ORIGINAL PAPERS

Adv Clin Exp Med 2016, **25**, 2, 317–325
DOI: 10.17219/acem/36597

ISSN 1899–5276

Volha Kistsen[1, B–D], Victor Evstigneev[2, A, F], Boris Dubovik[3, A, E], Vladimir Ulashchik[4, A, E]

The Effects of Repetitive Transcranial Magnetic Stimulation on Picrotoxin-Induced Convulsions in Mice*

[1] Department of Neurology and Neurosurgery, Belarusian Medical Academy of Post-Graduate Education, Minsk, Belarus
[2] Belarusian Medical Academy of Post-Graduate Education, Minsk, Belarus
[3] Department of Pharmacology, Belarusian State Medical University, Minsk, Belarus
[4] Institute of Physiology, National Academy of Sciences of Belarus, Minsk, Belarus

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Clinical and experimental results show that repetitive transcranial magnetic stimulation (rTMS) is effective and safe in the treatment of epilepsy. The characteristics of the impulse magnetic field (IMF) are empirical in these studies, making impossible to compare their effectiveness.
Objectives. The article presents the results of a study of the anticonvulsive effects of different modes of IMF on the picrotoxin seizure model, which is important for studying GABAergic brain mechanisms activated by rTMS.
Material and Methods.The experiments were performed on outbred male mice (n = 597; 450 in the experimental group and 147 in the control group). The picrotoxin was injected in a dose of 2.5 mg/kg subcutaneously after either rTMS or a placebo. The rTMS regimes varied in frequency (0.1, 0.3, 0.5, 1.0 and 10 Hz), intensity (10, 20 and 40% of maximal magnetic induction [MMI] of the big ring coil) and the number of procedures (1, 3, 10). The dependence of the criterial characteristics on the TMS parameters was shown in this convulsive model.
Results. The analysis of the data obtained showed that 10 rTMS sessions at an intensity of 20% of MMI in the range of frequencies from 0.5 to 1.0 Hz had the most stable and significant effect in terms of reducing the convulsive readiness of the brain, evaluated by the latent period of the myoclonuses in the picrotoxin test. In all the regimes of exposure, rTMS decreased the number of seizures in the picrotoxin model by 1.5–2 points; the most significant effect was obtained with 10 rTMS sessions in the 0.5–1.0 Hz frequency range and an intensity of 20% of the MMI ($p < 0.01$).
Conclusions. The study results support the use of IMF as therapy for blocking convulsive attacks (**Adv Clin Exp Med 2016, 25, 2, 317–325**).

Key words: repetitive transcranial magnetic stimulation, anticonvulsive effect, picrotoxin convulsions model.

Neurostimulation is gaining importance in the treatment of patients with pharmacoresistant epilepsy. Among the available neurostimulation techniques is repetitive transcranial magnetic stimulation (rTMS), a safe, noninvasive method for focal cortical stimulation [1]. rTMS induces lasting changes in cortical excitability [3]]. Repeated low-frequency stimulation (≤ 1 Hz) may induce suppression of excitatory synaptic transmission while high-frequency stimulation (> 3Hz) may potentiate it [4]. Therefore, long-term depression (LTD) and long-term potentiation (LTP)-like mecha-

* This study was carried out at the Department of Neurology and Neurosurgery of the Belarusian Medical Academy of Post-Graduate Education in the context of the research study entitled "The Regularities of the Anticonvulsive Action of Pulsed Magnetic Fields and Their Clinical Application", sponsored by the "Convergence" research program at the National Academy of Sciences of Belarus.

nisms have been considered effects of rTMS. LTD and LTP can affect both excitatory and inhibitory transmission [5].

It has been revealed that rTMS leads to a change in the distribution of some ions (sodium and calcium), and therefore affects membrane permeability [6]. Low-frequency rTMS was able to modify the levels of glutamate and GABA by upregulating GAD65 expression and downregulating NMDAR1 in the hippocampus [7].

Clinical results show that rTMS is effective and safe in the treatment of epilepsy. It can decrease seizure frequency with long-lasting effects, reduce spike generation and terminate ongoing seizures [8, 13].

A meta-analysis has shown that most antiepileptic effects occur mainly in patients with cortical dysplasia or neocortical epilepsy [14].

A series of studies have shown that low-frequency rTMS has an antiepileptic effect, and that this effect is frequency dependent [15]. It is necessary to carry out preliminary experimental investigations to find a reasonable approach to adjusting certain rTMS parameters to suit particular therapeutic aims. The anticonvulsant effect of different magnetic stimulation frequencies has been studied using different convulsive models [15–17]. A comparative analysis of different stimulation frequencies was performed only in some studies. Rotenberg et al. demonstrated that EEG-guided rTMS could suppress seizures in a kainic acid seizure model in rats in a frequency-dependent manner: 0.75 and 0.5 Hz were effective, but 0.25 Hz did not affect the seizure duration [19]. Using a pilocarpine seizure model, Huang et al. investigated a range of rTMS frequencies, and "demonstrated that pretreatment with TMS at 0.3, 0.5, 0.8, and 1.0 Hz all led to a longer latency of seizure onset, but 0.5 Hz and 0.8 Hz rTMS treatment engendered the longest latency for seizure development and conspicuous anticonvulsive effects" [18].

Data on investigating the effectiveness of different transcranial magnetic stimulation frequencies in picrotoxin-induced spasm patterns are not available in the literature. Picrotoxin is a $GABA_A$ and $GABA_C$ receptor Cl-channel antagonist. Therefore, a picrotoxin model allows the anticonvulsive possibilities of low-frequency rTMS in a GABA-deficit state to be evaluated, which is important for understanding the mechanisms of the anticonvulsive action of rTMS and its implementation in clinical practice.

The aim of this study was to investigate the mechanisms of the anticonvulsant action of rTMS on picrotoxin-induced seizures. In this paper the effects of different parameters of rTMS on picrotoxin-induced spasms are presented. The selection of the impulse magnetic field (IMF) frequency was based on an analysis of the results from a number of investigators and the authors' own experimental data. The rTMS frequencies used were 0.1, 0.3, 0.5, 1.0 and 10.0 Hz.

As Kammer et al. wrote: "In order to reach the stimulation threshold of neurons, a total field of 30–100 V/meter is needed" [20]. The selection of rTMS intensity was based on mathematic modeling results which showed that the total electrical field had subthreshold, threshold and overthreshold values when the circular coil was used with 10%, 20% and 40% of maximal magnetic induction (MMI), respectively.

Material and Methods

The Experimental Animals

The experiments were carried out on outbred sexually mature male white mice (n = 597) with a body mass of 24.0 ± 0.3 g according to a protocol for experiments developed by the authors investigating the anticonvulsive action of impulse magnetic fields in pentylenetetrazole and picrotoxin tests. The experiments were performed in accordance with all applicable national and international guidelines.

The mice were randomly divided into a control group and an experimental group. The experimental group (n = 450) was divided into 45 subgroups depending on the frequency (0.1, 0.3, 0.5, 1.0 and 10.0 Hz) and the intensity (10%, 20% and 40%) of the MMI of the ring coil, 0.125, 0.25 and 0.5 T, respectively) of the impulse magnetic field. The rTMS procedure was performed every day at the same time of the day for one, three and ten days.

The rTMS Procedure

A Neuro-MS magnetic stimulator (Neurosoft, Ivanovo, Russia) was used for the rTMS procedure. The circular coil with a diameter of 150 mm and 1.2 T MMI was used. Transcranial magnetic stimulation was performed for 5 min through the impulse magnetic field with bi-phase impulses of 250 μs width and a burst duration of 10 sec. A spectrum of frequencies (0.1, 0.3, 0.5, 1.0 and 10.0 Hz) and intensities (10%, 20% and 40% of MMI) were used for the stimulation. A device developed by the authors was used during the procedure, immobilizing the mouse in a plastic box on a special stand and positioning the inductor so that the rTMS effect was the same for each animal. This device also enabled the authors to carry out

the daily procedures as quickly as possible (10 min per subgroup every day).

The control group comprised of 147 animals. Picrotoxin was introduced into the control animals at the same time as the experimental group. The mice in the control group were placed in containers to replicate the experience of the experimental animals, to exclude the influence of systemic stress and the effects of immobilization. These animals also heard the clicks of the discharging condenser during rTMS, but without direct exposure to IMF; this was the placebo.

Seizure Induction

Picrotoxin (Sigma-Aldrich Corp., St. Louis, MO, USA) was subcutaneously administered in dosages of 2.5 mg/kg to the experimental animals (5 min after exposure to rTMS) and to the control animals (5 min after removal from the container). The latent period, the number and the duration of convulsive episodes of different severity were then registered visually, with each animal placed in a separate container for 30 min; the maximum severity of the convulsive events was noted.

Statistical Analysis

STATISTICA 6.0 for Windows Software (StatSoft Inc., Tulsa, USA) was used for the statistical analysis. *Post-hoc* one-way ANOVA and posteriori Duncan's multiple range tests were used for the parametric data; and Kruskal-Wallis with Dunn post-test was used for the nonparametric data. Multifactor dispersion analysis was used in the further processing of the results. Fisher's criterion was applied to estimate the qualitative indices. The results are presented in Tables 1 and 2 as means ± standard deviation for the parametric methods of calculation and as the median, 25 and 75 percentiles for the nonparametric methods. P-values < 0.05 were considered statistically significant.

Results

The main index reflecting the effectiveness of rTMS, particularly its ability to improve the threshold of the readiness for convulsions, is the latent period of the first spasm manifestations. This period was 142.4 ± 1.96 sec in the control group.

In the modes of influence investigated, rTMS increased the latent period of the myoclonuses (LPM) to a highly significant degree ($p < 0.02$), which indicated its evident effect.

The increase in the LPM varies considerably with different combinations of rTMS parameters, but the reduction of convulsive cerebral excitability revealed by the LPM under the influence of rTMS is constant, which is proved by the results of the integral estimate of the dependence of the rTMS influence upon the latent period of the myoclonuses on the number of the sessions, the frequencies and the capacity of the impulses.

The data presented in Fig. 1A illustrate clearly that when we perform an integral estimate of one-, three- and 10 sessions of rTMS, they lead to a 30–60% increase in the LPM ($p < 0.001$). It is notable that procedures with 10 sessions are twice as effective as one- and three-session procedures ($p < 0.001$).

The influence of the frequency of the impulses of the magnetic field on the LPM (Fig. 1B) is also unambiguous: The latent period increases con-

Table 1. The significance tests of the influence of the variations of rTMS parameters and their combinations

	SS	Degrees	MS	F	p
Free term	21830999	1	21830999	21978.17	0.0000
Sessions	334144	2	167072	168.20	0.0000
Frequency	26013	4	6503	6.55	0.0000
% of MMI	73442	2	36721	36.97	0.0000
Sessions* Frequency	25877	8	3235	3.26	0.0013
Sessions *% of MMI	45525	4	11381	11.46	0.0000
Frequency*% of MMI	38548	8	4819	4.85	0.0000
Sessions* frequency*% of MMI	125435	16	7840	7.89	0.0000
Error	402288	405	993		

Reliable levels of significance are displayed in bold ($p < 0.05$ from the multifactor dispersion analysis); Rtms – repetitive transcranial magnetic stimulation; MMI – maximal magnetic induction.

Table 2. The frequency of the seizure prevention with the development of the lateral position (%) after the one-, three- and ten-fold impacts of the rTSM in different models in mice

Groups	Parameters, Hz/% of MMI	1-fold rTMS		3-fold rTMS		10-fold rTMS	
		%	± Sx	%	± Sx	%	± Sx
1 (control)	0/0	0	0.0	0	0.0	0	0.0
2	10/40	80*	13.3	30*	15.3	90*	10.0
3	10/20	30*	15.3	10	10.0	70*	15.3
4	10/10	40*	16.3	60*	16.3	50*	16.7
5	1/40	10	10.0	20	13.3	80*	13.3
6	1/20	10	10.0	20	13.3	30*	15.3
7	1/10	10	10.0	70*	15.3	50*	16.7
8	0.5/40	50*	16.7	0	0.0	40*	16.3
9	0.5/20	30*	15.3	20	13.3	50*	16.7
10	0.5/10	40*	16.3	90*	10.0	30*	15.3
11	0.3/40	10	10.0	10	10.0	20	13.3
12	0.3/20	60*	16.3	30*	15.3	40*	16.3
13	0.3/10	0	0.0	10	10.0	20	13.3
14	0.1/40	50*	16.7	10	10.0	80*	13.3
15	0.1/20	0	0.0	90*	10.0	50*	16.7
16	0.1/10	50*	16.7	30*	15.3	30*	15.3

The asterisk (*) indicates $p < 0.05$ in comparison with the control group by Duncan's rank a posteriori criterion. There were 10 animals in each experimental group; there were 147 animals in the control group; rTMS – repetitive transcranial magnetic stimulation; MMI – maximal magnetic induction.

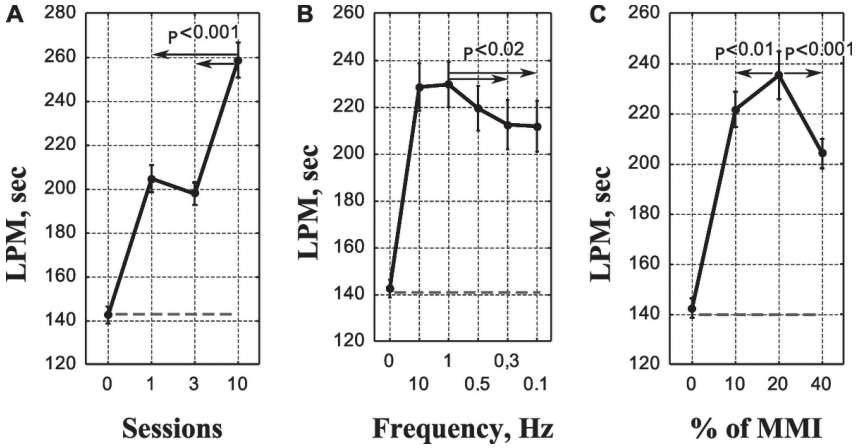

Fig. 1. The integral effects of the rTMS parameters – the number of sessions (A), the frequency of the impulses (B) and the capacity of the impulses (C) – on the latent period of the development of the myoclonuses (LPM) in picrotoxin tests in mice

The vertical lines are 95% confidence intervals. The control level is shown by a dotted line. The level of the statistical significance of the differences in comparison with the control (p, Duncan's criterion) in all the variants of the rTMS parameters presented in the picture is < 0.0001. rTMS – repetitive transcranial magnetic stimulation; MMI – maximal magnetic induction.

siderably at all the tested frequencies ($p < 0.001$), but at the frequencies of 1 and 10 Hz the increase is the greatest in comparison with other frequencies ($p < 0.02$).

The effects on the LPM of the capacity of the impulses in the range from 10% to 40% of MMI (Fig. 1C) differed from one another significantly in the presence of the high statistical confi-

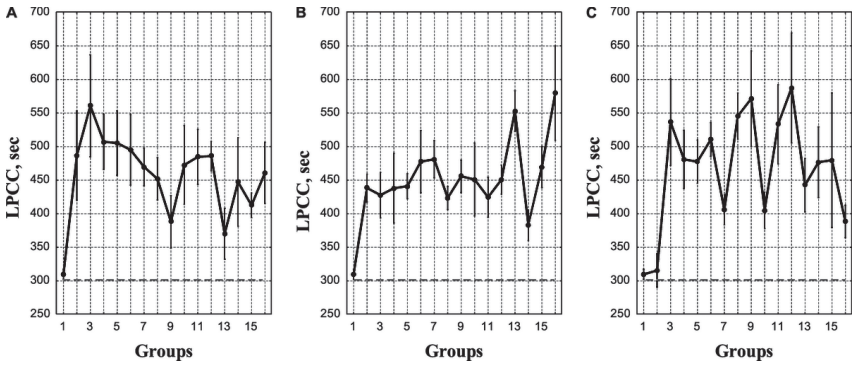

Fig. 2. The latent period of the development of the clonic convulsions (LPCC) in mice in picrotoxin tests after different modes of rTMS; A – one-fold rTMS; B – three-fold rTMS; C – ten-fold rTMS. 1 – control; 2 – 10 Hz, 40% of MMI; 3 – 10 Hz, 20% of MMI; 4 – 10 Hz, 10% of MMI; 5 – 1 Hz, 40% of MMI; 6 – 1 Hz, 20% of MMI; 7 – 1 Hz, 10% of MMI; 8 – 0.5 Hz, 40% of MMI; 9 – 0.5 Hz, 20% of MMI; 10 – 0.5 Hz, 10% of MMI; 11 – 0.3 Hz, 40% of MMI; 12 – 0.3 Hz, 20% of MMI; 13 – 0.3 Hz, 10% of MMI; 14 – 0.1 Hz, 40% of MMI; 15 – 0.1 Hz, 20% of MMI; 16 – 0.1 Hz, 10% of MMI

Vertical lines – 95% confidence intervals; the control level is shown by a dotted line; rTMS – repetitive transcranial magnetic stimulation; MMI – maximal magnetic induction.

dence of the influence of this parameter in general ($p < 0.001$). The intensity of 20% of MMI was the most effective ($p < 0.01$).

The multifactor dispersion analysis helped to determine that the variations of all the parameters of influence and their combinations were reliably significant in implementing the rTMS inhibitory effects on the convulsive cerebral readiness in mice, evaluated by the latent period of the myoclonuses (Table 1). Therefore, the anticonvulsive effect of rTMS in the given model is a result of the combined influence of the frequency of the pulsed stimulation, the capacity of the impulses and the number of rTMS sessions. If these parameters are varied, the effectiveness of rTMS will be changed significantly.

Thus, the results obtained received in the picrotoxin test by the criterion of the delayed development of myoclonuses confirm that the anticonvulsive effect of rTMS is a universal phenomenon. The analysis of the data shows that 10 sessions of rTMS at an intensity of 20% of MMI in the range of frequencies from 0.5 Hz to 1.0 Hz have the most stable and the most significant effect by the criterion of the reduction of the convulsive readiness of the brain as indicated by the latent period of the myoclonuses in the picrotoxin test.

In intoxication with picrotoxin, clonic convulsions appear after the development of myoclonuses, occurring at first with increasing and then with decreasing strength and frequency. In the control group, the latent period of the development of the clonic convulsions (LPCC) was 309.0 ± 3.8 sec. Repetitive TMS increased the LPCC by 1.5–2 times in most cases, with a high level of statistical significance (Fig. 2).

The results of the integral estimate of the influence of the rTMS variables investigated (the number of sessions, the frequency and the capacity of the impulses) on the LPCC are shown in Fig. 3.

The data presented testify with a high degree of significance ($p < 0.001$) that rTMS increases LPCC regardless of the number of sessions (Fig. 3A), with the predominant effect of 10 sessions manifesting itself as a tendency ($p > 0.05$).

The effects of the stimulation frequency on LPCC (Fig. 3B) are also unambiguous: rTMS increases the LPCC considerably throughout the range of tested frequencies ($p < 0.001$), without significant differences in the efficiency of different frequencies.

IMF at the intensity of 20% of MMI was the most effective at prolonging the latent period of clonic convulsions; this parameter had a high statistical significance overall ($p < 0.001$) (Fig. 3C).

The multifactor dispersion analysis showed that variations of triple and double combinations of the stimulation parameters, as well as isolated variations of impulse intensity of the impulses, were reliably significant in the implementation of the rTMS inhibitory effects on the convulsive readiness of the brains of mice as indicated by the LPCC ($p < 0.0001$). At the same time, the influence of variations in the number of sessions and the frequencies of the impulses didn't reach the level of statistical significance ($p = 0.06$).

The results of the analysis showed that when the testing of the convulsive readiness was carried out after a short period of time after the rTMS (5–10 min), the sum effect of the repeated impacts was relatively weak in this experimental design. On the other hand, the lack of essential significance of variations in the stimulation frequency in the 0.1–10.0 Hz range indicates that the frequency effect levels out at low rTMS intensities in the capacity range of 0.12–0.48 T.

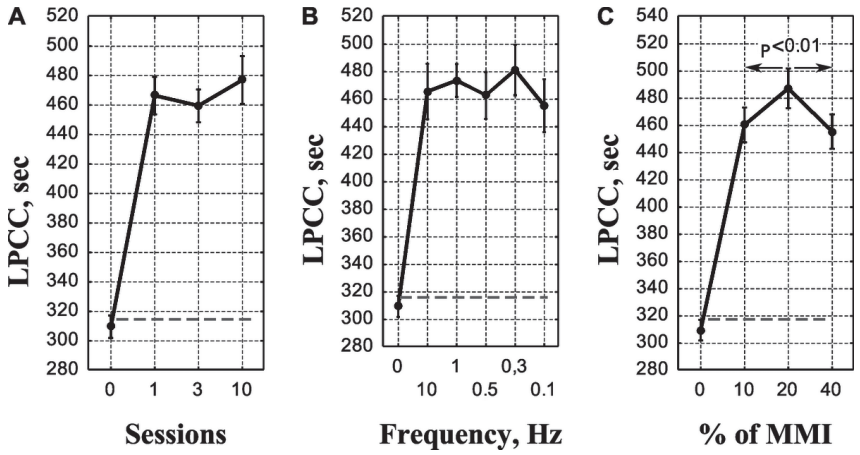

Fig. 3. The integral effects of the rTMS parameters – the number of sessions (A), frequency (B) and capacity of the impulses (C) – on the latent period of the development of the clonic convulsions (LPCC) in picrotoxin tests in mice

Vertical lines – 95% confidence intervals; the control level is shown by a dotted line. The level of statistical significance of the differences in comparison with the control (p, Duncan's criterion) in all the variants of the rTMS parameters presented in the picture is < 0.001; rTMS – repetitive transcranial magnetic stimulation; MMI – maximal magnetic induction.

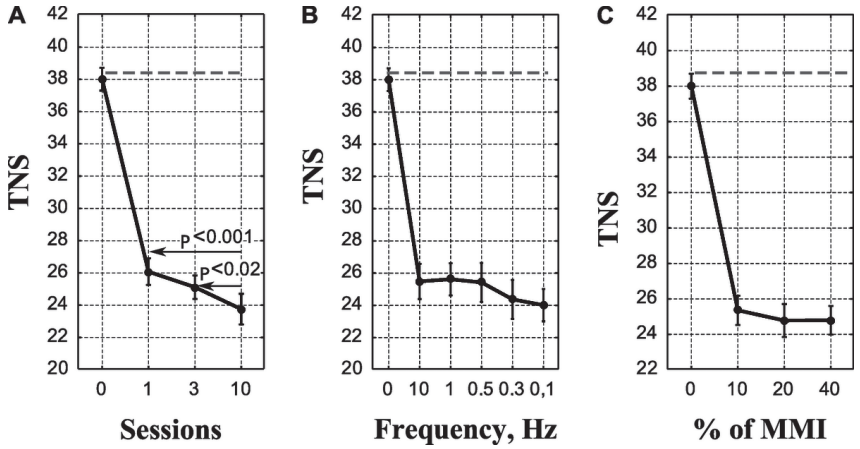

Fig. 4. The integral effects of the rTMS parameters parameters – the number of sessions (A), frequency (B) and capacity of the impulses (C) – on the total number of the seizures (TNS) in picrotoxin tests in mice

Vertical lines – 95% confidence intervals; the control level is shown by a dotted line. The level of statistical significance of the differences in comparison with the control (p, Duncan's criterion) in all the variants of the rTMS parameters presented in the picture is < 0.001; rTMS – repetitive transcranial magnetic stimulation; MMI – maximal magnetic induction.

If compared according to the speed of with which picrotoxin-induced convulsions develop, the rTMS effects are uniform and characterized by similar correlations to the parameters.

The indices characterizing the intensity of picrotoxin-induced convulsions are the frequency of the development of convulsions with a loss of orthostatic reflex (a lateral position), the total number of seizures and the total duration of a convulsive period.

The frequency with which rTMS in different modes prevented seizures of maximum severity (with a lateral position) is presented in Table 2.

The data in Table 2 show that the protection from convulsions of maximum severity varies considerably in different rTMS modes; however, the number of effective modes during 10-fold stimulation is the highest (13 out of 15), which indicates the high confidence of the presence of the rTMS anticonvulsive effect according to the criterion of Fisher's exact test ($p < 0.001$).

The results of the integral estimate of the influence of various rTMS parameters on the total number of seizures (TNS) in mice in the picrotoxin tests are presented in Fig. 4. The presented data show that the dependence of the effect of rTMS sessions on the total number of seizures is qualitatively unambiguous: one, three and ten stimulation sessions lead to a reduction in the number of seizures – on average from 38 to 24–26 (Fig. 4A) – and the effect of 10-fold stimulation is the greatest, with a high level of significance in comparison with the effects of one- and three-fold stimulation ($p < 0.02$).

The influence of the frequency of the magnetic field impulses on the number of seizures is presented in Fig. 4B: rTMS decreases the number of seizures considerably throughout the whole range of the tested frequencies ($p < 0.001$) and the effects of frequency variations are insignificant.

The effects of pulse power variations in the range of 10–40% of MMI on the total number of seizures (Fig. 4C) did not differ significantly when this parameter's influence had a high statistical significance ($p < 0.001$).

The multifactor dispersion analysis demonstrated that variations in the number of sessions, in combination with variations in the frequency and the capacity of the impulses, as well as combined variations of these three parameters were reliably significant in producing the anticonvulsive effects of rTMS as indicated by the criterion of the total number of seizures ($p < 0.003$). Isolated effects of variations in pulse frequency and power were of little importance (Table 4).

Thus, according to the most important criterion – convulsion manifestation in the picrotoxin test – the pattern due to which rTMS has an equal anticonvulsive effect at pulse powers of 0.12–0.48 T was confirmed. The ANOVA test established that according to the total number of seizures – which is the most important criterion for seizure severity – the greatest neurodepressive activity is achieved by 10 sessions of rTMS at subthreshold intensity.

The total duration of a convulsive period in the picrotoxin tests is also a very important indicator of the brain's convulsive readiness. When inhibited convulsions appear and disappear at high convulsant concentrations in target areas, that results in reducing the duration of hyperkinesis, because the pharmacokinetic curve of convulsant concentrations in the brain has an undulating form. The parameter of the total number of seizures is a direct indicator of brain convulsive excitability and the state of inhibitory mechanisms. The statistical analysis showed that the total duration of the convulsive period correlated reliably with the number of the seizures ($r = 0.67$, $p < 0.05$) and had similar deviation regularities after rTMS in different modes, including similar profiles of dependence on rTMS characteristics.

Discussion

The analysis of the data obtained in this study shows that rTMS in 10 sessions at an intensity of 20% of MMI in the range of frequencies from 0.5 to 1.0 Hz has the most stable and the most significant effect in terms of reduction of the convulsive readiness of the brain, as indicated by the latent period of the myoclonuses in the picrotoxin test. In all the modes of exposure, rTMS decreased the number of the seizures in the picrotoxin model by 1.5–2 points, with the greatest effect achieved by 10 sessions at 0.5–1.0 Hz frequency and an intensity of 20% of MMI intensity ($p < 0.01$).

Convulsive discharges occur in picrotoxin poisoning when a threshold depolarization of neurons due to the blockade of chloride channels is achieved, and the frequency of the discharges depends, on the one hand, on the value of the threshold potential at which discharge occurs (fast sodium current) and, on the other hand, on the initial polarization of the neurons (the resting potential) [21]. The first of these components is determined by the state of voltage-gated sodium channels; the second is determined by the function of brake mechanisms directed at the increase in the electronegative resting potential, regulated mainly by GABA-ergic brain mechanisms by controlling the conductivity of chloride channels.

Thus, it is evident that rTMS activates the brain's inhibitory functions, decreasing the total number of seizures and the duration of the convulsive period.

The investigations conducted allowed the authors to identify the effective modes of rTMS in terms of reduction of different manifestations of picrotoxin-induced seizures and to observe a number of specific features of the action of rTMS.

Nowadays there are some hypotheses concerning the mechanism of the anticonvulsive effects of rTMS. It is thought that rTMS has a direct modulating influence on the processes of excitation and inhibition of cortical neurons [4].

The data obtained shows that rTMS can modulate the ratio of excitation and inhibition processes in different types of cells and in local neural networks depending on the frequency and/or intensity of the impulse magnetic field, and also on the number of sessions. It can be assumed that the anticonvulsive reactions detected after one session of magnetic stimulation are connected with the selective increase in the function of the quick-responding interneurons that inhibit pyramidal cells, controlling the level and the temporal pattern of their spike activity. The same interneurons are detected in the medial septal area, the main subcortical input to the hippocampus. In the medial septal area, these interneurons are the main source of GABAergic hippocampal afferents and the targets of the action of picrotoxin [24]. In the hippocampus this class of interneurons forms reticular synaptic contacts on projecting septal neurons, controlling the activity of hippocampal pyramidal cells. Picrotoxin injected in medial septal

area reduces the convulsive threshold by blocking $GABA_A$-receptor inhibition of neurons. As a result, the pyramidal cells of the hippocampus are disinhibited. This class of interneurons, generating theta- and gamma-frequency oscillations, responds to magnetic stimulation, and it has been shown that the expression of the protein parvalbumin, which is specific for these interneurons, remains modified as much as a week after rTMS [25].

The second class of interneurons has irregular, adaptive, low-threshold volley activity and modulates dendritic integration of the input signal to pyramidal neurons. This class of interneurons is more sensitive to rTMS with a frequency of 1 Hz, and the expression of the protein calbindin, which is specific to this type of interneurons, is restored within 24 h [27].

It is possible that rTMS influence on the two types of hippocampal interneurons described above is one of the mechanisms responsible for the increase in the convulsive threshold in the picrotoxin tests and the other experimental convulsive tests carried out by the present authors.

Several research studies have investigated the neurotransmitter mechanisms of the effects of rTMSs. It has been shown that the expression of products of the early genes (c-Fos, zif268), the GABA-synthesizing enzymes (GAD65 and GAD67) and also the expression of NMDA-1 receptors in the hippocampus changed under the influence of rTMS in the neocortex of rats, which indicated the initiation of the process of synaptic plasticity [28]. The presence of immediate and long-term reactions to rTMS is confirmed by the results of the current study.

In the opinion of some authors who have studied the modulatory effects of rTMS, the reduction of the expression of the proteins that are specific to interneurons is connected with a decrease in the signals arriving from excitable systems as a result of long-term postsynaptic depression occurring during magnetic stimulation [5].

A study of the influence of rTMS on voltage-dependent ion channels showed that magnetic stimulation could change the flow rate and the distribution of sodium and calcium ions, which could affect the excitability of the membranes of neurons [26]. Moreover, the long influence of the impulse magnetic field (two weeks) reduces the expression of the components of sodium channels (SCN1A) and increases the expression of components of potassium channels (Kcal 1.1), and these effects persisted for six weeks [6]. Thus, the effect of rTMS can be influenced on the genomic level.

The present study of the anticonvulsive effects of rTMS at different frequencies, intensities and numbers of sessions in picrotoxin tests supports the presence of a universal mechanism of impulse magnetic fields to maintain the balance of inhibitory and excitable processes in the brain, aimed at arresting the development of convulsive attacks. The selective anticonvulsive effectiveness of some modes of magnetic stimulation is probably related to different mechanisms of neuronal structure that are mediated by resonance characteristics inherent to certain structures participating in epileptogenesis.

The study results support the use of IMF as therapy for blocking convulsive attacks, using a special protocol based on the experimental data acquired.

Acknowledgements. Thanks for reviewing this work at the initial stage go to Harvard Medical School researchers Alexander Rotenberg and Roman Gersner. The authors also thank Natalia Kistsen and Natalya Plotnikova, English language consultants, and Igor Doroshenko, a computer image designer, for their help with the manuscript.

References

[1] **Fitzgerald PB, Brown TL, Daskalakis ZJ:** The application of transcranial magnetic stimulation in psychiatry and neuroscience's research. J Acta Psychiatr Scand 2002, 105, 324–340.

[2] **Rossi S, Hallett M, Rossini PM, Pascual-Leone A:** Safety of TMS Consensus Group. Clin Neurophysiol 2009, 120, 2008–2039.

[3] **Bae EH, Schrader LM, Machiiet K, Alonso-Alonso M, Riviello Jr JJ, Pascual-Leone A:** Safety and tolerability of repetitive transcranial magnetic stimulation in patients with epilepsy: A review of the literature. Epilepsy Behav 2007, 10, 521–528.

[4] **Chen R, Wassermann EM, Hallet M:** Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997, 48, 1398–1403.

[5] **Funke K, Benali A:** Modulation of cortical inhibition by rTMS – findings obtained from animal models. J Physiol 2011, 589, 4423–4435.

[6] **Theodore WH:** Transcranial magnetic stimulation in epilepsy. Epilepsy Curr 2003, 3, 191–197.

[7] **Zhang JQ, Yu JM, Wang XM, Zhao HN, Huang M, Zhang XD:** Effects of pretreatment with low-frequency repetitive transcranial magnetic stimulation on expressions of hippocampus GAD65 and NMDAR-1 in rats with pilocarpine-induced seizures. Chin J Neuroimmunology and Neurology 2008, 15, 430–433.

[8] **Cantello R, Rossi S, Varassi C, Ulivelli M, Civardi C, Bartalini S:** Slow repetitive TMS for drug-resistant epilepsy: Clinical and EEG findings of a placebo-controlled trial. Epilepsia 2007, 48, 366–374.
[9] **Fregni F, Otachi PT, Do Valle A:** A randomized clinical trial of repetitive transcranial magnetic stimulation in patients with refractory epilepsy. Ann Neurol 2006, 60, 447–455.
[10] **Rotenberg A, Bae EH, Takeoka M:** Repetitive transcranial magnetic stimulation in the treatment of epilepsia partialis continua. Epilepsy Behav 2009, 14, 253–257.
[11] **Rotenberg A, Depositario-Cabacar D, Bae EH, Harini C, Pascual-Leone A, Takeoka M:** Transient suppression of seizures by repetitive transcranial magnetic stimulation in a case of Rasmussen's encephalitis. Epilepsy Behav 2008, 13, 260–262.
[12] **Sun W, Mao W, Meng X, Wang D, Qiao L, Tao W:** Low-frequency repetitive transcranial magnetic stimulation for the treatment of refractory partial epilepsy. Clin EEG and Neurosci 2011, 42, 40–44.
[13] **Fregni F, Thome-Souza S, Bermpohl F:** Antiepileptic effects of repetitive transcranial magnetic stimulation in patients with cortical malformations: An EEG and clinical study. Stereotact Funct Neurosurg 2005, 83, 57–62.
[14] **Hsu WY, Cheng CH, Lin M-W, Shih YH, Liao KK, Lin YY:** Antiepileptic effects of low frequency repetitive transcranial magnetic stimulation: A meta-analysis. Epilepsy Res 2011, 96, 231–240.
[15] **Akamatsu N, Fueta Y, Endo Y, Matsunaga K, Uozumi T, Tsuji S:** Decreased susceptibility to pentylenetetrazol-induced seizures after low-frequency transcranial magnetic stimulation in rats. Neurosci Lett 2001, 310, 153–156.
[16] **Anschel DJ, Pascual-Leone A, Holms GL:** Anti-kindling effect of slow repetitive transcranial magnetic stimulation in rats. Neurosci Lett 2003, 351, 9–12.
[17] **Godlevsky LS, Kobolev EV, Egidius van L:** Influence of transcranial magnetic stimulation on spike–wave discharges in a genetic model of absence epilepsy. Indian J Exp Biol 2006, 44, 949–954.
[18] **Huang M, Yu JM, Wang XM, Wang L:** The effects of pretreatment with low frequency transcranial magnetic stimulation on rats with pilocarpine-induced seizures. Chin J Phys Med Rehabil 2009, 3, 228–231.
[19] **Rotenberg A, Muller P, Birnbaum D, Harrinqton M, Riviello JJ, Pascual-leone A, Jensen FE:** Seizure suppression by EEG-guided repetitive transcranial magnetic stimulation in the rat. Clin Neurophysiol 2008, 119, 2697–2702.
[20] **Kammer T, Beck S, Thielscher A, Laubis-Herrmann U, Topka H:** Motor thresholds in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. Clin Neurophysiol 2001, 12, 250–258.
[21] **Newland CF, Cull-Candy SG:** On the mechanism of action of picrotoxin on GABA receptor channels in dissociated sympathetic neurones of the rat. J Physiol 1992, 447, 191–213.
[22] **Todd G, Flavel SC, Ridding MC:** Low-intensity repetitive transcranial magnetic stimulation decreases motor cortical excitability in humans. J Appl Physiol 2006, 101, 500–505.
[23] **Fitzgerald PB, Fountain S, Daskalakis ZJ:** A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 2006, 117, 2584–2596.
[24] **Freund TF:** GABAergic septal and serotonergic median raphe afferents preferentially innervate inhibitory interneurons in the hippocampus and dentate gyrus. Epilepsy Res 1992, 7, 79–91.
[25] **Freund TF:** Interneuron diversity series: Rhythm and mood in perisomatic inhibition. Trends Neurosci 2003, 28, 489–495.
[26] **Funke K, Benali A:** Cortical cellular actions of transcranial magnetic stimulation. Restor Neurol Neurosci 2010, 28, 399–417.
[27] **Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C:** Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 2004, 5, 793–807.
[28] **Benali A, Trippe J, Weiler E, Mix A, Petrasch-Parwez E, Girzalsky W, Eysel UT, Erdmann R, Funke K:** Theta-burst transcranial magnetic stimulation alters cortical inhibition. J Neurosci 2011, 31, 1193–1203.

Address for correspondence:

Volha Kistsen
Belarusian Medical Academy of Post-Graduate Education
P. Brovka 3/3
Minsk
Belarus
E-mail: kistsen@tut.by

Conflict of interest: None declared

Received: 28.10.2014
Revised: 7.01.2015
Accepted: 14.01.2015