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EEG PECULIARTITIES AND KINDLED SEIZURES UNDER CONDITION OF TRANSCRANIAL MAGNETIC STIMULATION

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SUMMARY

Transcranial magnetic stimulation (TMS) at low frequency (2/s for 10 s, 0.1 Tl at the height of the impulse peak) induced an increase in delta bandwidth power and a marked reduction in theta and alpha rhythms in the basal EEG in rats kindled via amygdalar electrical stimulation (ES). A marked reduction in beta and gamma bandwidth power was also seen. All effects were seen within half an hour of TMS and were brain structure-dependent. Amygdalar ES did not induce generalized clonic-tonic fits when applied to kindled rats after TMS. The duration of generalized epileptoform activity was also shorter after TMS. A reduction in theta, beta, and gamma rhythms and an increase in delta rhythm at the moment of cessation of epileptiform discharge (ED) (last 16 s of discharge) in TMS-kindled rats were observed. Thus, a suppressive antiseizure effect can be seen when relatively low intensity TMS is used, and the effects might be mediated through delta rhythmogenesis activation.

KEY WORDS: transcranial magnetic stimulation, amygdalar kindling, EEG, seizures

INTRODUCTION

An increase in the threshold of after-discharge (AD) induction after high-frequency (20Hz for 3 s) transcranial magnetic stimulation (TMS) has been seen in experiments on rats and quenching of the threshold of kindled amygdalar seizures after low-frequency (1Hz) TMS had been demonstrated [3, 17].

The mechanisms implicated in the effects of TMS on seizures/seizure susceptibility are controversial and have been suggested to involve either the inhibition of seizures [5, 13, 14, 15] and reduced cortical excitability or the breaking down of pair-pulse inhibition in the hippocampus.

Seizures can be induced by TMS [19] and this effect might be explained by additional depolarization of epileptisized neurons as a result of relatively strong currents induced by TMS. Thus, it is reasonable to suggest that the general principle of artificial stimulation of brain structures in epileptology, namely, a decrease in the risk of facilitation and an increase in inhibition during the course of a reduction in the intensity of high-frequency electrical stimulation, might also be applicable to TMS [2, 16]. The aim of the present work was therefore to investigate whether less intense TMS (0.1 Tl instead of 1 Tl) could induce antiepileptic effects in amygdalar kindled rats.

MATERIALS AND METHODS

Animals

Male Wistar rats with a starting weight of 180-250 g were used and were kept under standard conditions (constant temperature of 23°C, 60% relative humidity, 12 h dark/light cycles, standard diet and tap water given *ad libitum*).

Procedures involving animals and their care were conducted in conformity with the University guidelines that comply 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).

General surgery

Animals were anesthetized with nembutal ("Ceva", France, 35 mg/kg, i.p.) and implanted stereotaxically with bipolar electrodes (nichrome wires insulated except for the tips, wire diameter 0.12 mm, interelectrode distance 0.25 mm) in the left basolateral amygdala (AP=2.2; L= .7; H=8.5, according to the rat brain atlas [9]. Reference monopolar electrodes were implanted in the right basolateral amygdala, right ventral hippocampus (AP=-4.3; L=4.5; H= 8.0), left frontal cortex (AP=1.7; L=2.0; H=1.0), and left occipital cortex (AP=-6.3; L=3.0; H=1.0). Indifferent electrodes were fixed to the nasal bones. Electrodes were fixed to the skull with dental cement. Starting one week after surgery, the rats were handled daily and adapted to the experimental setup.

Kindling procedure

Kindling was started 10-14 days after surgery. Electrical stimulation of the amygdala was performed using an ESU-2 universal electrostimulator (former Soviet Union). Electrical stimuli (60 Hz, duration 1 ms) were applied for a total duration of 1 s. For kindling, the intensity of electrical current used was 80-140 μA, depending on its ability to induce after-discharge [10]. Generalized clonic-tonic seizures were seen following daily stimulation for 20-27 days. The severity of convulsions was evaluated according to the scale described by Racine.

EEG acquisition and analysis

The EEG signals were sampled at 256 samples/s using a data acquisition board (National Instruments, USA), and stored for off-line analysis. The signals were filtered with the bandpass set at 0.5-40 Hz. Fast Fourier Transform analysis was performed on 16-s samples ("Labview-5.0" software modified for EEG). The polygraph records were inspected visually and epochs containing artifacts discarded. The post-TMS change in the power of the different bandwidths of EEG (μV^2) registered at the beginning and at the end of ES-induced epileptic discharge was calculated with respect to corresponded indices in sham- TMS kindled rats and presented as the percentage increment or decrement of different bandwidths power. The frequencies were grouped into 5 bands of 0.5-4, 4-8, 8-12, 12-25, and 25-40 Hz.

TMS

The magnetic impulse generator, based on the electronic chain theory, was constructed in the Department of Biophysics, Informatics, and Medical Equipment. Theoretical analysis showed that the parameters of the impulses were a duration of 1 ms, an amplitude of magnetic induction between peaks of 0.2 Tl, and a current velocity in the coil of 1.0A/μs. The frequency of impulses was 2 per second, and the duration of stimulation 10 s. The shape of the impulse was sinusoidal, with the heights of the positive and negative deviations being 0.12 and 0.08 Tl, respectively. The wire diameter was 2.0 mm and the coil contained 60 turns of wire.

To minimize the effect of current induction in electrodes during TMS, the coil was placed such that the lines of magnetic field were directed in parallel to the electrodes and all wires were disconnected from the plugs during TM stimulation. Therefore, stimulation of the temporo-parietal zones was performed with a coil surface to skull surface distance of 2.5 sm. During stimulation, locomotor components resulting from single impulses were not observed.

TMS was performed 24 h after the last kindled ES. Sham-TMS kindled rats were used as controls when effects on seizures and epileptiform activity in brain structures were investigated.

Histology

At the end of the experiments, the rats were anesthetized with pentobarbital sodium and perfused with paraformaldehyde. Frozen slices (32 μm) of the brain were then prepared and every alternate section mounted on gelatin-coated slides, stained with neutral red, covered with a cover-slip, and examined by light microscopy. In all the rats used in the analysis, the electrodes were shown to be inserted at the appropriate location.

Data analysis

The bandwidth power data were analyzed by 1-way ANOVA, followed by the Newman-Keuls test. Numbers of rats showing seizures were analyzed using the Fisher test.

RESULTS AND DISCUSSION

EEG of kindled rats before TMS

As shown in the Table, in kindled rats, the greatest index seen in all brain areas before TMS was for the delta bandwidth. Theta and beta activities were in second and third positions, respectively, in the majority of structures, while the power of alpha and gamma frequency bands was less pronounced. It should be noted that the power of the delta, alpha, and theta bandwidths was least pronounced in the right (unstimulated) amygdala and hippocampus. There were no structure-dependent differences in beta rhythm power, while gamma rhythm was lowest in the frontal cortex.

EEG of kindled rats after TMS

TMS was followed by changes in the power of delta activity, which increased by 24.8% in the hippocampus in comparison with the pre-TMS level ($P<0.05$). It should also be noted that the difference in delta bandwidth power seen between the unstimulated amygdala and both cortical zones was lost following TMS.

Theta rhythm in the hippocampus did not change significantly after TMS ($P>0.05$), whereas, in all other structures, a marked reduction in theta activity was clearly seen, this being most marked in the frontal cortex (54% reduction) and least in the stimulated amygdalar zone (27% reduction) ($P<0.05$).

Marked reduction in the power of alpha activity was seen in the post-TMS period, with a 50% reduction in the frontal cortex and a 26% reduction in the hippocampus ($P<0.05$). The non-stimulated

amygdala and the frontal cortex showed least alpha activity after TMS.

Beta activity was reduced by 26% and 39% (P<0.05) in the right amygdala and frontal cortex, respectively, after TMS. Gamma activity was reduced by 31% in the stimulated amygdala and by 28% in the hippocampus (P<0.05).

Table

EEG effects of TMS in kindled rats (M±SEM)

	Amydala (left)	Amygdala (right)	Frontal cortex (left)	Occipital cortex (left)	Hippocampus (right)
DELTA RHYTHM					
Pre-TMS (n=6)	69.0±4.0	47.8± 3.1 *	67.7± 7.9 #	65.8± 6.1 #	43.6± 1.9 *@&
Post-TMS (n=7)	68.7± 2.0	56.3± 3.3	59.9± 3.7	62.8± 3.8	54.4± 2.4* (!)
THETA RHYTHM					
Pre-TMS (n=6)	45.3± 2.6	34.4± 2.2*	53.3± 4.7*#	56.2± 4.4*#	31.9± 1.8*@&
Post-TMS (n=7)	33.1± 1.5 (!)	24.2± 1.6* (!)	24.7± 1.7* (!)	34.7± 2.6#@ (!)	29.0± 1.6
ALPHA RHYTHM					
Pre-TMS (n=6)	22.0± 1.5	17.7± 1.3	21.8± 1.5	24.4± 2.0	19.1± 2.3#&
Post-TMS (n=7)	15.2± 0.8 (!)	11.1± 1.3 (!)	10.8± 0.5 (!)	17.3± 1.4 #@ (!)	14.0± 0.8 (!)
BETA RHYTHM					
Pre-TMS (n=6)	35.0± 2.6	36.3± 1.8	35.0± 2.7	38.6± 1.9	37.7± 4.5
Post-TMS (n=7)	26.4± 1.2	26.8± 1.5 (!)	21.1± 1.2 (!)	31.1± 0.4 @	28.5± 2.6
GAMMA RHYTHM					
Pre-TMS (n=6)	13.4± 1.3	15.3± 1.0	10.7± 0.3	14.7± 0.6 @	16.4± 1.4 @
Post-TMS (n=7)	9.2± 0.6 (!)	12.5± 1.0	9.2± 0.3	14.9± 0.4 *@	11.8± 0.9 (!)

Note: all data are presented in μV^2
* P<0.05 compared with the left amygdala, #- P<0.05 compared with the right amygdala, @- P<0.05 compared with the frontal cortex, &- P<0.05 compared with the occipital cortex. (!)- P<0.05 compared with the control group.

Effects of TMS on ES-induced seizures and epileptiform discharges

When amygdalar ES was carried out in the 30 min following TMS, generalized clonic seizures of body muscles were seen in 4/7 rats, the remaining 3 rats showing rearing and serial clonic seizures of the forelimbs. In the control group (sham TMS), all 6 animals demonstrated generalized clonic-tonic fits, the animals falling and showing postseizure depression (P<0.025). The duration of the ES-induced epileptiform activity was 58.7± 6.0 s in the sham-TMS rats and 32.3± 4.7 s in the TMS group (P<0.05).

The dynamics of the average power of different bandwidths is presented in Fig. 1. A reduction in the power of delta activity was seen in almost all structures at the beginning of the ED, while, at the end, all indices returned to the level of those in the control group (Fig. 1A). The power of theta activity decreased at the end of ED in all structures studied, while, after TMS (before amygdalar ES), a prevalence of theta activity power was seen in the left amygdalar zone (Fig. 1B). The power of alpha activity was also prevalent in the left amygdala; during the course of ED development, this index did not change in most structures with the exception of the occipital cortex, which showed marked reduction of the average power of alpha band activity (Fig 1C). In TMS-treated rats, the power of beta activity was prevalent in the left amygdala and occipital cortex, and a marked reduction in the average power of beta activity was seen in all structures studied at the end of ED (Fig. 1D). A similar marked reduction was seen in almost all structures when the dynamics of the power of gamma activity was investigated, the left amygdala being the only structure not affected (Fig. 1E).

Thus, during the course of kindled ED development under conditions of TMS, a marked reduction in the power of theta, beta, and gamma rhythm was seen, while delta rhythm increased. Alpha activity was not affected by TMS.

Our data show that TMS caused profound EEG changes in kindled rats. The most marked effect was the general reduction in the power of alpha activity in all brain structures. Reduced theta activity was also seen in all structures, except the hippocampus. In contrast the reduction in the power of the beta and gamma bandwidths was less widespread, and delta activity even increased in the hippocampus.

It is of interest to note that delta-type activity is associated with increased inhibition in the neocortex [1] and seizure susceptibility would therefore be expected to be reduced. The same is true of the slow-wave component of spike-wave epileptiform activity [4]. The reduction in alpha activity might also be regarded as abolition of the thalamic-derived facilitation of epileptic discharge in cortical structures [7]. The reduction in the higher frequencies of EEG activity might also contribute to the TMS-induced EEG changes of epileptogenesis, since EEG desynchronization is determined by activation of the ascending reticular formation during the course of which seizure susceptibility is reduced [8, 12].

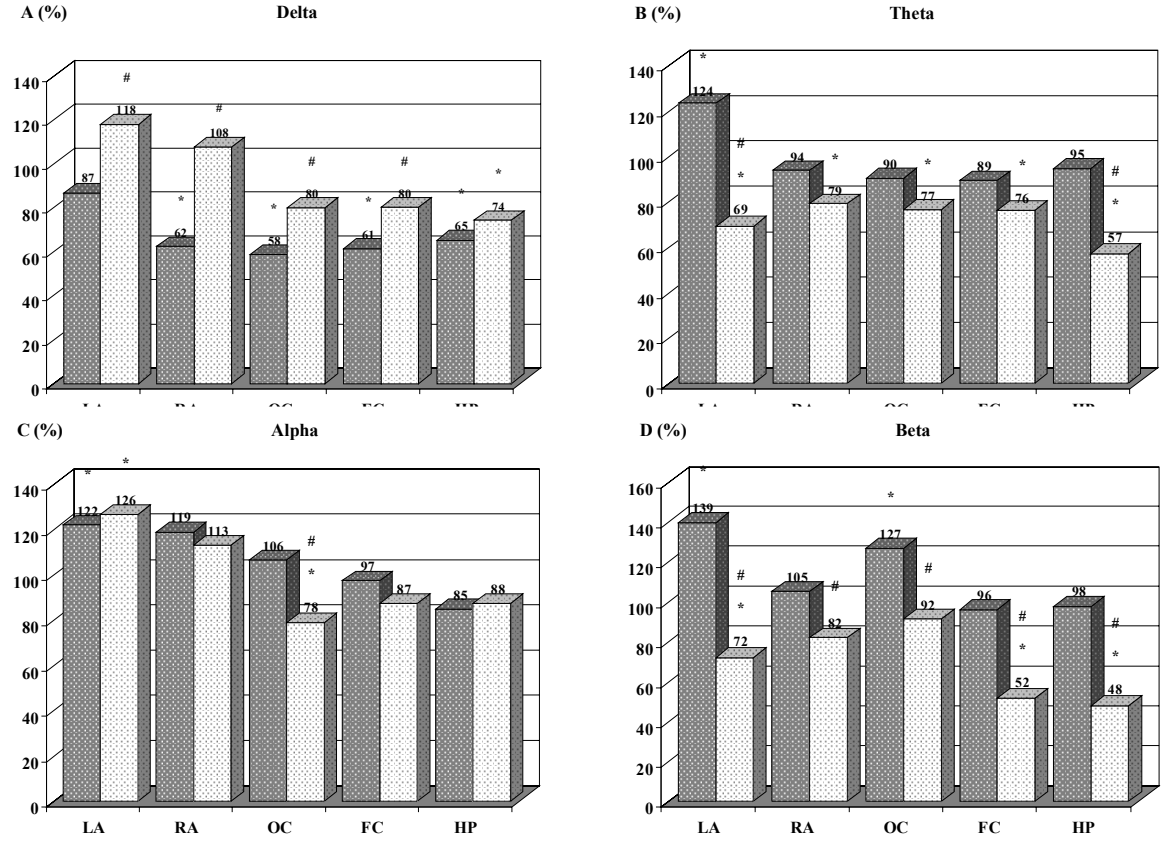

Fig. Effect of TMS on the dynamics of power of different EEG bandwidths in brain structures at the beginning (dark column) and end (light column) of kindled epileptiform discharge. LA and RA, left and right amygdala; OC and FC, occipital and frontal cortex; HP hippocampus. The power index is shown as a percent of that in sham-TMS rats (100%).
*P<0.05 compared with the control group.
#P<0.05 compared with the same index at the beginning of epileptiform discharge.

When EEG analysis was performed after amygdalar ES, the observed results conflicted with the above interpretation of the different roles played by certain mechanisms of rhythmogenesis. Thus, at the beginning of ED, a marked reduction in the power of the delta band was seen, while the power of alpha band activity was unchanged. Such dynamics, the opposite of what might be expected, might be explained both by the shift to new harmonics during the course of ED generation and by the "overcoming" of the antiepileptic effect of delta rhythmicity and the intensification of proepileptogenic alpha rhythmogenesis by powerful epileptogenic drives. Thus, the initial phase of ED development might be considered as an insufficiency of the "antiepileptic" state of the brain reflected by net changes in the different bands of EEG activity. Such an explanation is supported by the subsequent reversal of the above picture at the end of ED development, namely, the restoration of a high level of delta activity.

Together with shortening of ED, a decrease in behavioral seizures was seen when amygdalar ES was performed after TMS, supporting the idea that TMS initially affects central mechanisms of generation of epileptogenic excitation. This suggestion

is in good agreement with the results of Ebert and Ziemannn [3] who demonstrated raising of the threshold of kindling AD precipitation in rats. In addition, our data demonstrate the efficacy of low-frequency TMS of relatively low intensity under conditions of kindling development. In terms of the low frequency and low intensity our data extend previous results and show prevention of the spreading of ED to be a central mechanism in antiepileptic TMS action. Taking together our results and those of other authors, it seems that TMS has two principal modes of antiepileptic drugs action [18], the raising of the threshold for the generation of epileptic phenomena and a reduction in propagation of epileptogenesis via suppression of the neuronal chains involved in the generation of ictal activity.

Since kindled seizures are a model for complex partial seizures [6], relatively low intensity TMS might be considered as a possible therapeutic approach for this form of epilepsy, as previously shown for higher intensity TMS [13].

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ОСОБЛИВОСТІ ЕЕГ ТА КІНДЛІНГОВИХ СУДОМ ЗА УМОВ ТРАНСКРАНІАЛЬНОГО МАГНІТНОГО ПОДРАЗНЕННЯ

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РЕЗЮМЕ

Транскраниальне магнітне подразнення (ТМП) низької частоти (2/с на протязі 10 с, 0,1 Тл на висоті імпульсу) индукувало збільшення потужності дельта- ритму і суттєво редукувало тета та альфа- ритми фонової ЕЕГ у щурів з кіндлінгом, який було викликано електричними подразненями (ЕП) мигдалика. Суттєва редукція потужності бета- та гамма ритмів також мала місце. Всі зазначені ефекти ровзивались на протязі

півгодини з моменту ТМП і їх виразність в структурах мозку була різною. ЕП мигдалика не супроводжувалась розвитком генералізованих клоніко- тонічних нападів у кіндлінгових щурів після ТМП. Тривалість генералізованих епілептиформних проявів також була зменшена після ТМП. У кіндлінгових щурів з ТМП спостерігалась редукція тета, бета і гамма ритмів при одночасному збільшенні дельта ритму в момент припинення епілептиформного розряду (останні 16 с розряду). Таким чином, відносно низькоінтенсивна ТМП супроводжується розвитком протисудомного ефекту, який супроводжується підсиленням дельта ритму.

КЛЮЧОВІ СЛОВА: транскраніальна магнітна стимуляція, мигдаликовий кіндлінг, ЕЕГ, судоми

ОСОБЕННОСТИ ЭЭГ И КИНДЛИНГОВЫХ СУДОРОГ В УСЛОВИЯХ ТРАНСКРАНИАЛЬНОЙ МАГНИТНОЙ СТИМУЛЯЦИИ

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РЕЗЮМЕ

Транскраниальная магнитная стимуляция (ТМС) низкой частоты (2/с в течение 10 с, 0,1 Тл на пике импульса) индуцирует увеличение мощности дельта- ритма и существенную редукцию тета и альфа- ритмов в фоновой ЭЭГ у крыс с киндлингом, вызванным электрической стимуляцией (ЭС) миндалины. Существенная редукция мощности бета- и гамма ритмов также имела место. Все указанные эффекты развивались в течение получаса с момента ТМС и их выраженность в структурах мозга была разной. Амигдалярная ЭС не сопровождалась развитием генерализованных клонико- тонических приступов у киндлинговых крыс после ТМС. Длительность генерализованных эпилептиформных проявлений также была короче после ТМС. У киндлинговых крыс с ТМС отмечалась редукция тета, бета и гамма ритмов при одновременном увеличении дельта ритма в момент прекращения эпилептиформного разряда (последние 16 с разряда). Таким образом, относительно низкоинтенсивная ТМС сопровождается развитием противосудорожного эфекта, развивающегося на фоне усиления дельта ритма.

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