EFFCTS OF 50Hz, 300mT SINUSOIDAL MAGNETIC FIELDS ON MICE BEHAVIOR AND WIDE-SCALE GENE EXPRESSION IN THE WHOLE BRAIN

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ABSTRACT

To evaluate the effect of the power frequency magnetic fields (MF; 50 Hz, 300mTrms) on central nervous system, inbred mice (C57BL, male, 6 weeks old) were used. Both behavior and gene expression in the whole brain were studied. In the behavior experiment, the results showed no reproduced significant difference in evasion, startle response, grooming time, eating time and number of exploring behavior between control and MF exposure groups. In the gene expression experiment, at least 4,800 genes and expression sequence tags (ESTs) were expressed in the whole brain. No gene was listed as a reproduced responsive gene to the MF exposure.

INTRODUCTION

Public concern regarding the health effects of power frequency electric and magnetic fields (EMFs) has been increasing since an epidemiological study in 1979 reported an increased cancer risk in children living near power lines. Mechanisms of biological effects, however, have not been forthcoming, and most laboratory results are in disagreement with each other. In our previous studies, we have used strong magnetic fields (MFs; 50Hz, up to 300mTrms) to find conspicuous biological effects in model organisms. However, mutagenic or co-mutagenic effects, protein synthesis including stress response proteins, and genome scale gene expression change in microbial cells were not found. These results suggest that the power frequency MF could not affect the fundamental biological system.

The International Commission on Non-Ionizing Radiation Protection (ICNIRP) summarized an agreement that the central nervous system (CNS) is one of reliable organs to respond an exposure to the low frequency MFs [1]. Therefore, we have investigated the effects on CNS of rodents. If the serious effects on CNS are caused by the exposure to the strong magnetic field, the effects could be detected by the change in behavior of mouse and/or by the change in genome-wide gene expression in the CNS.

MATERIALS AND METHODS

Exposure System and Animals

The MF exposure system (Fig. 1) is possible to generated high density of 50 Hz up to 300mTrms MF for vertical axis. The field variation was below 5% in the 10cm x 10cm x 10cm-cube exposure space. Vibration and heat from the coil were carefully removed by using a separated incubator stand and using water jacketed plastic incubator.

We used inbred mouse (C57BL, male, 6 weeks old) purchased from CLEA Japan, Inc.. For each experimental set, ten mice were used. The weight was in the range of 17g to 21 g. For quarantine and acclimatization, the mice were kept for one week before experiments. All of the animal experiments were performed with the approval of the local committee on animals' experiments, installed by the Central Research Institute of Electric Power Industry according to the Japanese Law for Handling of Experimental Animals.

Fig.1 MF exposure system

Exposure Conditions

Ten mice were randomly divided into two groups for MF exposure and control, after quarantine. The mice were kept without food or water for 24 hr before the exposure

experiment, and a piece of 1.5 % agar (ca.10cm^3) was supplied as food or water source during the MF exposure. Each mouse in the MF exposure group was exposed individually to 50Hz, 300 mTrms MF in clear incubator (10cm x 10cm x 10cm-cube incubation space). The calculated induced current in brain and eye was 47.1 - 70.7 mA/m^2 (0.2 S/m, 1.0 -1.5 cm diameter) and 14.1 mA/m^2 (0.2 S/m , 0.3cm diameter), respectively. The temperature in incubator was controlled at 25 ºC, and the incubator was located in the center of the MF generation system. Each mouse in the control group was sham exposed (no MF exposure) in the same incubator. For behavior experiment, four experimental sets were used. For gene expression analysis, two experimental sets were used. The exposure design was shown in Fig. 2.

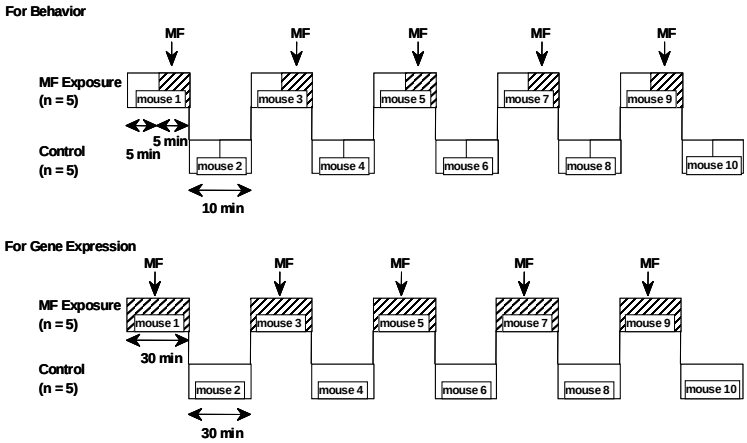

Fig.2 MF exposure design for each experiment

Observation of Behavior

The exposure condition was shown in Fig.2. This 5 min exposure was enough to study mouse behavior focused in this research, because the mouse behavior was very stable between 5min and 30min incubation. The behavior of each mouse was observed and recorded by using video camera. After the video recording, we checked escape or startle behavior by the video observation. We also checked eating time, grooming time and number of exploration by blind trial. The differences, between pre-exposure and exposure in same mouse, or between exposure and control groups in same experimental set, were estimated by a statistical analysis of two sample t-test with Welch's correction. Additionally, when the behavior was apparently changed by the exposure, the mouse was exposed to second MF to check the reproducibility and the existence of hyper sensitive individuals.

Gene Expression Analysis

After the 30min MF exposure shown in Fig. 2, the mouse was immediately scarified by cervical dislocation, and the whole brain was removed. The brain was frozen in liquid nitrogen until RNA extraction. Total RNA was extracted by acid-guanidine-phenol-chloroform method. The five brain samples in each group were separately minced and mixed by grinder. The quality of the RNA was determined by A260/280 and denatured gel electrophoresis. Messenger RNA was obtained by using OligoTex dT (TaKaRa Co., Japan). The mRNA samples for two experiment sets were sent to Incyte Genomics Inc., USA, to get gene expression data via KURABO Corp., Japan. We used three microarrays (Array 1 to 3) for the two experiment sets (Exp.1 and 2) to check the reproducibility. The gene expression data was obtained by Mouse GEM I (Gene Expression Microarray I; Incyte Genomics Inc., USA). The spotted microarray has around 9,000 spots which include 2,800 known genes, 5,900 ESTs and control genes. We obtained gene expression data as an Excel data sheet from the company.

Data analysis was done with the following estimation criteria; (1) signal was normalized as Balanced Signal, (2) data points with S/N < 2.5 in both fluorescence was deleted, (3) pick genes which have the expression difference above 1.74 in all array as a candidate of respondent gene to MF exposure. The company suggests that expression differences above 2.0 times may be significant. Company also shows that 1.74 times difference was obtained from same sample comparison. We think that the value would be minimum threshold for the expression difference in this experiment. After normalization, other bioinformatic analysis (such as principal component analysis;PCA) were done by using Excel and GeneSpring (Silicon Genetics Inc.,USA)

RESULTS

Effects on Behavior

No significant change in mouse behavior, such as evasion, astonishment, and exploration was found by video observation, except for one mouse. However, the mouse's behavior was not reproduced in the second MF exposure. Fig.3A shows the effect of the MF exposure on the total eating time. Some statistical differences were found between

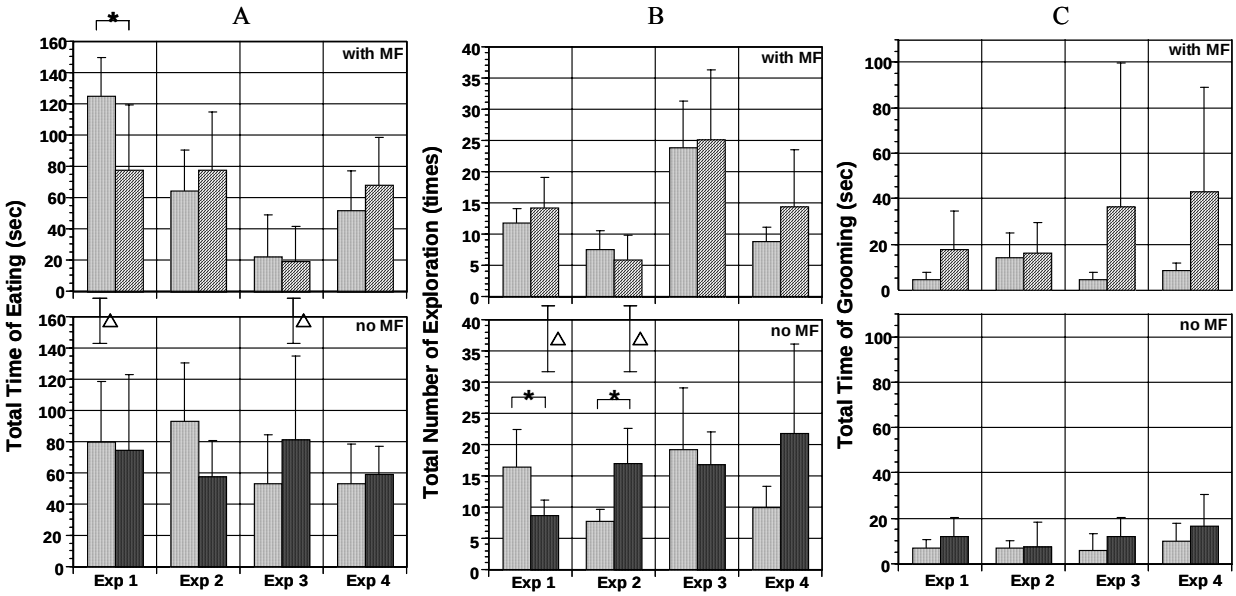

Fig.3 Comparison of mouse behavior with or without MF exposure

A; total eating time, B; exploration number, C; grooming time

Upper (with MF) ;no MF in first 5 min, ; MF exposure in second 5 min

Lower (no MF) ;no MF in first 5 min, ; no MF in second 5 min

*；P<0.05, △；P<0.05 , significant difference between exposure and control groups.

MF exposure and no exposure condition, but the differences were not reproduced. No clear trends of the differences were found by the exposure. Fig.3B shows the effect of the MF exposure on the number of exploration. Some statistical difference between MF exposure and no exposure condition was found. However, the difference was not reproduced, and the direction of the change was inconsistent. Fig.3C shows the effect of the MF exposure on the total grooming time. In the MF exposure group, the total time of grooming tended to increase by the MF exposure. However, there was no statistically significant difference in the grooming time. These results showed that no statistical significant or reproduced effect on mouse behavior can be found by the exposure to 50Hz, 300mT MF for 5 min.

Effect on Gene Expression

Approximately 5,900 genes (ca. 95% of expected total genes) could have enough signal intensity to be analyzed. After normalization, around 4,400 to 5,500 genes or ESTs still remained for further analysis. These transcripts were glyceraldehyde-3-phosphate dehydrogenase, staufen (RNA-binding protein) homolog 2 (Drosophila), and tubulin etc..
There were some genes which had over 1.74 times expression difference (Exposure/Control) in at least one array experiment. For the candidates of up-regulated transcripts by the MF exposure, ten genes and ESTs were found as shown in Table 1. However, there was no transcript which had over 2-fold difference of the expression level in all three arrays. For the candidates of down-regulated transcripts by the MF exposure, eighteen kinds of transcripts were found as shown in Table 2. Similarly, there was no transcript which had a reproduced expression difference with over 2-fold in all three arrays. Principal component 1 (PC1) showed that expression pattern of the control genes on each array were

Table 1 Candidates as up-regulated gene by MF exposure

GeneName	Incyte Gene ID	Balanced Ratio		
		Array 1, Exp.1	Array 2, Exp.1	Array 3, Exp.2
ESTs	-137114	nd	nd	1.7
sulfotransferase, hydroxysteroid preferring 2	-136559	2.4	1.9	-1.3
ESTs	-130398	2.3	1.4	-1.1
ESTs	-133655	2.1	2.1	1
Murine (DBA/2) mRNA fragment for gag related peptide	-135825	2	1.8	-1.1
histocompatibility 2, Q region locus 7	-133559	1.9	1.8	1
ESTs	-137227	1.8	nd	1
ESTs	-128919	1.7	1.6	-1.1
xanthine dehydrogenase	-128746	1.5	1.9	-1
Mus musculus clone L5 uniform group of 2-cell-stage gene family mRNA,	-133149	1.5	1.7	1.1

nd; not detected

Table 2 Candidates as down-regulated gene by MF exposure

GeneName	Incyte Gene ID	Balanced Ratio		
		Array 1, Exp.1	Array 2, Exp.1	Array 3, Exp.2
staufen (RNA-binding protein) homolog 1 (Drosophila)	-133074	-3.4	-1.1	1
adrenomedullin receptor	-136038	-2.3	-3.1	-1
ESTs	-130143	-2.2	-1.6	-1.8
ESTs	-133432	-1.9	-1.7	-1.4
ESTs	-134753	-1.8	-1.4	-1.4
peripheral myelin protein, 22 kDa	-134852	-1.8	-1.5	-1.5
ESTs, Weakly similar to IAP3_MOUSE INHIBITOR OF APOPTOSIS PROTEIN 3	-133224	-1.8	-1.6	-1.4
T-cell specific GTPase	-135036	-1.8	-1.6	-1.5
RAB1, member RAS oncogene family	-133870	-1.8	-1.7	-1.7
interferon gamma receptor	-135254	-1.7	-1.4	-1.2
Public domain EST	-135025	-1.7	-1.4	-1.3
ESTs, Highly similar to Y274_HUMAN HYPOTHETICAL PROTEIN KIAA0274 [H.sapiens]	-135247	-1.7	-1.5	-1.2
Public domain EST	-135065	-1.7	-1.5	-1.3
ESTs, Highly similar to p20-CGGBP [H.sapiens]	-136529	-1.7	-1.7	-1.2
kinesin heavy chain member 1A	-132856	-1.7	-1.8	1
Public domain EST	-131677	-1.5	-1.5	-1.7
coproporphyrinogen oxidase	-130603	-1.5	-1.7	-1.2
Zinc finger protein 118	-133996	nd	nd	-1.7

nd; not detected

very similar. The expression ratio in Array 1-Exp.1 and Array 2-Exp.1 was also very similar to each other because both arrays used same mRNA sample. No gene was listed as a reproduced responsive gene to the MF exposure.

DISCUSSION

There are some reports on animal behavior by the exposure to extremely low frequency MFs as shown in the NIEHS's report [2]. The report showed that no experimental evidence that mammals can perceive MFs at an environmentally relevant flux density, and also showed that too few experiments are available to reach a conclusion on the effect of MFs on avoidance and aversion reactions. In this study, we have investigated the effect of short time exposure to a strong MF (300mT, 50Hz) on the behavior of the inbred male mice. The results show no apparent effects on startle and escape, and no reproduced statistical significant effects on eating time, grooming time and numbers of exploration.

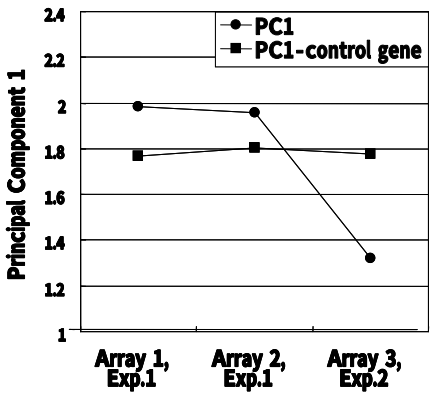

Fig.4 Principal component analysis of each gene expression data.

Many reports have shown inconsistent results on the effect of MFs on gene expression as shown in the NIEHS's report [2]. In our previous study, genome scale gene expression in yeast did not change by the strong MFs (10-300 mT, 50Hz) [3]. So, the power frequency MF could not affect the fundamental biological system such as stress response. Moreover, no such gene expression difference was found in mouse brain by the MF in this study. While Lai and Singh reported the DNA strand breaks in brain cells of rat was induced by the MF exposure (0.5mT, 60Hz, for 2h). If a similar phenomenon was found by the exposure to the stronger MF (300mT, 50Hz, for 0. 5h), the gene expression difference in DNA repair enzymes could be detected. However, in this study, the expression level of such type of enzymes could not be increased by the MF exposure. Around 1/3 of mouse transcripts were tried to test for the responsive to the MF, however no transcript was listed as reproduced significant ones. These results suggest that the 50Hz, 300 mT MF, which could be at least several ten thousands times greater than that at residential environment, could not affect the behavior and the genome scale gene expression of mouse CNS.

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