УДК: 615.213.015.2

THE INFLEUNCE OF TRANSCRANIAL MAGNETIC STIMULATION (TMS) ON EPILEPTIFORM ACTIVITY IN RATS WITH PHARMACOLOGICAL KINDLING

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SUMMARY

TMS of low frequency (1/s, during 10 s) and relatively high induction of magnetic field at the peak of its development (1,0-1,5 Tl) prevented the precipitation of kindled generalized clonic- tonic fits and reduced the duration of epileptic discharge (25,7±2,9 s while in sham-TMS rats it was 44,5±5,2 s (P<0,05). Investigation of the total power of EEG in the course of epileptiform activity (EpA) development revealed the decrease of total power of EEG in all investigated structures in TMS- rats (by 18% in comparison with the kindled sham-TMS rats) during the initial part of EpA development (first 15 s). At the end of EpA (last 15 s) such differences were kept in hippocampus, occipital, and frontal cortical zones. Analysis of the different bandwidths contribution to the total power of EEG showed that at the moment of EpA cessation most contributive was the reduction of tetha, beta and gamma rhythms while delta rhythm was developed in counterpart to these changes. Hence, the correspondance of gauned data to that one which were received under conditions of electrical kindling are under discussion.

KEY WORDS: transcranial magnetic stimulation, pharmacological kindling, ECoG, seizures

INTRODUCTION

TMS causes effect upon excitability of neuronal populations [4, 5, 7]. The increasing of the threshold of afterdischarge induction after high–frequency (20 Hz for 3 s) TMS was shown in experiments on rats [5]. Earlier the quenching of the threshold of kindled amygdalar seizures after low- frequency (1 Hz) TMS was established [15]. Mechanisms of TMS action upon seizures/seizure susceptibility are obscure and along with the inhibition of seizures [7], and decreasing of the cortical excitability [6, 14, 15], breaking down of pair- pulse inhibition in hippocamapus [4, 5] were described.

As far as rhythmogenesis of bioelectrical activity is based on certain neurophysiological mechanisms [1, 2, 3], we decided to investigate different bandwidths of EEG defined on widely-accepted clinician basis, in rats with pharmacological kindling and to analyze effects of TMS.

Seizures might be induced as a result of TMS [16], and such an effect is connected with an additional depolartization of epileptisized neurons with relatively strong currents induced by TMS. Hence, it is reasonable to suggest that general principle of artificial stimulation of brain structures in epileptology, namely,- decreasing of the risk of facilitation along with increasing of inhibition in the course of decreasing of the intensity of high- frequency electrical stimulation (ES) [13], might be applyable to the TMS as well [7]. That is why the aim of the present work was to investigate the question if less intensive (ten times) TMS could induce antiepileptic effects in pharmacologically kindled rats.

MATERIALS AND METHODS

Male Wistar rats with a start weight of 180-250 g were used. Animals were kept at standard conditions (constant temperature-23^{o}C, and relative humidity- 60%, 12 hrs dark/light cycles, standard diet and tap water were given ad libitum). Procedures involving animals and their care were conducted in conformity with 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 ("Ceva", France, 35 mg/kg, i.p.) and implanted with monopolar electrodes (nichrome wires isolated till tips, diameter of wire- 0,12 mm) stereotaxically into the right basolateral amugdala (AP=2,2; L=4,7; H=8,5), according to the rat brain atlas [10]. Reference monopolar electrodes were also implanted into right ventral hippocampus (AP= -4,3; L= 4,5; H= 8,0), left frontal (AP=1,7; L=2,0; V=1,0) and left occipital cortex (AP=-6,3; L=3,0; H=1,0). Indifferent electrodes were fixed in nasal bones. Electrodes were fixed to the skull by dental cement. Starting one week after surgery, the rats were daily handled and adapted to the experimental setup.

Kindling was started 10-14 days after the surgery and was induced via intraperitoneal administration of picrotoxin (0,2 mg/kg) daily during 21 days. Those rats which demonstrated generalized seizures were used for further observations. Severity of seizures was evaluated according to the scale of [3].

The EEG signals were sampled at 256 samples/s, and were stored for off- line analysis.

Signals were filtered with the bandpass set at 0,5-40 Hz. Fast Fourier Transform analysis was performed on 16-s samples. The polygraph records were visually inspected and epochs containing artifacts were discarded and the total power of the EEG (μV^2) was calculated and presented as the percentage of increment or decrement of (0,5-40,0 Hz) total power over the control baseline (100%). The frequencies were grouped into the following bands: 0,5-4, 4-8, 8-12, 12-25, 25-40 Hz. The absolute power of every frequency band was determined and the relative band power of the EEG power spectrum was calculated (in percents).

The "Avimp" device was used as a generator of magnetic impulses. To minimize the effect of induction of currents in electrodes during TMS, the coil was located in such a fashion that lines of magnetic field were directed in parallel fashion to electrodes and all wires were disconnected from the plugs during TM stimulation. Therefore, the stimulation of temporo-parietal zones was achieved- distance from the coil to the skull surface was 2,5 sm. During stimulation clonic- like locomotor components resulted from single impulse were observed. TMS was performed 24 h from the moment of first clonic-tonic fit precipitation. Sham- TMS kindled rats were used as a control.

After the end of the experiments rats were anesthetized with pentobarbital sodium and perfused with paraformaldehyde.Frozen sections were cut at 32 μm, stained with Nissle method and examined under light microscope. Rats used in the data presented here had electrodes in appropriate places.

Power of bandwidth data were analyzed by means of 1-way ANOVA, followed by Newman-Keuls. Seizures were analyzed using Fisher test.

RESULTS AND DISCUSSION

Administration of picrotoxin (0,2 mg/kg) in half an hour from the moment of TMS was followed by the development of generalized clonic seizures of body muscles in 10 out of 13 rats. The rest 3 rats demonstrated rearings, serial clonic seizures of forelimbs. In control group (sham- TMS) all animals (7 rats) demonstrated generalized clonic- tonic fits with fallings and postseizure depression (P<0,025). The duration of provoked ED in sham- TMS rats was 44,5±5,2 s, while in the group with TMS it was shortened up to 25,7±2,9 s (P<0,05) (Fig 1).

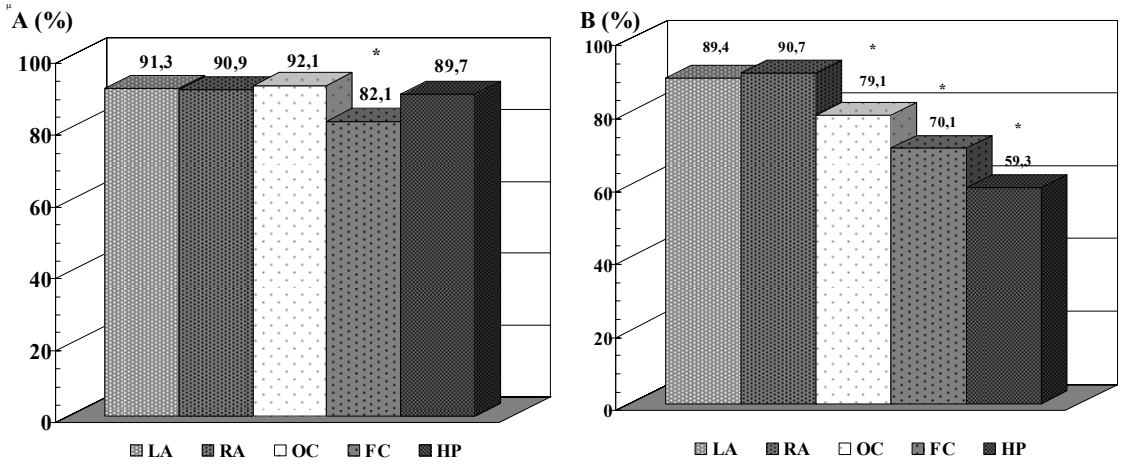

Fig. 1. Dynamic of total power of EEG in brain structures of kindled rats during periods of beginning (A) and cessation (B) of ED.
LA-, RA- left- and right- amygdali; OC-, FC- occipital and frontal cortex correspondingly; HP- hippocampus.
The index of power in percents pertaining to the analogous one in the sham -TMS rats
*-P<0,05 in comparison with the data in control group.

The initial period of the beginning of epileptic activity (EpA) (first 15 seconds from the start of EpA) was characterized by the decrease of the total power of EEG in all investigated structures in TMS- rats (by 18% in comparison with the control group of rats). The frontal cortex was the exclusion where the total power of EEG was reduced by 25,6% (P<0,05). The apparent differences were observed during the last 15 s of EpA in both groups. Thus, the absence of diferences was observed in both amygdalar zones where the total mean power was reduced also by 11% (P>0,05). But, in hippocampus the total EEG power was almost two- times less than in control group (P<0,05). Significant reduction in comparsion to the control group was seen also in occipital (by 19,6%) and frontal (by 28,5%) cortical zones (P<0,05).

The dynamic of the average power of different bandwidths revealed that at the stage of beginning of EpA the reduction of the power of delta band of activity was observed almost in all

structures, while at the end of EpA all indices restored up to the level of those ones in control group (Fig 2). The power of tetha band activity was characterized by the decreasing at the end of EpA in all investigated structures, while at the beginning of EpA the prevalence was seen in left amygdalar zone. The power of alpha band of activity was also prevalent in left amygdala while this index was not changed in most structures in the course of AD development. Occipital cortex was an apparent exclusion, where the marked reduction of average power of alpha band of activity was seen. The power of beta band of activity was prevalent in TMS- treated rats in the left amygdalar and in the occipital cortex, while drastic reduction of the average power of beta activity in all investigated structures was seen at the end of ED. Similar pronounced reduction was observed in almost all structures while the dynamic of the power of gamma activity was investigated - left amygdala was the only structure which was not affected.

Our data permit to come to conclusion that TMS causes suppressive antiepileptic effects upon behavioral and EEG component of pharmacologically kindled seizures in rats.

It is of interest to note that delta type of activity is associated with the increased inhibition in the neocortex [1, 2, 3]. It means that the development of the state of decreased seizure susceptibility is most probably expected. The same is true for slow - wave component of spike-wave epileptiform activity [3]. The reduction of alpha activity also might be regarded as an abolishment of the thalamic- derived facilitation of epileptic discharge in cortical structures [3]. The reduction of higher frequencies of EEG activity might be also contributive to the final effect of TMS- induced EEG changes of epileptogenesis because of desynchronization of EEG is determined by activation of ascending reticular formation in the course of which the decreased seizure susceptibility is precipitated [2, 3, 12].

When EEG analysis was performed after epileptogen administration the above mentioned interpretation of the different role played by certain mechanisms of rhythmogenesis came into some discrepancies. Hence, at the beginning of the EpA drastic reduction of the power of delta- band was registered while the power of alpha band activity was relatively rigid to the TMS. Such an opposite to expected dynamics might be explained both by the shift to new harmonics appearance in the course of EpA generation and by "overcoming" of the defensive potency of delta rhythmicity along with the intensification of proepileptogenic alpha rhythmogenesis by powerful epileptogenic drives. Thus, the initial phase of EpA development might be considered as an insufficiency of the "antiepileptic" state of brain reflected by net changes of different bands of EEG activity. Such an explanation is supported by the consequent substitution of described picture by the opposite one at the end of ED development. Namely, restoration of high level of delta activity was observed.

Along with the shortened character of EpA, the decreasing of behavioral seizures were observed when epileptogen was administered after TMS. This is in favor for the idea that TMS initially affects central mechanisms of generation of epileptogenic excitation. This suggestion is in good correspondence to showed by [5] heightening of the threshold of kindling ED precipitation in rats. Besides, our data shows the efficacy of low- frequency TMS of relatively low intensity under condition of kindling development. In these two last respects our data extends previous data and show the prevention of spreading of ED as a central mechanism of antiepileptic TMS action. Altogether it looks like TMS possesses two principal modes of antiepileptic drugs action [16]- heightening of thresholds of generation of epileptic phenomena and decrease of propagation of epileptogenesis via suppression of neuronal chains involvement into generation of ictal activity.

It is of interest to note that similar dynamic of delta and theta activities was observed after intensification of DOPA- regulation via MAO B inhibitor L-deprenyl or L-amphetamine administrations [3]. Such a correspondence is in favor for the antidepressant action of TMS: effects which were shown both in patients suffered from depression [11, 12], and in experimental animals [5], and mechanisms of which might be realized via activation of central dophaminergic system. Besides, D_2 receptors activation might be contributive to antiepileptic effects precipitation [8].

Taking into consideration that kindled seizures reflects main features of complex partial seizures [9], TMS of relatively low intensity might be regarded as a method of certain therapeutic significance under condition of this form epilepsy which was previously shown for higher intensity of TMS [11].

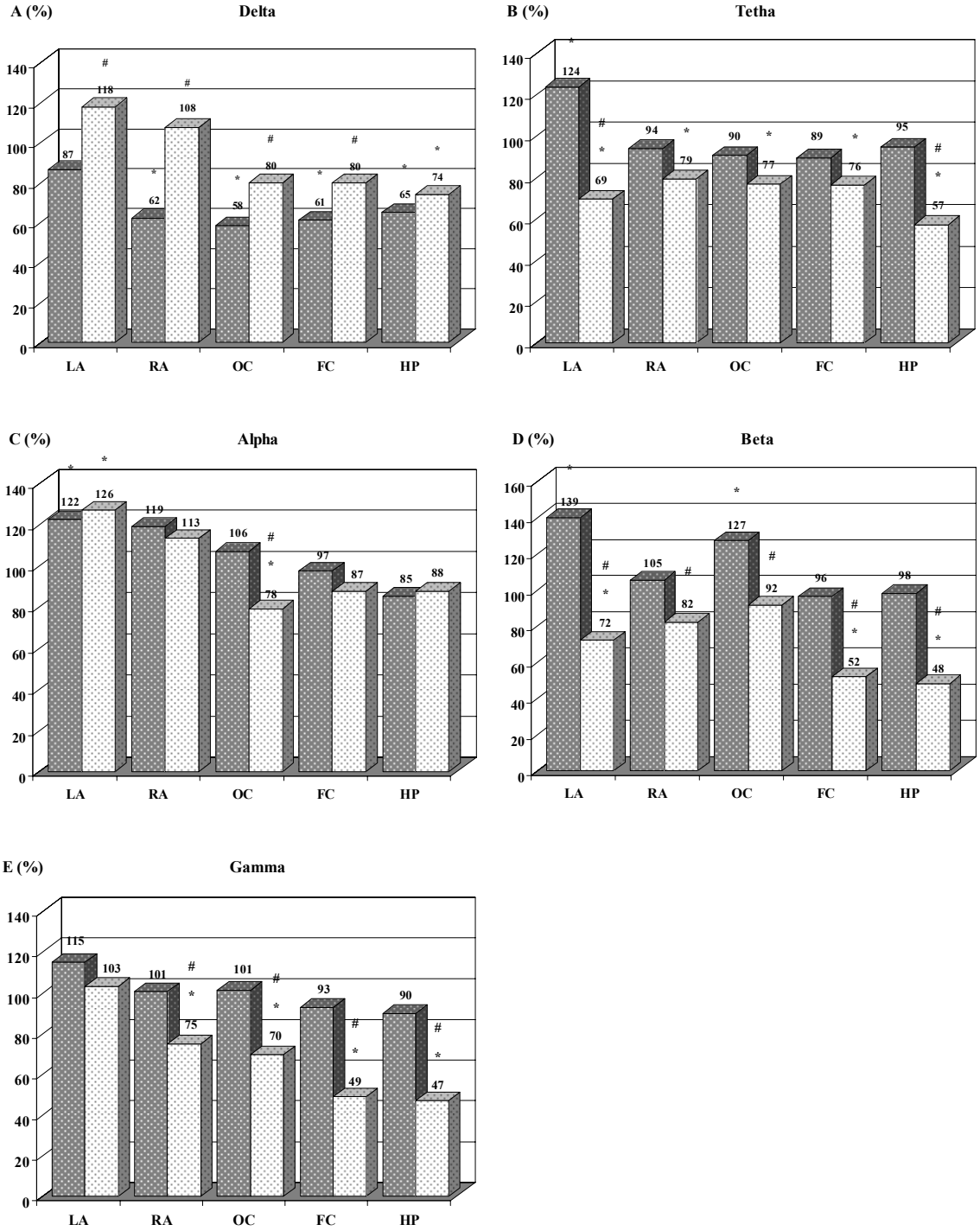

Fig. 2. Dynamic of power in different bandwidth of EEG in brain structures of kindled rats during periods of the beginning (columns N1) and cessation (columns N2) of ED. All marks are the same as in Fig.1.
#-P<0,05 in comparison with the same index at the beginning of AD.

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ВПЛИВ ТРАНСКРАНІАЛЬНОЇ МАГНІТНОІ СТИМУЛЯЦІЇ (ТМС) НА ЕПІЛЕПТИФОРМНУ АКТИВНІСТЬ У ЩУРІВ З ФАРМАКОЛОГІЧНИМ КІНДЛІНГОМ

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РЕЗЮМЕ

ТМС низької частоти (1/с, на протязі 10 с) і відносно високої інтенсивності індукції магнітного поля на висоті розвитку імпульсів (1,0-1,5 Тл) попереджала розвиток кіндлінгових генералізованих клоніко-тонічних судом и редукувала тривалість епілептичних розрядів до 25,7±2,9 с (в контролі - 44,5±5,2 с, P<0,05). Дослідження загальної потужності ЕЕГ на протязі розвитку епілептиформного післярозряду покзаало зниження потужності загальної потужності ЕЕГ в усіх досліджуваних структурах мозку під впливом ТМС (на 18% у порівнянні до групи контролю) на протязі початкової частини епілептичного післярозряду (перші 15 с). Наприкінці розвитку післярозряду (останні 15 с) така різниця утримувалась в устворенних гіпокампу, окципітальної кори, лобних відділів кори. Аналіз вкладу різних частотних діапазонів в ефекти, які спостерягались, показав, що наприкінці розряду найбільший виразний вклад вносила тета-, бета- та гама- активність, в той час як дельта ритм змінювався протилежним чином. Проводиться співставлення результатів з такими, які було тримано за умов електростимуляційного кіндлінгу.

КЛЮЧОВІ СЛОВА: транскраніальна магнітна стимуляція, фармакологічний кіндлінг, електрокортикограма, судоми

ВЛИЯНИЕ ТРАНСКРАНИАЛЬНОЙ МАГНИТНОЙ СТИМУЛЯЦИИ (ТМС) НА ЭПИЛЕПТИФОРМНУЮ АКТИВНОСТЬ У КРЫС С ФАРМАКОЛОГИЧЕСКИМ КИНДЛИНГОМ

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РЕЗЮМЕ

ТМС низкой частоты (1/с, в течение 10 с) и относительно высокой индукции магнитного поля на высоте развития импульсов (1,0-1,5 Тл) предупреждала развитие киндлинговых генерализованных клонико-тонических судорог и редуцировала длительность эпилептических разрядов до 25,7±2,9 с (в контроле - 44,5±5,2 с, P<0,05). Исследования общей мощности ЭЭГ в течение развития эпилептиформного послеразряда показало снижение общей мощности ЭЭГ во всех исследованных структурах мозга под влиянием ТМС (на 18% в сравнении с группой контроля) на протяжении начальной части эпилептического послеразряда (первые 15 с). В период окончания послеразряда (последние 15 с) такие различия сохранялись в образованиях гиппокампа, затылочной коры, лобных отделов коры. Анализ вклада разных частотных диапазонов в наблюдаемые эффекты показал, что в период окончания послеразряда наибольший вклад вносили тета-, бета- и гамма- активность, в то время как дельта ритм изменялся противоположным образом. Проведен сравнительный анализ результатов с таковыми, полученными при электростимуляционном киндлинге.

КЛЮЧЕВЫЕ СЛОВА: транскраниальная магнитная стимуляция, фармакологический киндлинг, электрокортикограмма, судороги