induces a hypoexcitability of cortex. This mode of TMS action upon SWD may be represented by an involvement of neuronal chains, as supposed to happen in the course of "antiepileptic system" activation[25].

The present data are the first which show that TMS decreases SWD bursts in WAG/Rij rats. Taking into consideration parallel fashion of development of minor behavioral manifestation of absence-like seizure[19,21], it may be proposed that TMS possesses an efficacy against absence epileptiform activity. Meanwhile, the question of fine mapping of the zones of TMS-induced excitation, as a basis of neurophysiological consequences of the effects of TMS, is of principal interest and may comprise future line of investigations.

References

1 Anand S & Hotson J, Transcranial magnetic stimulation: neurophysiological applications and safety, *Brain Cognition*, 50 (2002) 366.

2 Fitzgerald P B, Benitez J, de Castella A, Daskalakis Z J, Brown T L, Kulkarni J A, Randomized, controlled trial of sequential bilateral repetitive transcranial magnetic stimulation for treatment-resistant depression, *Am J Psychiatry*, 163 (2006) 88.

3 Bender S, Oelkers R Ax, Weisbrod M, Does the EEG response to single pulse transcranial magnetic stimulationc represent a model for spike-wave complexes? *Epilepsia*, 46 (2005) 328.

4 Varrasi C, Civardi C, Boccagni C, Cecchin M, Vicentini R, Monaco F & Cantello R, Cortical excitability in drug-naive patients with partial epilepsy, *Neurology*, 63 (2004) 2051.

5 Chen R, Gerloff C, Classen J, Wassermann E M, Hallett M, & Cohen L G, Safety of different inter-train intervals for repetitive transcranial magnetic stimulation and recommendations for safe ranges of stimulation parameters, *Electroenceph Clin Neurophysi*, 105 (1997) 415.

6 Tergau F, Low-frequency repetitive transcranial magnetic stimulation improves intractable epilepsy, *Lancet*, 353 (1999) 2209.
7 Menkes D I & Gruental M, Slow-frequency repetitive transcranial magnetic stimulation in patient with focal cortical dysplasia, *Epilepsia*, 41 (2000) 240.
8 Theodore W H, Hunter K, Chen R, Vega-Bermudez F, Boroojerdi B, Reves-Tyer P, Werhahn K, Kelly K R & Cohen L, Transcranial magnetic stimulation for the treatment of seizures, A controlled study, *Neurology*, 59 (2002) 560.
9 Shandra A A, Godlevsky L S, Antiepileptic effects of cerebellar nucleus dentatus stimulation under different conditions of brain epileptisation, *Indian J Exp Biol*, 28 (1990) 158.
10 Gloor P, Generalized epilepsy with spike and wave discharges:reinterpretation of its electrographic and clinical manifestations, *Epilepsia*, 20 (1979) 571.
11 Kostopoulos G & Gloor P, A mechanism for spike-wave discharges in feline penicillin epilepsy and it's relationship to spindle generation, in *Sleep and epilepsy*, edited by M B Sterman, M N Shouse & P Passonaut (Academic Press, New York) 1982, 11.
12 Blumenfeld H, Cellular and Network Mechanisms of Spike-Wave Seizures, *Epilepsia*, 46 (2005) 21.
13 Fromm G H, Concepts of the neurophysiological action of antiepileptic drugs, *Am J EEG Technol*, 28 (1988) 185.
14 Ebert U & Ziemann U, Altered seizure susceptibility after highfrequency transcranial magnetic stimulation in rats, *Neurosci Lett*, 273 (1999) 155.
15 Godlevsky L S, Barnyak E M, Matsko A M, Mandel A V, Oleynik A A, Zhylinskaya A V & Brusentsov A I, The influence of transcranial magnetic stimulation on epileptiform activity in rats with amygdalar kindling, *Neurophysiologiya* (in Russian), 33 (2001) 129.
16 Paxinos G & Watson Ch, The rat brain in Stereotaxic Coordinates, Fourth ed., (Academic Press, San Diego, CA) 1988.
17 Coenen A M L, Drinkenburg W H I M, Peters B W M M, Vossen J M H & van Luijtelaar E L J M, Absence epilepsy and the level of vigilance in rats of the WAG/rij strain, *Neurosci Biobehav Rev*, 15 (1991) 259.
18 Van Luijtelaar E L J M & Coenen A M L, Two types of electrocotrical paroxysms in an inbred strain of rats, *Neurosci Lett*, 70 (1986) 393.
19 Coenen A M L & van Luijtelaar E L J M, Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats, *Behav Genet*, 33 (2003) 635.
20 Midzianovskaia I S, Kuznetsova G D, Coenen A M, Spiridonov A M & van Luijtelaar E L J M, Electro-physiological and pharmacological characteristics of two types of spike-wave discharges in WAG/Rij rats, *Brain Res*, 911 (2001) 62.
21 Meeren H K M, van Luijtelaar E L J M, Lopes de Silva F H, Berdiev R K, Chepurnova N E, Chepurnov S A & Coenen A M L, The cotico-thalamic theory for generalized spike-wave discharges, *Achievements Physiological Sciences* (in Russian), 35 (2004) 3.
22 Pinault D, Vergnes M & Marescaux C, Medium-voltage 5-9-Hz oscillations give rise to spike-and-wave discharges in a genetic model of absence epilepsy: *in vivo* dual extracellular recording of thalamic relay and reticular neurons, *Neuroscience*, 105 (2001) 181.
23 Shandra A A & Godlevsky L S, Pentylenetetrazol-induced kindling as a model of absence and convulsive forms of epilepsy, in *Kindling 6*, edited by M E Corcoran & S L Moshe (Spinger, New York) 2005, 49.
24 Rudiak D & Marg E, Finding the depth of magnetic brain stimulation: a reevaluation. *Electroenceph Clin Neuro-physiol*, 93 (1994) 358.
25 Godlevsky L S, van Luijtelaar E L J M, Shandra A A & Coenen A M L, Cause and effect relations in disease; lessons from epileptic syndromes in animals, *Med Hypotheses*, 58 (2002) 237.