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Effect of weak combined static and extremely low-frequency alternating magnetic fields on spatial memory and brain amyloid- β in two animal models of Alzheimer's disease

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ABSTRACT

Subchronic effect of a weak combined magnetic field (MF), produced by superimposing a constant component, 42 μ T and an alternating MF of 0.08 μ T, which was the sum of two frequencies of 4.38 and 4.88 Hz, was studied in olfactory bulbectomized (OBE) and transgenic Tg (APPswe, PSEN1) mice, which were used as animal models of sporadic and heritable Alzheimer's disease (AD) accordingly. Spatial memory was tested in a Morris water maze on the following day after completion of training trials with the hidden platform removed. The amyloid- β (A β) level was determined in extracts of the cortex and hippocampus of mice using a specific DOT analysis while the number and dimensions of amyloid plaques were detected after their staining with thioflavin S in transgenic animals. Exposure to the MFs (4 h/day for 10 days) induced the decrease of A β level in brain of OBE mice and reduced the number of A β plaques in the cortex and hippocampus of Tg animals. However, memory improvement was revealed in Tg mice only, but not in the OBE animals. Here, we suggest that in order to prevent the A β accumulation, MFs could be used at early stage of neuronal degeneration in case of AD and other diseases with amyloid protein deposition in other tissues.

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Introduction

Amyloid- β (A β) is a key pathogenic agent in Alzheimer's disease (AD). The abnormal amyloidogenesis, leading to A β protein deposition in the extracellular and perivascular spaces of the brain, is one of the main causes of neuron death in AD. Therefore, many recent studies have focused on investigation of the methods for preventing A β deposition and removing the senile plaques, formed by A β . The efficiency of this approach was demonstrated in transgenic (Tg) animals carrying the inserted human gene of A β precursor protein. Cleaning of their brain from amyloid plaques using antibodies against A β protein was accompanied by recovery of spatial memory (Solomon, 2009). However, this method has a number of negative side effects in patients with AD (Holtzman, 2008). Therefore, the problem of removing A β aggregates from the brain remains quite important.

In previous research, we studied the mechanisms of the effect of weak combined magnetic fields (MFs) on properties of aqueous solutions of various biologically

active ions, as well as proteins and peptides (Novikov, 1998; Novikov & Fesenko, 2001; Novikov et al., 2010; Novikov & Zhadin, 1994; Zhadin et al., 1998). We used a low-frequency variable component with the magnitude of about 10 nT and a constant component with the magnitude comparable to the geomagnetic field. According to our proposed approach, the frequencies of the variable component of the MFs formally corresponded to the cyclotron frequencies of ionic forms of a number of amino acids and a ratio of the magnitudes of the constant and variable components lied within 500–3000 range. Such MFs combination has an extremely high biological activity. In particular, it was shown that it can accelerate the decomposition of the A β into soluble peptide fragments with a decreased neurotoxic effect and with less capability of forming the insoluble aggregates (Fesenko et al., 2003; Novikov & Fesenko, 2001; Novikov et al., 2010). In this work, we used a weak combined variable MF of 0.05 μ T with frequencies 3.58–4.88 Hz and constant magnetic field of 42 μ T. The region of the A β molecule most sensitive to the weak MF was located between residues Asp7 and Ser8.

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In this region, the hydrolysis of A β under the action of the MFs took place (Fesenko et al., 2003; Novikov et al., 2010).

In this work, we used weak combined MFs with the parameters close to those mentioned above to study the effect of MFs *in vivo* in two animal models of AD. One was the well characterized B6C3-Tg (APP^{swe}, PSEN1Delta E9) 85DBO/J mice, i.e. double transgenic mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) both directed to CNS neurons. Both mutations are associated with early-onset AD. The other was the olfactory bulbectomized (OBE) mice, which showed the behavioral, morphological, immunological and biochemical signs similar to sporadic AD. They have pronounced impairment of spatial memory, increased A β level in the brain, pathology in the cholinergic system and demonstrate a loss of neurons in the brain structures responsible for memory (Aleksandrova et al., 2004; Bobkova et al., 2004, 2001; Hozumi et al., 2003; Nesterova et al., 2008, 1997; Yamamoto et al., 1997).

Materials and methods

Subjects

The experiments were carried out on 3-month-old male NMRI mice and 8-month-old male transgenic B6C3-Tg (APP^{swe}, PSEN1DeltaE9)85DBO/J mice (JacksonLab, USA) with 25 ± 0.6 g weight, which co-express a chimeric mouse/human APP695 harboring the Swedish K670M/N671L mutations (Mo/HuAPP^{swe}) and human PS1 with the exon-9 deletion mutation (PS1dE9). This model was generated by co-injection of MoPrP.Xho (mouse prion protein) expression plasmids for each gene; the two transgenes co-integrated and segregated as a single locus. These Tg mice were purchased from the Jackson Laboratories (stock # 004462; Bar Harbor, ME). C3H mice (phenotypically non-mutant mice) from the colony were the controls.

Olfactory bulbectomy was performed under Nembutal anesthesia (40 mg/kg, i.p.) using a 0.5% Novocain solution for local anesthesia in scalping. We used a soft holder and a fastener for the front teeth to fix the head horizontally (without angle). Careful and accurate bilateral aspiration of the olfactory bulbs was performed by a water pump coupled plastic tip. At the end of each experiment, morphometric testing was carried out to check if these structures were completely removed. Single burr hole of 2 mm diameter was drilled over the olfactory bulbs, using the stereotaxic coordinates: AP-3; L0; H 3.5 in The Mouse Brain Library @ www.mbl.org (Rosen et al., 2000). The control to OBE mice was sham-operated (SO) animals,

subjected to the same procedures except the olfactory bulb ablation. Animals were allowed food and water ad libitum and housed in groups of eight in standard laboratory cages under 12:12 h light-dark conditions (light from 8:00 AM) at 21–23°C. The OBE and SO animals were exposed to weak combined MF five weeks after bullectomy. Tg mice and control C3H mice were exposed to the same MF at the age of 8 months. The scheme of the experiment is shown in Figure 1.

All animal experiments were carried out in strict accordance with the “Regulations for Studies with Experimental Animals” (Decree of the Russian Ministry of Health of 12th August 1997, No. 755). The protocol was approved by the Commission on Biological Safety and Ethics of the Institute of Cell Biophysics, Russian Academy of Sciences.

Characterization of MFs and the methods of their formation

The animals were located in the central zone of the Helmholtz coil system. Mice from two groups (OBE + MF and SO + MF, or Tg + MF and C3H + MF) were exposed to MF simultaneously. The animals were set into the organic glass box with an open top which was divided into two equal parts, the size of the box was $36.7 \times 20.7 \times 14.0$ cm. Four mice were kept in each group.

The setup for generating the MFs consisted of two pairs of coaxial Helmholtz coils oriented along the geomagnetic field (GMF) vector (Figure 2). The diameter of each coil was 140 cm; the distance between the coils in the pairs was 70 cm. Each Helmholtz coil had

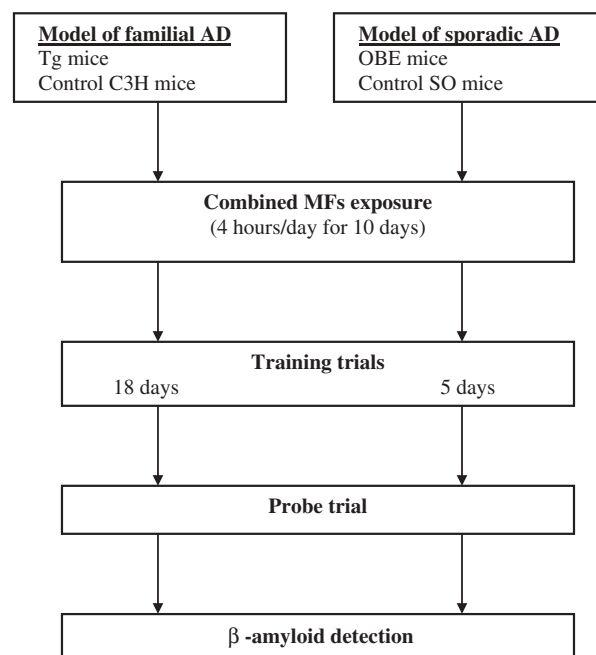


Figure 1. The scheme of the experiment.

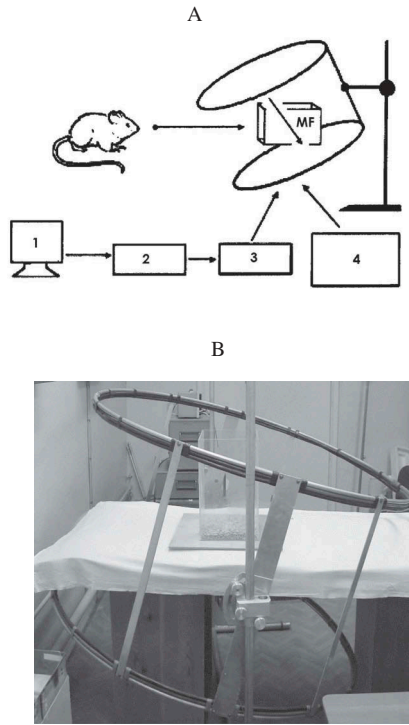


Figure 2. The experimental setup for combined MFs exposure. (A) Block diagram of the experimental setup. Biological objects are in the zone of action of MFs formed by Helmholtz coils. The coils are oriented so that the vector of the artificial MF is parallel to the GMF vector. To form the preset value of the static MF component, use is made of a compensator (4) electrically linked with the Helmholtz coil system. The compensator is a source of DC. With its help the static component of GMF is amplified, to be used in the experiment as the MF static component. A personal computer (1) with a digital-analog converter (2) generate an electrical signal with preset frequency-amplitude characteristics, which through an amplifier (3) is fed to the coil system and forms the alternating component of the MF. (B) Photograph of the exposure system.

100 turns of the isolated copper wire 0.1 cm in diameter. The coil was covered with insulating tape. Two identical coils were connected in series. The resistance of a pair of coils was 22.5Ω . A support (supporting framework) for coils was made of duralumin (due to the disruption effects through dielectric layer no closed circuits occurred in the supporting framework). The local GMF ($\sim 37 \mu\text{T}$) partially adjusted to $42 \pm 0.1 \mu\text{T}$ using one pair of Helmholtz coils served as a DC component of MFs. For this reason, the direct current of 33.8 mA was sent to this pair of coils.

The alternating component collinear to the DC field was formed using the second pair of Helmholtz coils. An alternating current signal produced by a programmable sinusoidal current generator was fed to another pair of coils to create a variable component of MFs with induction amplitude of 80 nT. The current signal was

the sum of two frequencies of 4.38 and 4.88 Hz. These frequencies correspond to the cyclotron frequencies of glutamic and aspartic acids, respectively, as calculated by the standard expression:

$$f_c = qB_{DC}/2\pi m,$$

where q and m are the charge and mass of an ion, respectively, and B_{DC} is the induction of the DC magnetic field.

The parameters of AC (frequencies and amplitudes) were preset by the digital method using a digital-analog converter (DAC) of an L 791 plate (L-Card Company, Moscow, Russia). This provided a high accuracy of setting the parameters. For generation of the alternating magnetic field to the second pair of coils, the alternating current of the following type was applied:

$$I(t) = A_1 \cos(\omega_1 t) + A_2 \cos(\omega_2 t),$$

where $\omega_1 = 2\pi 4.38 \text{ s}^{-1}$, $\omega_2 = 2\pi 4.88 \text{ s}^{-1}$, $A_1 = A_2$, that produced the alternating magnetic field changing over time (Figure 3).

The collinear AC and DC magnetic fields were at an angle of 26 degrees with the horizontal plane. Note that MFs (DC and low frequency AC) used in our experiments completely penetrate through the biological object (Liboff, 1985).

The MFs were measured with a Mag-03 MS 100 three-axial MF sensor (Bartington Instruments Ltd., United Kingdom). The animals were exposed to MFs in 4-h daily sessions for 10 days. We chose to use a single 4 h exposure because, according to our preliminary data, *in vitro* exposure to MFs accelerates hydrolysis of protein (Novikov & Fesenko, 2001) (the process rate reached notable values within this period of time). The number of days (10 days) was determined from previous experimental results obtained *in vivo* using

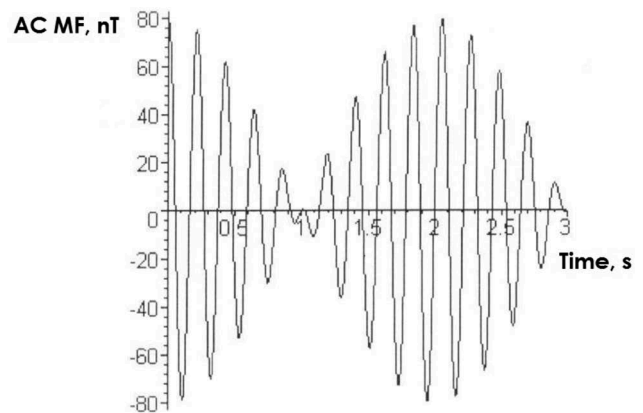


Figure 3. Form of changes over time of the alternating magnetic field.

tumor-bearing mice with Ehrlich ascite carcinoma (Novikov et al., 2009) (this number of exposure sessions cause significant effect of MFs on mice).

The experiments were carried out in the presence of the natural and technogenic magnetic backgrounds with an induction of 50-Hz component of 20–40 nT in the daytime between 10 and 18 h at room temperature (18–22°C) under natural illumination. The SO, OBE, Tg and C3H animals without exposure to the weak combined MF were groups of active controls. They were under action of natural geomagnetic field with 42 μ T induction with the same magnetic noise level as the test groups.

The sham exposure system was represented by the MFs exposure device with the static DC MF level ($42 \pm 0.1 \mu$ T) the same as applied in the experiments, but the alternating current power supply was not used. In this case physical conditions were similar to those for the main control groups. Note that the control group and the experimental group were kept in the same environment, under DC MF of 42 μ T, with the same natural and technogenic magnetic backgrounds, at the same temperature, humidity, lightness and so forth. We used the sham exposure system in separate series of experiments to compare the parameters of the main control groups and sham exposure groups.

Training trials and memory tests

After exposure to the MFs, the mice were trained in a Morris water maze for 5 days (four trials per day) for the olfactory bulbectomy experiment and for 18 days in the Tg-mouse experiment. Experiments were performed in a test room with extra-maze cues to facilitate spatial learning. A circular swimming tank (80 cm diameter and 40 cm wall height with an escape platform of 5 cm-diameter) was filled to 30 cm depth with water at 23°C and rendered opaque by adding powdered milk. The tank was mentally divided into four sectors: the escape platform was located in the middle of the third quadrant during training. A preliminary control set of experiments with visible platform conducted prior to the training trials demonstrated that all animals (SO, OBE, C3H and Tg; 8 mice in each groups) can swim, do not have motor or visual impairments, since control and MF-treated mice were able to find visible target platform in Morris water maze within similar time intervals (12 ± 3.9 ; 15 ± 3.8 ; 14 ± 4.5 s and 16 ± 2.7 sec for SO, OBE, C3H and Tg accordingly as well as 10 ± 2.5 ; 13 ± 1.9 ; 11 ± 3.6 ; 15 ± 3.9 s for SO, OBE, C3H and Tg, treated by MF). After that the target platform was submerged to a depth of 0.5 cm so as to be invisible to the swimming animal during the whole period of

training trials. Latency to reach the invisible platform was then determined. If the animals failed to locate the platform within the test period of 60 s, they were placed on the platform for 10 s. The animals were trained daily in a random order. Each animal in each training session started from different sectors of the Morris water maze, while the order of the starting sectors in each subsequent session was not similar to the one in previous session. The Morris water maze and animal housing facility were in the same building but in different rooms 5 m apart from each other. Spatial memory (probe trial) was tested with the hidden platform removed on the following day after completion of training. During the probe test period (60 s), occupancy time spent in each sector was recorded. After termination of behavioral experiments, intracardial perfusion was carried out with cooled physiological solution under ethyl ether narcosis.

β -Amyloid detection

Brains of OBE and SO mice were removed, frozen on dry ice and stored at -80°C for biochemical studies. The brains of Tg animals and C3H mice were immersion-fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS pH 7.4) and were cut in the coronal plane in 10 μ m sections on a cryostat. Then, the fixed tissues were kept in sucrose solution in phosphate-buffered saline (PBS, pH 7.4; -20°C) until histological assay.

The A β level was determined in extracts of the cortex and hippocampus of OBE and SO mice using a modified DOT analysis described earlier (Aleksandrova et al., 2004). In this method, a nitrocellulose membrane was pretreated for 1 min with 40% ovalbumin in phosphate buffer and then for 10 min with 2.5% glutaraldehyde, samples were applied to the membrane, and the membrane was kept for 1 h in 4% ovalbumin in phosphate buffer with 0.1% NaN_3 .

Density and sizes of amyloid plaques

Estimation of amyloid plaque loads was performed in Tg and Tg + MF by counting amyloid plaques in brain slices, staining with thioflavine S, in 10 fields of view (magnification $\times 20$) in the each sixth brain sections (a slice thick 10 μ m) of the next brain areas: in the CA1 and CA3 fields of the hippocampus and temporal cortex. Images were captured by digital photography. The amyloid deposits contained within fields of view were counted separately by their sizes (magnification $\times 40$): big plaques with maximal diameter $>30 \mu$ m, medium plaques $18 \mu\text{m} < \text{maximal diameter} < 30 \mu\text{m}$ and small ones with maximal diameter $<18 \mu\text{m}$. The number of

plaques of each size in a sample were summed and then averaged for each group of animals.

The experiment was run under a blind condition. All tests were conducted according to the protocol of “blind control.”

Statistical analysis

Differences in memory parameters were evaluated by a two- and one-way ANOVA (statistical package “Statistica 6.0”). The statistical significance of preference for the target sector as compared with other indifferent sectors was evaluated using a Post-hoc analysis with the LSD criterion. The water-maze acquisition latencies were evaluated by repeated measures ANOVA (Statistica 6.0 statistical package). The level of cerebral A β in OBE mice, differences in number of amyloid plaques with different sizes, and their density in Tg animals were evaluated using the two-tailed Student’s test. All data were expressed as mean $\pm$ SEM.

Results

The effect of subchronic MFs on learning rate and spatial memory

The rate of learning was determined basing on the time spent by animals looking for the safety platform emerged in water clouded with milk (latency period). Repeated measures ANOVA analyses with Tukey’s post-hoc analysis for multiple comparisons using groups and trial day number as variations revealed a significant principal effect of the trial day on the dependent variable-escape latency ($p < 0.001$). Between subjects, the MFs treatment factor had no significant effect on the escape latency. One-way ANOVA demonstrated a significant difference between the OBE and SO groups ($p < 0.05$), but not for C3H and Tg mice ($p > 0.05$) for escape latency. The data in Figure 4 (A,B) show that the latencies to reach the escape platform were increased significantly in OBE and OBE + MF groups in comparison to SO mice in 4th day of training as well as in Tg animals in comparison to Tg + MF mice in first day of training and Tg animals in comparison to C3H mice in last days of training. In general, this points to the fact that the OBE animals have a decreased ability to gain spatial skills in comparison to SO, and the exposure to MFs did not have effect on latency in SO, OBE, C3H and Tg animals (Figure 4 A and B). We suggest that the MFs do not affect the learning rate in OBE, C3H and Tg mice and that the SO animals have an increased sensitivity to the MF. It is necessary to

indicate that C3H mice needed more training sessions than SO animals to acquire the spatial skills.

At 24 h after the last training session, spatial memory was tested in a probe trial during which search patterns were monitored in the absence of save platform. Two-way ANOVA analysis of spatial memory used one independent measure, i.e. the time spent by the animals in each sector for probe trials and groups. A significant main effect of the Factor Sector ($p < 0.01$), but not the Factor Group ($p > 0.05$) on the dependent variable, occupancy time was revealed. However, in both cases interaction of factors Sector $\times$ Group were significant, namely $F(9, 63) = 7.379$ $p < 0.000$... for SO, SO + MF, OBE, OBE + MF and $F(9, 63) = 4.245$ $p < 0.0004$ for C3H, C3H + MF, Tg, Tg + MF. The results of the one-way ANOVA analysis presented in Table 1 demonstrate that the factors of sector preference became statistically significant for Tg, but not for OBE groups after MFs exposure. The SO animals exposed to the same MFs demonstrated a significant increase in the factor of sector preference. The results of the Post hoc analysis are presented in Figure 5 (A,B). The comparison of memory testing results obtained in SO-OBE and C3H-Tg groups after MFs exposure demonstrated that in general OBE and Tg mice have deteriorated spatial memory which agrees with many other literature data demonstrating cognitive decline in the OBE and Tg mice. OBE animals both treated and non-treated by MFs could not find the target sector which contained the safety platform during training trials. In contrast, MFs exposure significantly improves spatial memory in Tg animals.

Thus, the behavioral study revealed that the OBE mice did not remember the sector, in which the saving platform was located during training. It supports our previous data on the impairment of the spatial memory in OBE animals (Aleksandrova et al., 2004). The subchronic exposure to the weak MFs did not affect spatial memory of these animals. However, the MFs improved the memory not only in SO mice, but also in Tg animals.

Effects of MF exposure on the brain a β in OBE mice

Table 2 presents the absolute values of A β level in the extracts of the neocortex and the hippocampus in OBE and SO mice after exposure of MFs. The sensitive DOT analysis revealed that the A β level in the extracts of the OBE animals was more than five times higher ($p < 0.001$) in comparison to SO mice. The exposure to the weak combined MFs induced reliable decrease in the A β level almost threefold ($p < 0.01$), but it was higher than in SO mice

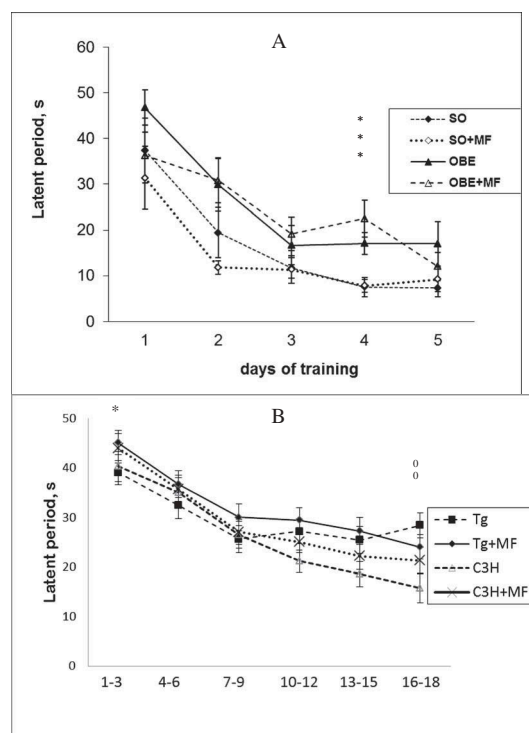


Figure 4. Exposure of MFs shows no effect on spatial learning of Tg and OBE mice in the Morris water maze. Spatial learning was assessed as function of training day with respect to latent period using repeated measures ANOVA with followed post hoc analysis. Data are presented as the mean $\pm$ SEM.

(A) OBE + MF, OBE and control SO + MF, SO groups were training over 5 days to find the hidden platform and show no differences in dynamic of spatial learning. One way ANOVA revealed significant differences in latent period at 4th day ($***p < 0.001$ OBE vs SO). (B) Tg + MF, Tg mice and control nTg groups C3H + MF, C3H were training over 18 days. One-way ANOVA revealed significant differences at 1st block (1–3 days, $*p < 0.05$ Tg vs Tg + MF) and at last block of training trials (16–18 days; $**p < 0.01$ Tg vs C3H).

Table 1. The means of factor of the recognizing of the Morris water maze sector by time spent there in OBE, SO, Tg and C3H mice exposed to the weak MF.

Groups of animals	Mean of the factor	
	F	p Value
SO	$F(3, 12) = 3.73$	0.042*
SO + MF	$F(3, 12) = 30.18$	0.000***
OBE	$F(3, 12) = 2.18$	0.140
OBE + MF	$F(3, 12) = 0.64$	0.600
C3H	$F(3, 12) = 4.12$	0.034*
C3H + MF	$F(3, 12) = 3.98$	0.039*
Tg	$F(3, 12) = 2.07$	0.210
Tg + MF	$F(3, 12) = 3.11$	0.049*

$n = 4$ (in each group four mice).

* $p < 0.05$ and *** $p < 0.001$.

($p < 0.05$). When OBE animals were sham-exposed, no differences in the amyloid contents were found between the sham-exposed and the control animals ($A\beta$ ng/g 32.14 ± 4.23 and 34.02 ± 4.46 , respectively).

Effect of MFs on $A\beta$ deposits in the brain of Tg mice

Figure 6 (A) exemplifies microphotographs of slices of the cortex and areas of the Hippocampus stained with thioflavine S. Treatment with MFs had positive effects on $A\beta$ plaques in Tg+MF group. Quantitative analysis of plaques with different size (Figure 6B) showed the decreased density of plaques with small and middle sizes in the cortex and with large and middle size in the CA3 area of the hippocampus. In CA1 field the tendency for increase of small plaques was observed followed by a decreased density of middle-size plaques.

Discussion

We revealed the reduction of the $A\beta$ level in the brain of OBE mice and a decrease in the number of large and middle amyloid plaques in the cortex and hippocampus after exposure to the weak combined MFs. The spatial memory was improved in the group of Tg + MF mice, but not in OBE+MF animals. The different effects of the weak MFs on spatial memory in animals of these two models of AD may be explained by different causes. Positive influence of MFs in Tg mice agrees with the main amyloid hypothesis of AD genesis, in that it is sufficient to destroy the $A\beta$ plaques to improve the state of the brain and memory. It seems that in case of Tg mice the reduction of the $A\beta$ -plaques is really sufficient to improve their memory, because it is shown that $A\beta$ -deposits impair the memory in Tg animals due to impairment of impulse transmission in axons and dendrites. Interestingly, the high frequency electromagnetic field reverses cognitive impairment in AD Tg mice as well as decreases brain $A\beta$ aggregation (Arendash et al., 2010). The precise mechanisms of MF benefit will require more extensive research in the future. It is important to note that neurons survive in majority of Tg models of AD including the model used in our experiments (Takeuchi et al., 2000). However, in OBE animals, as our investigation showed earlier, there are the death of neurons especially in the brain areas such as the temporal cortex, the hippocampus, nuclei synthesizing the main neurotransmitters, which are responsible for learning and memory (Aleksandrova et al., 2004; Bobkova et al., 2004, 2001; Hozumi et al., 2003; Nesterova et al., 2008). Here, it is necessary to point out again why we decided to use the OBE mice in our study. Adequacy of the animal model usage is a key issue in any research since, if adequate, it allows drawing the correct conclusion from the results. Olfactory bulbectomy in animals elicits various behavioral abnormalities, such as increased motor activity

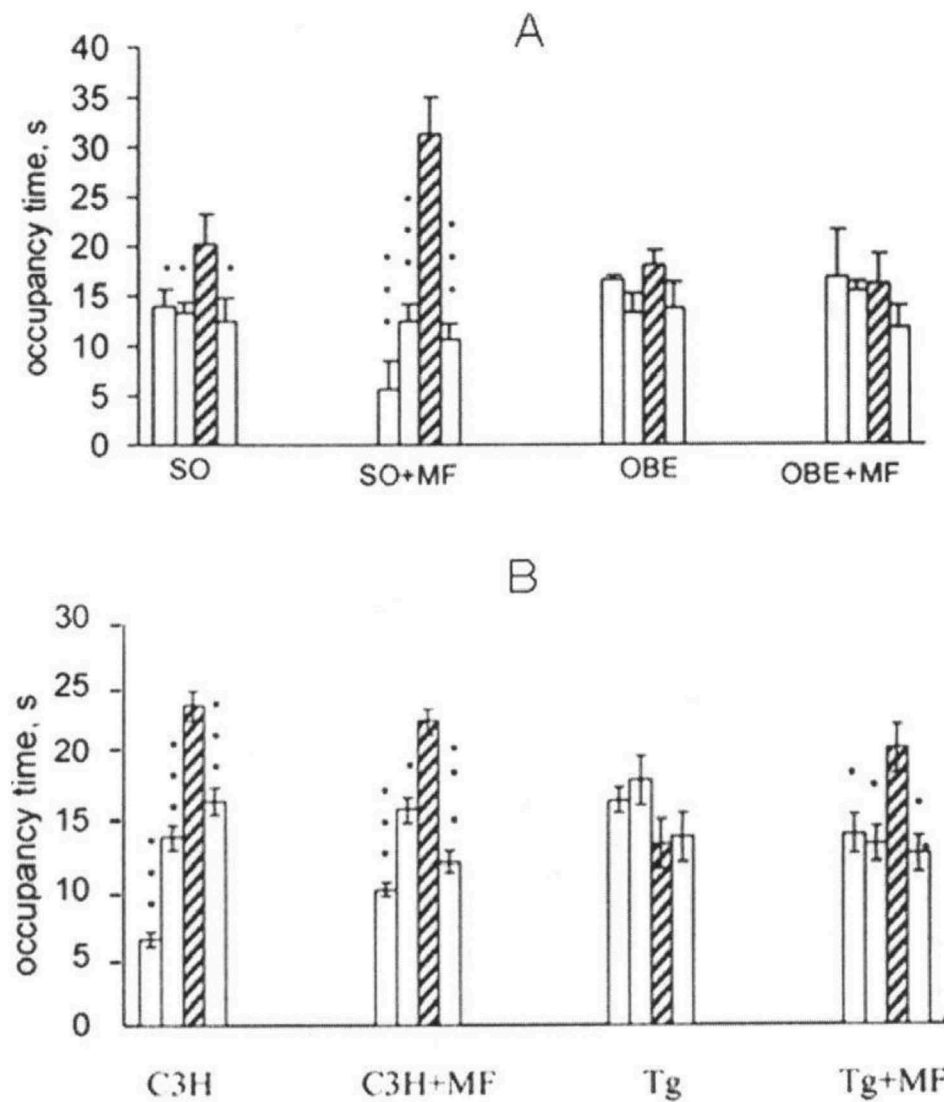


Figure 5. Effect of an exposure of the weak MFs on the spatial memory of OBE and SO mice (A), as well as Tg and C3H animals (B). The ordinate is the time spent in each sector of a Morris water maze.

The hatched bin represents the time in sector in which escape platform was located during training; the empty bins denote time in indifferent sectors of the water maze. The differences are shown between sector in which escape platform was located during training and other sectors * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 2. The level of the brain A β in OBE and SO mice exposed to the weak MFs.

Groups of animals	The level of the brain beta-amyloid (ng/g)
SO	5.03 $\pm$ 0.36
SO + MF	5.21 $\pm$ 0.37
OBE	34.12 $\pm$ 4.17***
OBE + MF	10.91 $\pm$ 2.17* ##

Average values $\pm$ standard error are given, $n = 4$ (in each group four mice). The significance of differences from the group of SO mice: * $p < 0.05$ and *** $p < 0.001$.

The significance of differences from the group of OBE mice: ## $p < 0.01$.

behavior (Sieck, 1972), impaired learning and memory (Hozumi et al., 2003; Nesterova et al., 1997; Yamamoto et al., 1997) and depressive-like behavior (Leonard & Tuite, 1981; Song & Leonard, 2005). Distinctive

features of OBE animals include a loss of neurons in the cortex and hippocampus as well as cholinergic system dysfunction in basal forebrain (Bobkova et al., 2004; Hozumi et al., 2003; Nesterova et al., 1997). They show the membrane pathology, free radical generation and apoptosis (Capurso et al., 1997; Koliatsos et al., 2004; Leung & Wilson, 2003), as well as impairment of brain asymmetry (Bobkova et al., 2008). The OBE mice were shown to have impaired hippocampal long-term potentiation (Han et al., 2008). As was mentioned in the Introduction, OBE mice have an increased level of the brain amyloid precursor protein APP and A β in extract of brain tissue and in mitochondria (Aleksandrova et al., 2004; Avetisyan et al., 2016).

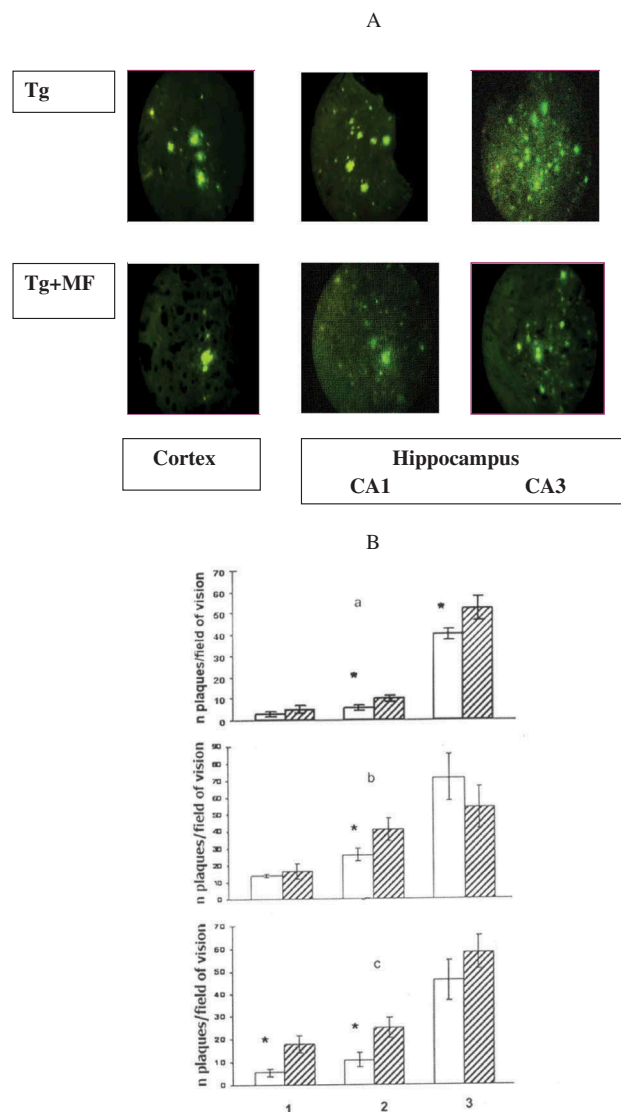


Figure 6. Density of the amyloid plaques in the temporal cortex (a), in CA1 (b) and in CA3 (c) fields of the hippocampus in transgenic mice – model of family AD after subchronic exposure of weak combined MFs.

(A) Representative microphotographs of the cortex, CA1 and CA3, respectively. (B) 1 – density of plaques with size $>30\ \mu\text{m}$; 2 – density of plaques with $18\ \mu\text{m} < \text{size} < 30\ \mu\text{m}$; 3 – density of plaques with size $<18\ \mu\text{m}$. The empty bins denote density of plaques in Tg + MF mice. The hatched bins represent the density of plaques in control group of Tg mice without exposure to MFs. The differences are shown between density of plaques with similar size in Tg + MF and Tg groups of mice in different brain structures. * $p < 0.05$.

Therefore, OBE mice have some features similar to AD, including memory impairment, depressive state, cholinergic system dysfunction, a loss of neurons and an increased $\text{A}\beta$ level in the brain and olfactory deficit (Moriguchi et al., 2006; Warner et al., 1986). It is important that alterations in neurotransmitter and receptor functions, mediating abnormal behavioral

effects of olfactory bulbectomized animals, are also similar to those in AD patients. Deficient serotonergic function was associated with depressive behavior in OBE rats (Nesterova et al., 2008; Van Der Stelt et al., 2005). The serotonergic system was profoundly affected in AD: a very low or undetectable serotonin concentration was observed in most cortical and subcortical areas in senile sporadic as well as in presenile familial-type AD (Herregodts et al., 1989). Moreover, olfactory bulbectomy-induced and AD-related memory deficits were suggested to share common cellular mechanisms including dysfunction of the cholinergic system and NMDA receptors (Moriguchi et al., 2006). Therefore, OBE mice are a suitable model of sporadic AD to investigate the MF effect on memory and the $\text{A}\beta$ level in the brain (Gulyaeva et al., 2017).

Therefore, we suggest that exposure to MFs is a useful procedure to decrease the level of brain $\text{A}\beta$ in family as well as in sporadic AD. However, we think it could be applied prior to the loss of neurons in the brain, especially at the earlier stage of the AD development. In this case the weak combined MFs can be useful in order to prevent the AD. The decrease of $\text{A}\beta$ in the brain of Tg and OBE mice may be consequence of $\text{A}\beta$ decomposition under exposure of the weak combined MF (Fesenko et al., 2003). Note that there are different points of view on the effect of MF on the neurodegenerative processes. Some researchers consider the exposure to MF as a potential risk factor for neurodegenerative diseases (Garcia et al., 2008; Hakansson et al., 2003; Hug et al., 2006), whereas the others deny it (Kheifets et al., 2009). Furthermore, there is evidence of a beneficial effect of MF on the cognitive processes and the visual memory in patients with AD, Parkinson's disease and multiple sclerosis (Sandyk, 1994, 1995; Sandyk & Iacono, 1994). However, some researchers suspect that such very low strength MFs can have much of a biological influence. The use of MF opens new opportunities of treating this severe disease. Another way to increase the MF efficiency is the variation of its parameters. MFs have a broad effect on biological systems. Mid-eighties were marked with the discovery by Blackman and Liboff (Blackman et al., 1985; Liboff, 1985) of a surprising phenomenon: a low frequency alternating (AC) MF (1–120 Hz) changed free calcium concentration in nervous tissue only in the presence of a simultaneously acting static (DC) MF. The most prominent effect was observed at the AC field frequency close to the cyclotron frequency of a calcium ion. There were three unexpected features in this phenomenon: (1) the necessity of simultaneous action of DC and AC MFs, (2) the resonance effect on cyclotron frequency and (3) very small values of acting MFs, measured with tens of μT , and extremely

low frequencies of AC MF, measured with several tens of Hz. Therefore, these results caused suspicion in the scientific community, but many confirmations of these data were later obtained in studies performed on different objects and under different experimental conditions (Liboff et al., 1987; Rochev et al., 1990; Lerchi et al., 1991; Persson et al., 1992; Yost & Liburdy, 1992; Lovely et al., 1993; Smith et al., 1993; Blackman et al., 1994; Zhadin et al., 1999) which captured the interest of the scientific community about the existence of the above effects. In the mid-nineties, a series of experiments on aqueous solutions of amino acids were performed (Novikov, 1994, 1996; Novikov & Zhadin, 1994; Zhadin et al., 1998). At the cyclotron frequencies measured by several Hz, which corresponded to the investigated amino acid ions, and short-term increase in the current caused by application of the voltage to the investigated solution was revealed at very weak alteration of MF measured by tens of nT. Afterwards, the experiment and results described in the above articles were successfully reproduced in Italy (Alberto et al., 2008; Comisso et al., 2006; Del Giudice et al., 2002; Giuliani et al., 2008) and in Germany (Pazur, 2004). These results established formal grounds to test biological activity of these parameters of the combined magnetic fields.

These works confirmed the existence of such effects. Now new effects of the weak combined MFs were obtained. It was shown that MFs inhibits malignant tumor growth in experimental animals (Novikov et al., 2009). Parameters of MF were found (frequency 1, 4.4, 16.5 Hz or the sum of these frequencies; intensity 300, 100, 150–300 nT, respectively) at which this MF in combination with a collinear static MF of 42 μ T has such an effect. It was very likely due to stimulation of tumor necrosis factor production (Novoselova et al., 2001). Such kind of MFs influences the formation of reactive oxygen species (Novikov et al., 2015, 2016a, 2016b, 2016c, 2017; Ponomarev & Novikov, 2009) and hydrogen peroxide production (Ponomarev et al., 2008), changes the microenvironment of protein macromolecules in aqueous solutions (Fesenko et al., 2000), accelerates the spontaneous hydrolysis of proteins and peptides to form peptide fragments (Novikov & Fesenko, 2001), influences the fission and regeneration of the planarian (Novikov et al., 2008). Among the most likely causes of a decrease of the brain A β level observed in our study, was accelerated decomposition of the A β into soluble peptide fragments with a decreased neurotoxic effect and smaller capability of forming the insoluble aggregates (Fesenko et al., 2003; Novikov & Fesenko, 2001; Novikov et al., 2010).

Many researchers suggest that the nervous system is very sensitive to weak MF (Persinger et al., 1999, 2010;

Thomas et al., 1986; Zhadin et al., 1999). There is evidence that MF selectively activates the limbic structures of the brain, which is damaged in AD patients. Therefore our results obtained in AD Tg and OBE mice suggest that weak combined MFs with low frequency could be used as a method for cleaning of the brain from A β in patients with AD. Moreover, we suggest that such MF can be applied in preventive purposes in humans not only with high AD risk, but also for other diseases involving amyloid protein deposition in other tissues.

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