

図 14 亜慢性曝露を行った脳のスライス像  
(上) HE 染色透過像  
(中) FITC-Albumin 蛍光像  
(下) RCA-1 蛍光染色像  
全て同一部位の像を示す。

## 研究成果の刊行に関する一覧表

## 書籍

著者氏名	論文タイトル名	書籍全体の 編集者名	書 籍 名	出版年	ページ
Suzuki Y, Baba M, Taki M, Fukunaga K, Watanabe S.	Visualization of energy absorption due to high frequency electromagnetic field within tissue equivalent gel phantom.		Bio-Dielectrics: Theories, Mechanisms and Applications, Annual Conference 2006 of the Dielectrics Group of the Institute of Physics, Leicester, UK	2006	21
Masuda H, Ushiyama A, Hirota S, Watanabe H, Wake K, Watanabe S, Yamataka Y, Taki M, Ohkubo C.	Real-time measurement of brain microcirculation during RF-EMF exposure using an "8"-shaped loop antenna.		Abstract Collection of the 27th Annual meeting of Bioelectromagnetic Society	2005	170
Ushiyama A, Masuda H, Hirota S, Watanabe H, Wake K, Watanabe S, Yamataka Y, Taki M, Ohkubo C.	Development of real-time measuring system for blood-brain barrier function and acute effects on BBB function by RF exposure to rat brain.		Abstract Collection of the 27th Annual meeting of Bioelectromagnetic Society	2005	510-511
Ushiyama A, Masuda H, Hirota S, Watanabe H, Wake K, Watanabe S, Yamataka Y, Taki M, Ohkubo C.	Acute effects on blood barrier function by RF-EMF exposure to rat brain in vivo.		Abstract book of the 28th General Assembly of International Union of Radio Science	2005	KP.6(01436)

Masuda H, Ushiyama A, Hirota S, Watanabe H, Wake K, Watanabe S, Yamanaoka Y, Taki M, Ohkubo C.	Real-time measurement of brain microcirculation during RF-EMF exposure using an "8"-shaped loop antenna.		Abstract book of the 28th General Assembly of International Union of Radio Science	2005	K03.5(097)
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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
大久保千代次	電磁界の健康リスク評価とWHOの動向	生体医工学	43(3)	369-374	2005
多氣昌生、渡辺聡一、和氣加奈子	携帯電話の生体安全性	生体医工学	43(3)	375-387	2005

## 5.4

### Visualization of energy absorption due to high frequency electromagnetic field within tissue equivalent gel phantom

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Safety of high frequency (HF) electromagnetic fields (EMF) is commonly discussed in terms of specific absorption rate (SAR) [1]. Tissue equivalent phantoms are useful for the dosimetry on energy absorption of HF-EMF exposures. We have developed a new technique to evaluate HF-EMF energy absorption based on the visualization of three-dimensional (3D) temperature distribution by means of micro-capsulated thermo-chromic liquid crystal (MTLC)

In this study, MTLC is used as temperature sensor. The diameter of the capsule, which is made of urea resin, is about 20 to 30 micrometer. When the visible light is projected on MTLC, the scattered light with proper wavelength, depends on the temperature of the capsule liquid crystal, is observed. The wavelength becomes shorter with the increase of temperature. In other words, the scattered light changes in its color from red to purple with the increase of temperature [2]. MTLC are used to suspend uniformly in the transparency substances such as liquid, gel or solid.

High-molecular gel constructed from "carrageenan", which is extracted from seaweed and has high transparency, is used as the substrate of the tissue equivalent phantom to prevent convection. The dielectric properties of the phantom are adjusted by mixing sucrose, propylene glycol (PG) and potassium chloride (KCl). Complex permittivity plots of the dielectric properties of phantoms are shown in Fig. 1. In this graph, horizontal axis and vertical axis indicate relative permittivity ( $\epsilon'(\omega)$ ) and loss ( $\epsilon''(\omega)$ ), respectively. Each marker shows the complex permittivity at 500MHz, 900MHz, 1.5GHz, 1.95GHz, and 2.45GHz, respectively. Solid line indicates muscle property, that is obtained by the parametric model of biological tissues [3]. Dielectric properties of the phantom based on carrageenan can be controlled by varying the concentration of sucrose, PG and KCl. We can adjust a value of complex permittivity of phantom to that of muscle at 900MHz and 1.5GHz.

We perform HF-EMF exposure experiments using tissue equivalent phantom. Figure 2 shows one of results exposed to HF-EMF. A dipole antenna is used to irradiate tissue equivalent phantom containing MTLC at the frequency of 1.5GHz. The temperature distribution, which is visualized by scattering light from red to purple, on the plane lit up by the slit light is obtained clearly. The temperature distributions on other planes are also visualized by moving the slit light location. This technique enables non-destructive and non-invasive real-time measurement. These observations imply the possibility measuring 3-D distribution of HF-EMF energy absorption within dielectric materials by reconstructing measured data at each plane.

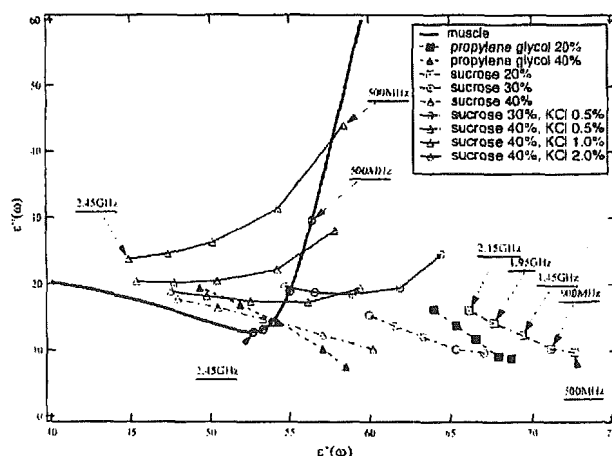


Figure 1: Complex permittivity plots for carrageenan phantoms.

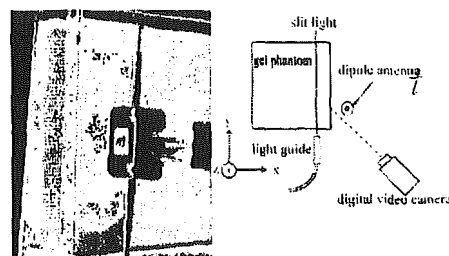
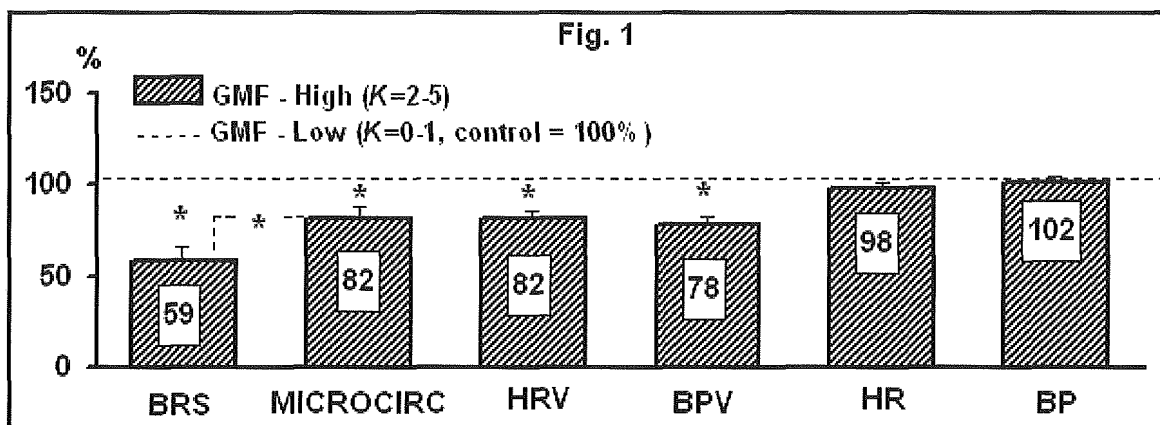


Figure 2: Visualization of temperature distribution in the tissue equivalent phantom.

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Microcirculatory blood flow, similarly with BRS, HRV and BPV, significantly decreased on days with intense geomagnetic activity ( $K = 2-5$ ) compared with quiet days ( $K = 0-1$ ), Fig 1. The decrease was significantly larger for BRS than for MPPG suggesting that GMF primarily attenuated arterial baroreflex vascular control mechanism followed by decrease in microcirculation. The reduction of the microcirculatory blood flow did not accompany changes in BP supporting GMF direct baroreflex-mediated microcirculatory response then systemic BP - microcirculatory blood flow relationship.

**CONCLUSIONS:** Most likely, GMF affects central hypothalamic and brain stem baroreflex regulatory centers [3] enhancing the effector limb of sympathetic response, leading to increase in vascular tone and to reduction of microcirculatory blood flow in target organs. We recommend in days with intense geomagnetic activity, and especially during geomagnetic storms, intensify the therapy of ischaemic cerebral and heart diseases to improve microcirculation in brain tissue and in myocardium. Verapamil, a  $Ca^{2+}$  channel blocking agent should be especially effective, due to its favorable effect on BP, HR, microcirculatory blood flow and for its potential specific magneto-protective properties [4].

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15-2

**REAL-TIME MEASUREMENT OF BRAIN MICROCIRCULATION DURING RF-EMF EXPOSURE USING AN "8"-SHAPED LOOP ANTENNA.** H. Masuda<sup>1</sup>, A. Ushiyama<sup>1</sup>, S. Hirota<sup>1</sup>, H. Watanabe<sup>2</sup>, K. Wake<sup>2</sup>, S. Watanabe<sup>2</sup>, Y. Yamanaka<sup>2</sup>, M. Taki<sup>3</sup>, C. Ohkubo<sup>1</sup>. <sup>1</sup>Dept of Environmental Health, National Institute of Public Health, Saitama, Japan., <sup>2</sup>Biomedical EMC Group, EMC Center, Wireless Communications Dept, National Institute of Information and Communications Technology, Tokyo, Japan., <sup>3</sup>Dept of Electrical Engineering, Graduate School of Engineering, Tokyo Metropolitan Univ, Tokyo, Japan.

**OBJECTIVE:** Few studies have directly observed brain microcirculation during exposure to radio frequency electromagnetic fields (RF-EMF). Our previous study showed no effects of RF-EMF

exposure on blood-brain barrier (BBB), plasma velocity or vessel diameter, as microcirculatory parameters in rat brain using a cranial window method. However, the changes in these parameters were measured just after RF-EMF exposure, but not during exposure. To simultaneously perform measurements of rat brain microcirculation and RF-EMF exposure to a local cerebral region just under the cranial window, we developed a new type of antenna, "8"-shaped loop antenna. Aim of the present study was to investigate whether RF-EMF exposure induces reversible effects on brain microcirculation observable only during RF-EMF radiation.

**METHODS:** Twenty male Sprague-Dawley rats ( $456 \pm 7$  g) were used. The rats were divided into two groups: RF group was exposed to RF-EMF and Sham group was not exposed to any RF-EMF. All rats were subjected to cranial window implantation and intravital-microscopic observation under pentobarbital anesthesia with a cocktail of ketamine and xylazine. The pial microcirculation within cranial windows were observed using a fluorescent microscope equipped with an ICCD camera. In order to measure the microcirculatory parameters, several types of fluorescent dyes were administered via the tail vein. Rat heads were locally exposed to 1,439MHz electromagnetic near-field TDMA (time division multiple access) signal for PDC (Personal Digital Cellular, Japanese cellular telephone standard) systems by an "8"-shaped loop antenna placed 4 mm over the cranial window. RF-EMF exposure was maintained at a brain averaged SAR (2.0W/kg). During 80 min experimental period including 50 min RF-EMF exposure, three microcirculatory parameters (BBB-function, plasma velocity and vessel diameter) in pial venules were measured every 10 min, and the results were compared between RF and Sham group.

**RESULTS:** We were able to direct observe pial microcirculation through the cranial windows during RF-EMF exposure to rat brain. But no changes in any of the three microcirculatory parameters were elicited by RF-EMF exposure. No extravasation of fluorescence dye, FITC-Dx (MW: 70000) from the pial venule was detected in the two groups. Although plasma velocity increased during the experimental period, there were no differences between the two groups. Furthermore, vessel diameter showed no changes throughout experimental period.

**CONCLUSION:** These results reveal no effects of RF-EMF exposure, at least not on BBB-function, plasma velocity or vessel diameter in rat brain microcirculation, under these conditions.

*This study was financially supported by The Committee to Promote Research on the Possible Biological Effects of Electromagnetic Fields, Ministry of Internal Affairs and Communications, Japan.*

15-3

**EFFECTS OF 915 MHZ EXPOSURE ON THE INTEGRITY OF THE BLOOD- BRAIN BARRIER.** J. S. McQuade<sup>1</sup>, J. H. Merritt<sup>2</sup>, O. Rahimi<sup>3</sup>, S. A. Miller<sup>2</sup>, T. Scholin<sup>2</sup>, A. L. Salazar<sup>1</sup>, M. C. Cook<sup>1</sup>, P. A. Mason<sup>2</sup>. <sup>1</sup>General Dynamics, Advanced Information Engineering Services, Brooks City-Base, TX, <sup>2</sup>Air Force Research Laboratory, Human Effectiveness Directorate, Directed Energy Bioeffects Division, Brooks City-Base, TX, <sup>3</sup>Univ of Texas at San Antonio, San Antonio, TX.

**OBJECTIVE:** To determine whether exposure to 915 MHz alters the integrity of the blood brain barrier (BBB).

**METHODS:** Transverse Electromagnetic (TEM) cells made by Dr. Lars Malmgren that had the same dimensions as the TEM cells used by Salford et al. (1992) were used to expose freely moving Fisher male rats (250-300 gm) for 30 minutes to 915 MHz (continuous wave) at whole-body specific absorption rate (SAR) values of 20, 2, 0.2, 0.02, and 0.002 W/kg. Other animals were exposed to a 915



Methods: The dissected sciatic nerve bundle of a bullfrog, *Rana catesbeiana*, was placed on platinum electrodes in an acrylic moist nerve chamber to measure compound action potentials (CAPs). The chamber was positioned at the center of a superconducting magnet (Oxford, U.K.), 100 mm in diameter and 700 mm long, which produced magnetic fields of up to 8T at its center. The ambient temperature in the magnet was maintained at 24°C ± 0.2 °C by circulating temperature-regulated water in a coiled tube. The nerve bundle was then selectively stimulated under a stimulus intensity of 4.0 mA-16.0 mA for a duration of 1.0 ms and exposed to 8 T SMF for the long axis of the nerve bundle parallel to magnetic field and the CAPs such as A $\beta$ -fiber (37 m/s), A $\delta$ -fiber (3.3 m/s) and C-fiber (0.5 m/s) were measured during 8T SMF exposure. The CAPs were measured for two groups, the control group (without SMF) and the exposed group (with SMF for 3 hours)

Results and Discussion: The experimental results show that 8T SMF enhanced the CAP of A $\beta$ -fiber in the relative refractory period by 10% but did not affect conduction velocity of A $\beta$ -fiber, A $\delta$ -fiber and C-fiber, and nerve fatigue of A $\beta$  fiber. These results indicate that SMF affect the threshold of excitatory membrane during refractory period, hypothesizing that channels associated with nerve fibers are affected by SMF. We need more experiments using rats to investigate the effects of SMF on the nerve function of human.

P-C-123

#### DEVELOPMENT OF REAL-TIME MEASURING SYSTEM FOR BLOOD-BRAIN BARRIER FUNCTION AND ACUTE EFFECTS ON BBB FUNCTION BY RF EXPOSURE TO RAT BRAIN.

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**OBJECTIVE:** Use of cellular phones is increasing dramatically. Concerns about possible biological and health effects of radio-frequency (RF) signals have been raised, although they are based on limited scientific evidence. Particularly, effect(s) on the blood-brain barrier (BBB) is a controversial issue. BBB prevents high molecular weight substances in the blood from getting into the brain, while nutrients and metabolites are allowed to pass the barrier. A few positive effects of RF exposure are reported, however, most results appear to be due to thermal effects and attempts to replicate studies have been unsuccessful. In these reports, histological approaches were examined, however, the time delay after RF exposure to specimen fixation may produce artifacts. Therefore, we set about to develop a real-time measuring system for blood-brain barrier function using a micro-perfusion method, which enables us to collect cerebrospinal fluid (CSF) continuously. Moreover, we examined high intensity RF exposure to rat brains and monitored albumin concentration in CSF during exposure.

**METHODS:** *Animals and surgical procedure* Experiments were performed on male Sprague-Dawley rats (8-12 weeks old). All surgical procedures were performed under sterile conditions. Anesthesia was initiated with a mixture of ketamine and xylazine (9:1). Rats were set on a stereotaxic instrument, and an incision was made in the rat's scalp to expose coronal sutures. A hole was made at 0.8 mm caudal and 1.3 mm lateral to the sagittal suture. A guide cannula (NG-8FS, Eicom Co., Kyoto) was introduced to the hole to reach one of the lateral ventricles and secured to the skull with acrylic dental cement. A dummy cannula (ND-8, Eicom Co.) was inserted into the guide cannula until experiment. At the end of experiment, Evans blue dye was injected into the lateral ventricle to confirm the placement of the cannula.

**Experimental protocol** After a 48hrs recovery period, rats were anesthetized, and a push pull cannula (NDP-I-8-01FEP, Eicom Co.) inserted into the guide cannula. The in-line and the out-line of cannula were connected to the push-pull pump unit (EP-70, Eicom Co.). At the beginning of RF exposure, FITC-labeled albumin was injected into caudal vein and CSF perfusion was started at a rate of 1 µl/min. Fluorescence intensity of FITC-albumin in perfusate was monitored by spectrofluorometry (FP-6500, Jasco Co., Tokyo).

**RESULTS:** The sensitivity for FITC-albumin of this system was less than 1 ng/ml in CSF and is sufficient to detect albumin leaked from blood into CSF. After 1,439 MHz RF exposure for 30 minutes at 35W/kg (average local SAR for brain) using a loop antenna, the fluorescence intensity increased indicating BBB function affected by RF exposure.

**CONCLUSION:** In this study, we developed a new system, which enables us to quantify BBB function in vivo with high sensitivity, and can be applied during exposure. Using this system, we detected leakage of albumin from blood under high dosage conditions. More detailed study is currently in progress.

## **In vitro – cellular**

P-C-126

### **CHARACTERIZATION OF CONNEXINS IN NEURONES SUBMITTED TO APPLIED EMF.**

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**INTRODUCTION:** We have developed elsewhere a physical model which explains the ability of a sinusoidal magnetic field (AMF) to induce oscillatory, recruitment and eventual synchronizing activity in molluscan pacemaker neurones (1,2,3). Nonetheless we should take into account that the so called electric synapses could also be mediators of such an effect. We are trying to evaluate the relative contribution, of both parameters: magnetic field and gap-junctions in the synchronizing process.

**OBJECTIVES:** The objectives of this work were: 1) to characterize the expression of connexins and glial fibrillary acidic protein (GFAP) in control and exposed samples to ELF-AMF of *Helix aspersa* brain ganglia by immunostaining; 2) to study the ultrastructural interconnections with electron microscopy.

**METHODS:** The brain ganglia were placed between a pair of Helmholtz coils immersed in molluscs Ringer solution. The AMF exposure conditions were coincident with the ones applied to test the bioelectric activity (3) with exposure times of 30 min. The control samples were maintained in Ringer solution for times of 15min (Ha 104), 30min (Ha 114, Ha 12) and 60 min (Ha 109). The exposed samples were: Ha 16, 1mT, 4 Hz; Ha 24, 10 µT, 8.3 Hz; Ha 30, 10 µT, 217 Hz; Ha 54, 1 mT, 8 Hz. The immunocytochemistry (IC) EnVision® (DAKO) method was applied. Being the antisera specific for mammals there exists the possibility of a certain non-specific reaction, so that we are studying the expression of connexin 26 (Chemicon) (specific of neurons), enolase (Dako) (specific of neurons) and connexin 43 (Chemicon) and glial fibrillary acidic protein (GFAP, Dako), both specific for astrocytes.

**ACUTE EFFECTS ON BLOOD BARRIER FUNCTION DUE TO HIGH INTENSITY  
RF-EMF EXPOSURE OF THE RAT BRAIN *IN VIVO***

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**ABSTRACT**

To explore the biological effects on the blood barrier of radio-frequency electromagnetic field (RF-EMF) exposure, we developed a real-time measuring system during RF-EMF exposure, which was based on a micro-perfusion method and enables the monitoring of intensity changes in fluorescent-labeled albumin concentration from collected cerebrospinal fluid (CSF). Following 1.5 GHz RF-EMF exposure for 30 minutes at 35W/kg (average brain SAR), the fluorescent intensity was increased, suggesting that albumin from circulating blood leaked into CSF due to a blood barrier disorder. However, this effect was not observed in the sham exposure group. The effect was probably evoked by thermal effect due to high intensity RF-EMF exposure. Further study is ongoing.

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This study was financially supported by Ministry of Health, Labour and Welfare, Japan

## INTRODUCTION

Possible health effects of weak RF-EMF have been discussed worldwide. However, further scientific evidences are needed for better health risk assessment, particularly, the effects on the blood-brain barrier (BBB) which are still not clear due to the variety of experimental designs. One of the main problems on BBB study is that there are few methods which are able to monitor the barrier function on real time *in vivo*. In this study, we developed a real-time measuring system for blood-cerebrospinal fluid (CSF) barrier function which defined same as BBB in a broad sense. To collect CSF continuously, we employed micro-perfusion method to the measuring system. We examined high intensity RF-EMF exposure to the rat brain and monitored albumin concentration in CSF during exposure, as a pilot study.

## MATERIALS AND METHODS

### Animals and surgical procedure

Experiments were performed on male Sprague-Dawley rats weighing between 300 and 400 g. All surgical procedures were performed under sterile conditions. Anesthesia was initiated with a cocktail of ketamine and xylazine (9:1). Rats were set on a stereotaxic instrument, and an incision was made in the rat's scalp to expose coronal sutures. A hole was made at 0.8 mm caudal and 1.3 mm lateral to the sagittal suture. A guide cannula (NG-8FS, Eicom Co., Kyoto) was introduced to one of the lateral ventricles through the hole and secured to the skull with acrylic dental cement. At the end of experiment, Evans blue dye was injected through the cannula into the lateral ventricle to confirm the placement of the cannula.

### Experimental protocol

After a 48 hour recovery period, the rats were again anesthetized, and a push pull cannula (NDP-I-8-01FEP, Eicom Co., Kyoto, Japan; Fig.2) was inserted into a guide cannula. The inlet and the outlet of the cannula were connected to the push-pull micro perfusion pump unit (EP-70, Eicom Co.) which enabled the collection of CSF continuously. At the beginning of RF-EMF exposure, FITC-labeled albumin (FITC-albumin) was injected into caudal vein and CSF perfusion was started at a rate of 1  $\mu$ l/min. Fluorescence intensity of FITC-albumin in perfusate was monitored by using spectrofluorometry (FP-6500, Jasco Co., Tokyo) under the condition of excitation /emission wave length at 490/515nm . Experimental setup was summarized in Fig. 1.

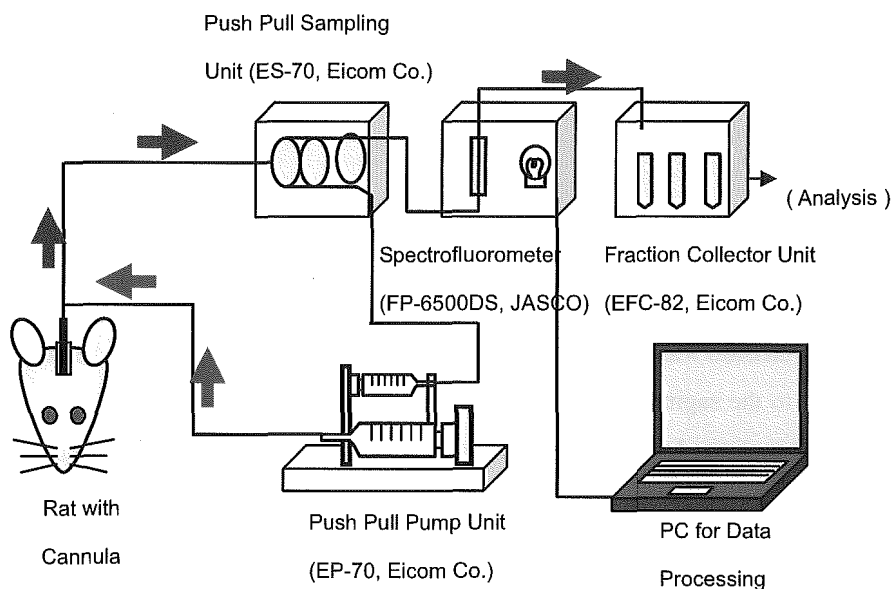
### Exposure conditions

Each rat was exposed to RF-EMF (1,439 MHz) at 35W/kg (average brain SAR) for 30 minutes using a loop antenna (Fig.3). In the sham exposure group, rats were kept in an anechoic chamber for 30 minutes without RF-EMF exposure. This exposure conditions are extremely higher than the radiofrequency safety guidelines, i.e., 2 W/kg for the general public. Fluorescence intensity in perfusate was monitored for 180 minutes from the beginning of the exposure.

## RESULTS and DISCUSSION:

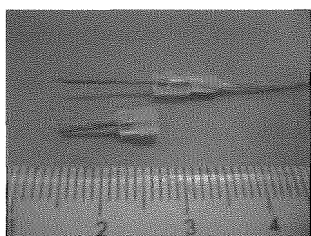
In this study, we developed a new system, which makes it possible to quantify blood barrier function in vivo with high sensitivity, and can be applied during exposure simultaneously. The fluorescence intensity observed by this system was purely dependent on the concentration of FITC-albumin in perfusate. Therefore, the fluorescence intensity can be recognized as one of the parameters for brain barrier abnormality because, under normal conditions, albumin

concentration in cerebrospinal fluid was physiologically limited and it was about 200 times lower than that in the blood. The fluorescence intensity increase was recognized in the exposure animals but was not in the sham animals. This indicates that blood barrier function was affected by high intense RF-EMF exposure probably due to its thermal effects. More detailed analysis is ongoing in order to clarify whether this effect is only a thermal effect or inherent to electromagnetic exposure.

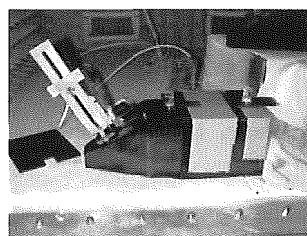


**Fig.1 Schematic diagram of developed system for real-time monitoring of blood barrier function**

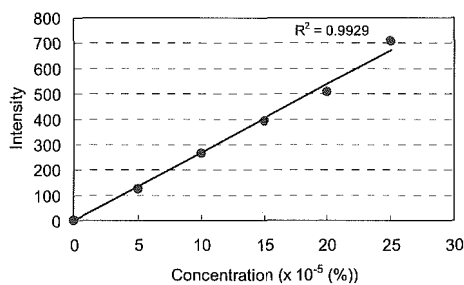
Artificial CSF flow into the lateral ventricle via inlet of cannula (red arrow) and perfusate flow out via outlet of cannula (blue arrow).



**Fig 2 Assembly of push-pull cannula for rat**  
Upper is an inner cannula which includes in/out route and lower is a guide cannula



**Fig 3 Overview of RF-EMF exposure setup**  
Rat under anesthesia is fixed in a folder and RF-EMF is exposed by using a loop-antenna.



**Fig 4 Relationship between the concentration of FITC-labeled Albumin and fluorescence intensity**  
Observed fluorescence intensity shows linearity against the concentration of FITC Albumin.

# REAL-TIME MEASUREMENT OF BRAIN MICROCIRCULATION DURING RF-EMF EXPOSURE USING AN “8”-SHAPED LOOP ANTENNA.

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## ABSTRACT

Aim of present study was to investigate whether radio frequency electromagnetic field (RF-EMF) exposure induces reversible effects on brain microcirculation observable only during exposure. Using our developed “8”-shaped loop

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antenna, we exposed rat equipped with a cranial window to RF-EMF at 2W/kg of averaged SAR of target area just under the window. Three microcirculatory parameters, BBB permeability, vessel diameter and plasma velocity showed no significant differences between sham and RF-EMF exposed rats even during exposure period. These results reveal no reversible effects of RF-EMF exposure, at least not on these three parameters in rat brain microcirculation, under these conditions.

## **INTRODUCTION**

Few studies have directly observed brain microcirculation during exposure to radio frequency electromagnetic fields (RF-EMF). Our previous study showed no effects of RF-EMF exposure on blood-brain barrier (BBB), plasma velocity or vessel diameter, as microcirculatory parameters in rat brain using a cranial window method<sup>[1]</sup>. However, the changes in these parameters were measured just after RF-EMF exposure, but not during exposure. To simultaneously perform measurements of rat brain microcirculation and RF-EMF exposure to a local cerebral region just under the cranial window, we developed a new type of antenna, “8”-shaped loop antenna<sup>[2]</sup>. Aim of the present study was to investigate whether RF-EMF exposure induces reversible effects on brain microcirculation observable only during RF-EMF radiation.

## **MATERIAL AND METHODS**

### **INTRAVITAL MICROSCOPY**

Twenty male Sprague-Dawley rats (456±7 g) were used. The rats were divided into two groups: RF group was exposed to RF-EMF and Sham group was not exposed to any RF-EMF. All rats were subjected to cranial window implantation and intravital-microscopic observation under pentobarbital anesthesia with a cocktail of ketamine and xylazine. The pial microcirculation within cranial windows was observed using a fluorescent microscope equipped with an ICCD camera. In order to measure the microcirculatory parameters, several types of fluorescent dyes were administered via the tail vein.

### **RF-EMF EXPOSURE**

Rat heads were locally exposed to 1,439MHz electromagnetic near-field TDMA (time division multiple access) signal for PDC (Personal Digital Cellular, Japanese cellular telephone standard) systems by an “8”-shaped loop antenna placed 4 mm over the cranial window. RF-EMF exposure was maintained at a brain averaged SAR (2.0W/kg).

### **EXPERIMENTAL PROTOCOL**

During 80 min experimental period including 50 min RF-EMF exposure, three microcirculatory parameters in pial venules, BBB-function, plasma velocity and vessel diameter were measured every 10 min, and the results were compared between RF and Sham group. At the end of experiments, we fixed the rat brains with 4% paraphormaldehyde

and examined albumin leakage using immunohistochemistry. The statistical analysis was performed by Student's *t*-test or ANOVA.

## RESULTS

### AVERAGED SAR OF TARGET AREA

Fig. 1 shows SAR distribution of rat phantom model at 1 W of input power into the 8-shaped loop antenna. The SAR distribution was localized at a parietal region of brain. When a averaged SAR of target area just under the cranial window was 2.0 W/kg, the whole brain and whole body averaged SARs were 0.37 and 0.02 W/kg, respectively.

### MICROSCOPIC OBSERVATION DURING RF EXPOSURE

Using an intravital fluorescence microscopy and an 8-shaped loop antenna enable us to directly observed rat pial microcirculation and exposed RF-EMF to rat, simultaneously (Fig. 2). Plasma image flowing in microvessels of the pia mater was visualized by FITC-Dx injected into vein.

### BBB PERMEABILITY

Blood-brain barrier (BBB) permeability was evaluated by extravasation of FITC-dextran of 70,000 molecules injected into a rat venule. Although fluorescence intensity of pia mater area decreased through an experimental period, there were no significant differences of the fluorescence intensity between sham and RF-EMF exposed grouped.

### VESSEL DIAMETER

Vessel diameters of sixteen different venules in each rat were measured every 10 min and were indicated as a percent diameter of initial value. No significant differences of the percent diameter were recognized between two groups.

### PLASMA VELOCITY

Velocities of plasma flowing in pial venules were measured by a dual-slit method and indicated as a percent velocity of initial value. No significant differences of the percent velocity were showed until the end of experiment.

### HISTOLOGY

To confirm whether serum albumin was accumulated in parietal region of rat brain, we observed an albumin leakage in rat brain section using an immunohistochemistry. No positive stain was recognized in two groups.

## DISCUSSION AND CONCLUSION

Our developed new type of loop antenna, an "8"-shaped loop antenna succeeded to concentrate SAR distribution into the target area just under the cranial window. This was convenience to evaluate dynamic changes in microcirculatory



parameters in the target area during RF-EMF exposure. In present experiment, we focused on three microcirculatory parameters, BBB permeability, vessel diameter and plasma velocity. However, no significant differences of these parameters were recognized between sham and exposed group even in during exposure period. Therefore, these results suggest no effects of RF-EMF exposure, at least not on three parameters in rat brain microcirculation, under these conditions.

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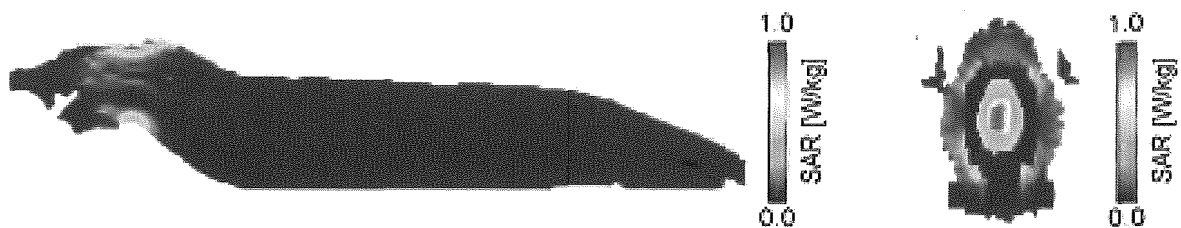


Fig. 1 SAR distribution

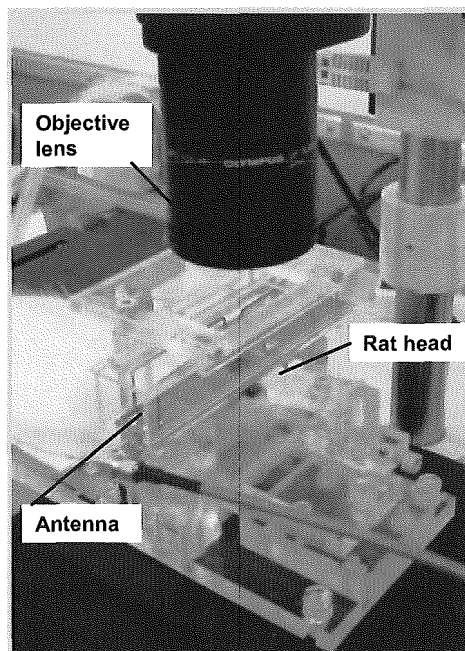


Fig. 2 RF-exposure and observation system

**THREE DIMENSIONAL VISUALIZATION OF THE TEMPERATURE DISTRIBUTION IN A PHANTOM FOR THE ASSESSMENT OF LOCALIZED EXPOSURE TO MICROWAVES.** M. Baba<sup>1</sup>, Y. Suzuki<sup>1</sup>, M. Taki<sup>1</sup>, K. Fukunaga<sup>2</sup>, S. Watanabe<sup>2</sup>. <sup>1</sup>Tokyo Metropolitan Univ, Tokyo, Japan, <sup>2</sup>National Institute of Information and Communications Technology, Tokyo, Japan.

**OBJECTIVE:** We have proposed a new method of specific absorption rate (SAR) measurement based on the visualization of three-dimensional (3D) temperature distribution by means of micro-capsulated thermo-chromic liquid crystal (MTLC) [1][2]. The objective of this paper is to report further developments of this method and to discuss application of this method to the assessment of local exposure especially of the microwaves and millimeter waves.

**METHODS:** Micro-capsulated thermo-chromic liquid crystal (MTLC) is used as a temperature sensor. The diameter of the capsule is about 20 to 30 micrometers and specific gravity is about 1.01g/cm<sup>3</sup>. When light is incident on MTLC, the wavelength of scattered light corresponds to the temperature of MTLC[3]. Therefore temperature of MTLC is visualized as the color of the scattered light. MTLC is suspended uniformly in the substance, such as liquid, gel, or solid. The substance needs to be transparent in order to allow observation of the scattered light. In our study we use  $\kappa$ -carrageenan $\pm$ , which forms gel and has high transparency. Complex permittivity of the carrageenan-based gel containing MTLC is adjusted to that of muscle at 1.45 GHz by mixing propylene glycol to use as a phantom. The visualized color is detected by a digital video camera. The camera which outputs uncompressed image data is used so that color of the image should be calibrated to a particular temperature. Color processing method adopted in this study utilizes H (Hue)-S (Saturation)-L (Lightness) color space that is transformed from RGB color space. The hue value basically represents the wavelength of the color and it is used as a calibration variable. We should note that the observed color or scattered wavelength and its intensity depend on the angle of measuring location

**RESULTS:** We have constructed correlation curves of hue versus temperature with its operating temperature range of 2, 5, and 10 degrees. The hue values are processed by a median filter with 6 x 6 pixels (0.9mm x 0.9mm). These curves show that hue and temperature have one-to-one correspondence.

**CONCLUSION:** We obtained calibration curves to relate the hue to the temperature. It enables quantitative measurements of temperature distribution. An improved temperature resolution is achieved by using MTLC with 2-degree temperature range and an uncompressed image camera. This method allows 3D measurement of temperature distributions with sufficient spatial resolution.

**DISCUSSION:** This method is applicable to the experimental studies to relate tissue temperature elevation with SAR and with incident power density in the microwave and millimeter wave region. Further studies are necessary to develop transparent phantom material with tissue equivalent electrical constants, and to analyze thermal properties of the phantom in comparison with those of human body.

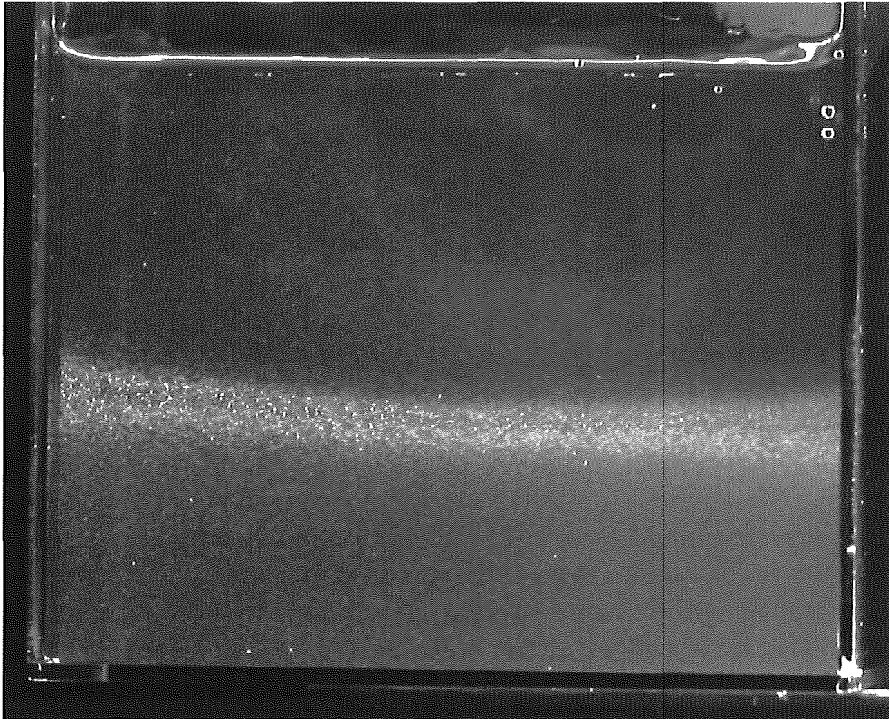


Fig.1 Temperature distribution is visualized when the phantom containing MTLC is heated from the bottom

#### References.

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**COMPUTATIONAL COMPARISON OF THE SAM PHANTOM TO ANATOMICALLY CORRECT MODELS OF THE HUMAN HEAD.** G. Bit-Babik, A. Faraone, C.K. Chou, M. Swicord. Motorola Florida Research Laboratories, Fort Lauderdale, FL. 33322. USA.

**INTRODUCTION:** The present compliance testing of mobile phones with respect to human RF exposure standards (IEEE C95.1-1999 *Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields from to 3 kHz to 300 GHz* and *International Commission on Non-Ionizing Radiation Protection (ICNIRP) 1998 Guidelines for Limiting Electrical, Magnetic, and Electromagnetic Fields (Up to 300 GHz)*) relies on Specific Absorption Rate (SAR) measurements in the Specific Anthropomorphic Mannequin (SAM) exposed to the RF energy from handset under the test in predefined positions. The SAM phantom, as defined in the current measurement standards (*IEEE Std 1528-2003 and IEC 62209 Part 1-2005*), was developed to provide substantially conservative SAR

## **Physiological Effects of Continuous Whole-Body Exposure to Extremely Low Frequency Electromagnetic Fields With or Without Transient Magnetic Fields on Cerebral Microcirculation in Mice With Brain Tumors**

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Many reports have suggested a variety of biological effects from extremely low frequency electromagnetic fields (ELF-EMF), however, few *in vivo* studies have focused on microcirculatory effects of ELF-EMF. Recently, the importance of noise components in EMF has also been recognized. Fifty or 60 Hz EMF in residential environments sometimes include various kinds of noise as transient waves, which arise from the use of electric devices such as switching regulators and inverters.

In this study, we explored the subchronic effects of whole body exposure to 50 Hz EMF with or without transient waves on cerebral microcirculation in brain tumors. For these purposes, we employed a mouse cranial window (CW) technique, allowing for the chronic observation of cerebral microcirculation, and a brain tumor implantation model using CW. SCID (Severe combined immuno-deficiency) mice were used in this study. After CW implantation, a small piece of human glioma U87 tissue was implanted into the CW prior to EMF exposure. Mice were subchronically exposed to plain 50 Hz time-varying EMF at 0.3 and 3.0 mT (rms), or combination of 50 Hz EMF at 3.0 mT (rms) with repetitive transient EMF (1 burst/s, 7.4 kHz waves with duration of 50 msec and peak magnet density of 162.6  $\mu$ T). Mice were continuously exposed to EMF for 16 days following tumor implantation. During and after the exposure, we measured tumor growth and quantified the microcirculatory parameters of angiogenic vessels in the growing tumor by real-time confocal microscopy, and measured permeability of rhodamine-labeled albumin from blood vessels using a photon counting system.

Although tumor size increased markedly following implantation, tumor growth did not show any significant difference among any of the exposure groups compared with the non-exposure group. Furthermore, ELF-EMF did not affect any of the microcirculatory parameters in the tumors, for instance, vascular density, mean diameter and branched numbers of vessels. These results indicate that no detectable effect on cerebral microcirculation was induced by exposure to plain 50 Hz EMF at 0.3 and 3.0 mT or by the combined exposure of 50 Hz at 3.0 mT and a transient magnetic field.

**Key words: electromagnetic fields exposure, transient magnetic fields, brain tumor, microcirculation, mouse**