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*Original Article*

# Exposure time-dependent thermal effects of radiofrequency electromagnetic field exposure on the whole body of rats

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**ABSTRACT** — We investigated the thermal effects of radiofrequency electromagnetic fields (RF-EMFs) on the variation in core temperature and gene expression of some stress markers in rats. Sprague-Dawley rats were exposed to 2.14 GHz wideband code division multiple access (W-CDMA) RF signals at a whole-body averaged specific absorption rate (WBA-SAR) of 4 W/kg, which causes behavioral disruption in laboratory animals, and 0.4 W/kg, which is the limit for the occupational exposure set by the International Commission on Non-Ionizing Radiation Protection guideline. It is important to understand the possible *in vivo* effects derived from RF-EMF exposures at these intensities. Because of inadequate data on real-time core temperature analyses using free-moving animal and the association between stress and thermal effects of RF-EMF exposure, we analyzed the core body temperature under nonanesthetic condition during RF-EMF exposure. The results revealed that the core temperature increased by approximately 1.5°C compared with the baseline and reached a plateau till the end of RF-EMF exposure. Furthermore, we analyzed the gene expression of heat-shock proteins (Hsp) and heat-shock transcription factors (Hsf) family after RF-EMF exposure. At WBA-SAR of 4 W/kg, some *Hsp* and *Hsf* gene expression levels were significantly upregulated in the cerebral cortex and cerebellum following exposure for 6 hr/day but were not upregulated after exposure for 3 hr/day. On the other hand, there was no significant change in the core temperature and gene expression at WBA-SAR of 0.4 W/kg. Thus, 2.14-GHz RF-EMF exposure at WBA-SAR of 4 W/kg induced increases in the core temperature and upregulation of some stress markers, particularly in the cerebellum.

**Key words:** RF-EMF exposure, Thermal effects, Core temperature, Heat-shock protein

## INTRODUCTION

Mobile phone telecommunication and Wireless-Fidelity (Wi-Fi) systems are highly popular in modern societies. However, there is a public concern regarding the health risk of radiofrequency electromagnetic fields (RF-EMFs) in these wireless telecommunications assigned from 0.8 to 5 GHz. As high-power RF-EMF exposure increases the body temperature of living organisms, many studies have been conducted on the thermal effects of RF-EMF (Adair *et al*., 2003; Foster and Morrissey, 2011). According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guideline for safety of RF-EMF (ICNIRP Guideline, 1998), the limits of whole-body averaged specific absorption rates (WBA-SARs) are 0.08 W/kg for the general public and 0.4 W/kg for occupational exposures. Exposure to low-intensity RF-EMF, not exceeding 0.4 W/kg, resulted in non-thermal effects and no or little biological effects. Moreover, in embryonic and juvenile rats subjected to long-term low-intensity RF-EMF exposure, embryo toxicity, teratology, and immunotoxicity have not been observed (Takahashi *et al*., 2010; Ohtani *et al*., 2015). On the other hand, high-intensity RF-EMF exposure at WBA-SAR of $\geq$ 4 W/kg causes thermal effects and behavioral disruption in rats, such as sleeping and spreading saliva on rat's tail (ICNIRP Guideline, 1998). Whole-body high-intensity RF-EMF exposure caused increases in skin, brain, and rectal temperatures

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in several animals (Adair *et al*., 2001; Jauchem and Frei, 1997). However, biological effects of RF-EMF exposure at WBA-SAR of 4 W/kg on thermal stress remain controversial, particularly, in real-time analyses of the variation in body temperature of free-moving animals. Therefore, assessment of core temperature and stress markers is required to understand the possible *in vivo* thermal effects of RF-EMF exposure at WBA-SAR of approximately 4 W/kg.

The correlation between physiological changes and increase in body temperature due to RF-EMF exposure is complex. Some stresses induced by high-intensity RF-EMF could deteriorate the cell infrastructure and alter cell homeostasis. Heat-shock proteins (Hsp) are induced by heat or other environmental stresses (Kregel, 2002) and are good biomarkers that are frequently employed in molecular toxicology. Therefore, expression analyses of these markers could play a significant role in the assessment of the thermal effects of RF-EMF exposure. In previous *in vivo* studies, changes in the Hsp family according to exposure intensity and duration were noted. In most of the cases, upregulation of the expression of *Hsp* was not observed when exposure duration and/or intensity was less than 3 hr/day and WBA-SAR was less than 2 W/kg (Sanchez *et al*., 2008; Paparini *et al*., 2008; Watilliaux *et al*., 2011). However, some *Hsp* genes were upregulated after exposure for more than 3 hr/day at WBA-SAR of 4 W/kg (Fritze *et al*., 1997; Yang *et al*., 2012). Thus, these negative or positive consequences of RF-EMF exposure on *Hsp* family have necessitated studies not only on the intensity of RF-EMF exposure at WBA-SAR of approximately 4 W/kg but also on the duration of RF-EMF exposure to determine the association between the thermal effects and stresses. However, it is difficult to define expression of *Hsp in vivo* because many different conditions, such as animal species, frequencies and intensity of RF-EMF, local or whole-body exposure, and with or without anesthesia, should be considered. Therefore, to investigate the biological effects of RF-EMF exposure on animals, an exposure system that allows adequate exposure assessment and transcriptional analyses of the stress markers is necessary.

Here, to determine the association between increase in body temperature and thermal stress derived from RF-EMF exposure at WBA-SAR of 4 W/kg, we established a reverberation chamber system emitting uniform high-intensity RF-EMFs for rats (Chakarothai *et al*., 2013), performed accurate exposure assessment with computer simulation (Shi *et al*., 2014), and exposed the whole body of rats to RF-EMFs. To reveal the possible thermal effects of 2.14-GHz RF-EMF exposure and confirm whether the thermal or non-thermal stresses were caused by RF-EMF exposure or not, we analyzed the changes in the core temperature in real time under free-moving condition and performed gene expression analyses of *Hsp* and heat-shock transcription factors (*Hsf*) family as stress markers after the exposure.

## MATERIALS AND METHODS

### Animals

All animal procedures were approved by the committee for animal experiments at the National Institute of Public Health, Japan. Sprague-Dawley rats were purchased from Japan SLC (Shizuoka, Japan). The 6-week-old male rats were housed in acrylic cages in an animal room with a 12-hr light/12-hr dark cycle and provided food and water *ad libitum*. The temperature and relative humidity were maintained at 23°C ± 1°C and 50% ± 5%, respectively. The rats were assigned to the RF-EMF-exposed group (n = 4) subjected to various durations and intensities of RF-EMF and the control (sham-exposed) group (n = 4).

### RF-EMF exposure

RF-EMF exposures were performed in a reverberation chamber system (Chakarothai *et al*., 2013), which was similar to the above-mentioned clean animal room, but with a shielded structure. The apparatus could uniformly emit high-power RF-EMF, and accurate SAR was calculated by computer simulations (Shi *et al*., 2014). The exposed frequency was a 2.14-GHz wideband-code division multiple access (W-CDMA) signal. An acrylic cage containing four rats per cage was placed at the center of the chamber. A series of operations of RF-EMF exposure was performed once for each group as shown in Fig. 1. The exposure durations were 1 day (6 hr/day) and 3 consecutive days (3 or 6 hr/day) at WBA-SAR of 4 and 0.4 W/kg, which is 50 and 5 times higher than that set by the ICNIRP guideline for general public, respectively. Each exposure was performed in daylight (sleep stage).

### Body temperature analyses

The core temperature was measured using iButton (DS1922L) data loggers (Dallas Semiconductors, Dallas, TX, USA), which can collect the temperature data without the need of a wire. The logger was surgically inserted into the abdominal cavity of rats 2 days before RF-EMF exposure. During three consecutive RF-EMF exposures, core temperature data were recorded every minute. After dissection of the rats, the loggers were removed and collected data were analyzed. To complement core temperature data, the rectal temperature was measured using a

fiber-optic thermometer (model FL-2000; Anritsu Meter Co., Tokyo, Japan) under similar condition for 6 min. Furthermore, as a positive control, the rats were placed at 38°C for 1 hr, and the core temperature in free-moving rats was also analyzed with loggers.

## Gene expression analyses

The brain tissues were removed from sham-exposed (control) and RF-EMF-exposed rats and soaked in RNAlater (Qiagen; Valencia, CA, USA). The total RNA from the cerebral cortex and cerebellum were extracted using RNeasy Midi and Mini Kits (Qiagen; Hilden, Germany), reverse-transcribed with High-Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the cDNAs obtained were used as template. Quantitative PCR was performed using Fast SYBR Green Master Mix (Thermo Fisher Scientific, Inc.). All primers (*Hsp* family: *Hsp27*, *Hsp40*, *Hsp60*, *Hsp70*, *Hsp90aa1*, *Hsp90ab1*, and *Hsp110*; *Hsf* family: *Hsf1*, *Hsf2*, and *Hsf4*; inflammatory cytokine: *Tnfa*, *Il6*, *Il1a*, and *Il1b*; and housekeeping genes: *Gapdh* and *Actb*) (Table 1) were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/input.htm) within the amplified fragment length of 150 bp and purchased from Exigen, Inc., Tokyo, Japan. PCR was performed at 95°C and 60°C for 45 cycles in a Stratagene Mx3000P quantitative PCR system (Agilent Technologies, Palo Alto, CA, USA). All samples were assayed in triplicates. The relative expression levels of each mRNA were determined according to the comparative cycle threshold method using the equation $2^{-\Delta\Delta CT}$.

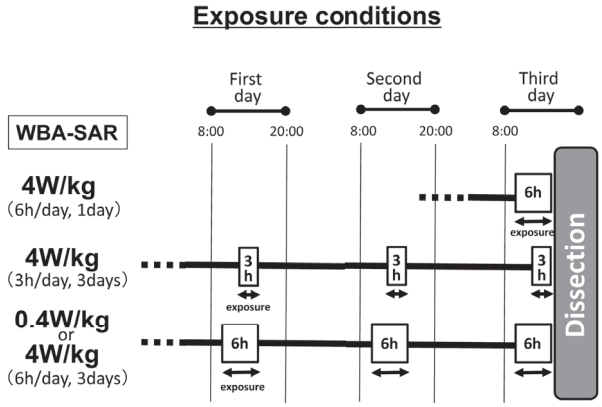


**Fig. 1.** Conditions of RF-EMF exposure. Sprague-Dawley rats were exposed to RF-EMF as follows: 1-day exposure (top), 3 consecutive days of exposure for 3 hr/day (middle) and 3 consecutive days of exposure for 6 hr/day (bottom) at WBA-SAR of 4 or 0.4 W/kg. The RF-EMF exposure was performed during daylight (indicated by arrows).

## Statistics

The relative mRNA expression levels of 14 target genes were compared between the control (n = 4) and RM-EMF-exposed (n = 4) animals using unpaired Student's *t*-tests (assuming equal variances) or Welch's *t*-test (assuming unequal variances). Differences were considered significant when $P < 0.05$ and $P < 0.01$.The statisti-

**Table 1.** Primer sets for the genes of *Hsp* family, *Hsf* family, and inflammatory cytokines.

| Gene | Forward sequence (5'→3') | Reverse sequence (5'→3') |
|---|---|---|
| *Hsp27* | TGTCAGAGATCCGACAGACG | GTTCATCCTGCCTTTCTTCG |
| *Hsp40* | AGGCTCTCTGTGGTTGCACT | GGGGATCCTATCAGGGAAGA |
| *Hsp60* | ACCTGTGACAACCCCTGAAG | TGACACCCTTTCTTCCAACC |
| *Hsp70* | GTCTCAAGGGCAAGATCAGC | AGAGTCCAGCCAGGAGATGA |
| *Hsp90aa1* | CAACCAATGGAGGAAGAGGA | AGCGTCTGAGGAGTTGGAAA |
| *Hsp90ab1* | GCTGGTGGATCCTTCACTGT | TCTTCACCACCTCCTTGACC |
| *Hsp110* | AGTTGCCCACTGGATTAACG | TTGACAAGAGCATGGCAGTC |
| *Hsf1* | GACAGCAGCTCAGCACACTC | TGGTGACAGCATCAGAGGAG |
| *Hsf2* | TGGCAAGCTTTGTGAGACAG | AACAGGGCCATCTCTTTCCT |
| *Hsf4* | GAGAGGTGCAAGCTTTGAGG | ACTGGATTAGCTTGCCGATG |
| *Tnfa* | TGCCTCAGCCTCTTCTCATT | GAGCCCATTTGGGAACTTCT |
| *Il6* | CCGGAGAGGAGACTTCACAG | CAGAATTGCCATTGCACAAC |
| *Il1a* | AGGCCATAGCCCATGATTTA | TGATGAACTCCTGCTTGACG |
| *Il1b* | AAAAATGCCTCGTGCTGTCT | GGGATTTTGTCGTTGCTTGT |
| *Gapdh* | ATGACTCTACCCACGGCAAG | TACTCAGCACCAGCATCACC |
| *Actb* | GCTCTCTTCCAGCCTTCCTT | CGGATGTCAACGTCACACTT |

cal analyses were performed using Excel Statistics 2008 (Social Survey Research Information, Inc., Tokyo, Japan)

## RESULTS

### Core temperature analyses

Increase in the core temperature was observed in RF-EMF exposed (Fig. 2) and sham exposed rats (Fig. 3), taking approximately 10 min to reach 38.5°C from baseline (approximately 37.5°C) after the onset of RF-EMF exposure. All four exposed rats showed same tendencies of increase in core temperature during RF-EMF exposure at WBA-SAR of 4 W/kg. As shown in Fig. 2, the intraperitoneal temperature remained 1.0-1.5°C higher than the baseline and reached a plateau till the end of RF-EMF exposure. To observe the change in core temperature closely, the averaged data of core temperature were extracted at various points during RF-EMF exposure (Table 2). The temperatures in each RF-EMF-exposed rat increased by 1.1-1.4°C. However, the increase in temperatures in each sham-exposed rat was ≤ 0.5°C. These data showed that the set point in the core temperature increased by 1.0-1.5°C during RF-EMF exposure. Following the exposure, the intraperitoneal temperature returned to the pre-exposure level and remained at approximately 37.5°C after approximately 30 min following turning off of the exposure system (Fig. 2). In contrast, there were no successive increases in the core temperature in the sham-exposed and low-intensity (0.4 W/kg) RF-EMF-exposed groups, i.e., the set point did not

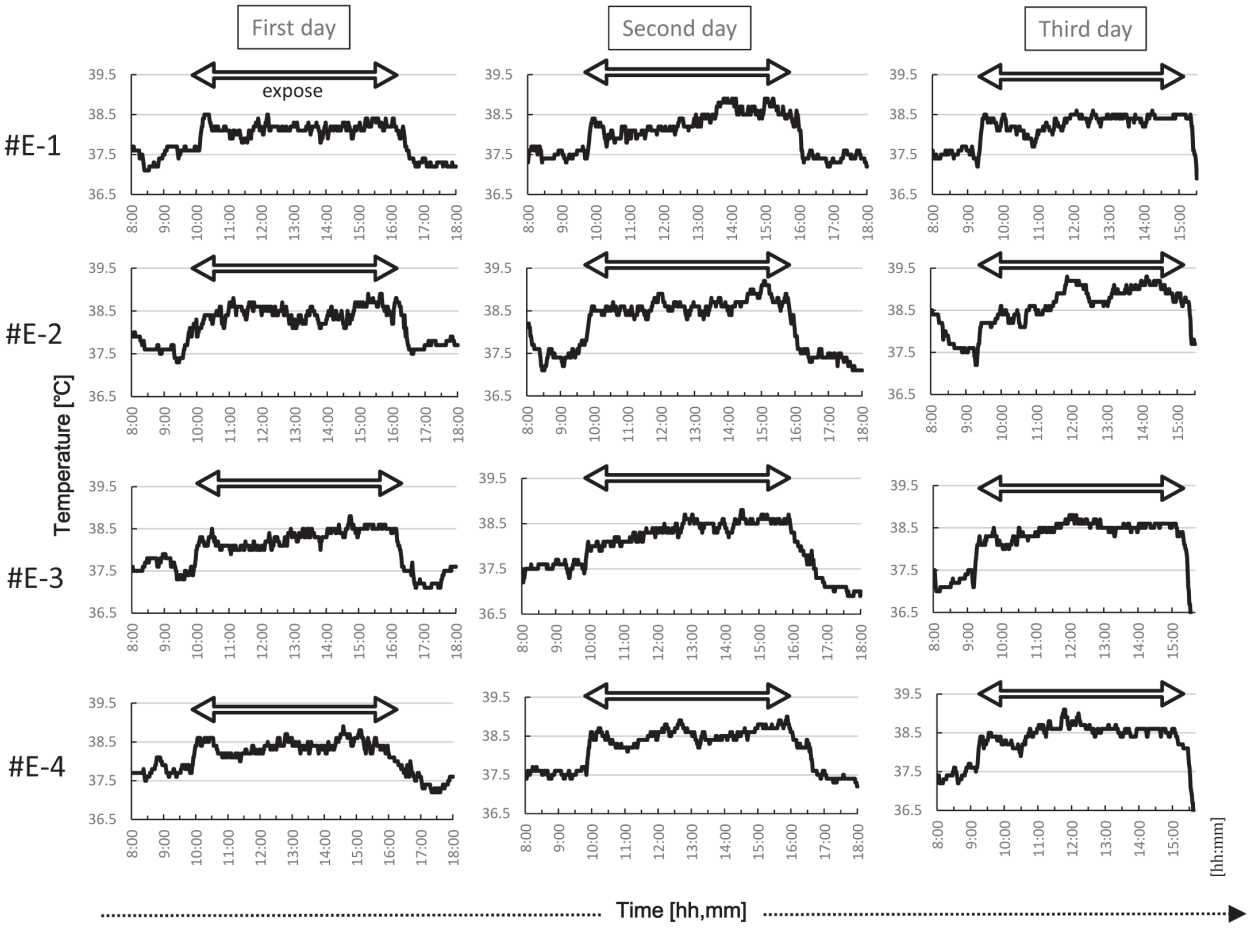


**Fig. 2.** Core body temperature of the RF-EMF exposed group. The intraperitoneal temperature of the Sprague-Dawley rats was measured using iButton data loggers. After dissection, the loggers were removed from the rat's body, and data were analyzed. RF-EMF exposure was started between 9:00 am and 10:00 am at WBA-SAR of 4 W/kg for 6 hr/day for 3 consecutive days.

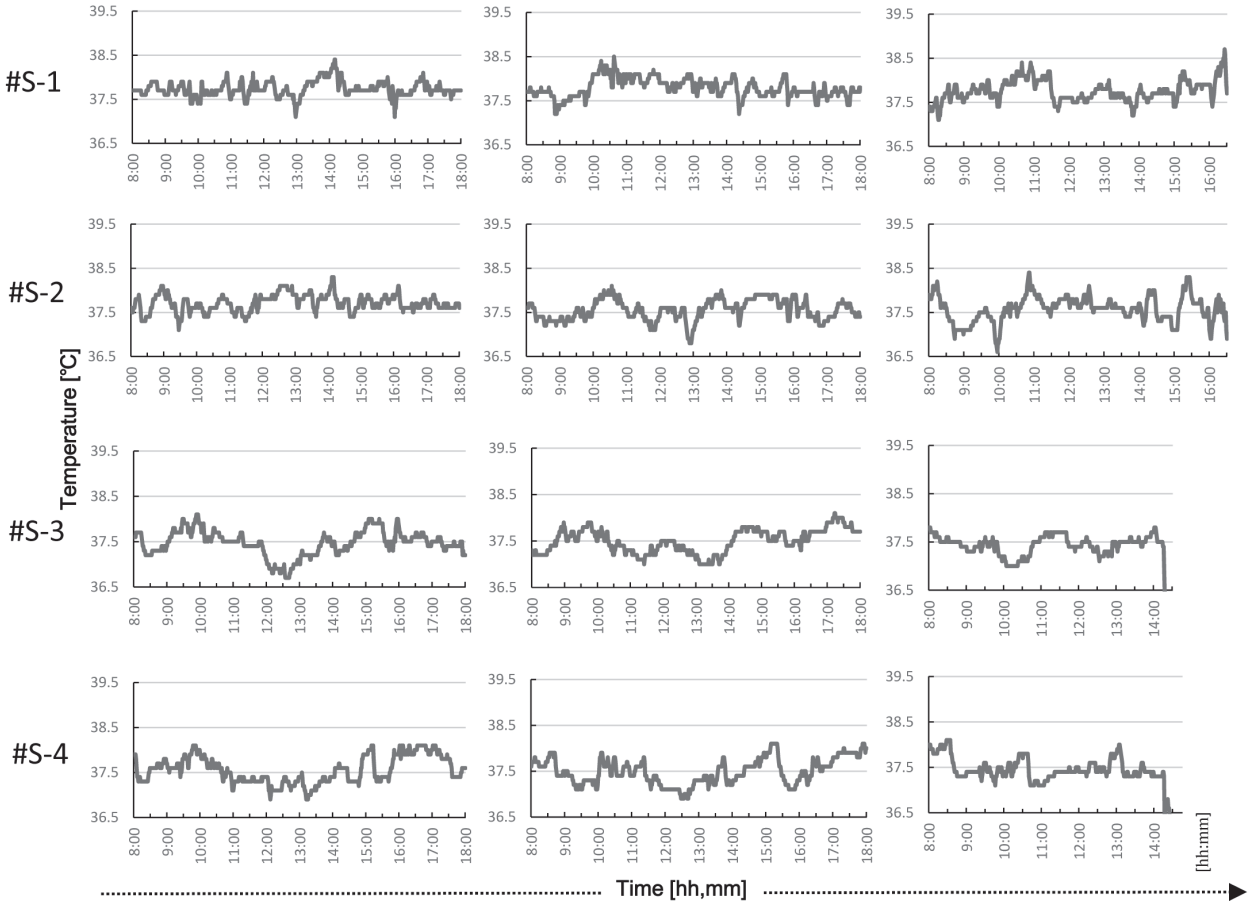


**Fig. 3.** Core body temperature of the sham exposed group. The intraperitoneal temperature of the Sprague-Dawley rats was measured using iButton data loggers. Sham exposure was performed in parallel with RF-EMF exposure.

change. In all rats, slight variations in the core temperature were observed throughout the measurement. Furthermore, as a positive control, we also analyzed the core temperature of free-moving rats under thermal condition. When the rats were caged at 38°C for 1 hr, the core temperature linearly increased from 37.5°C to 42°C (Fig. 4).

To confirm the reliability of iButton data loggers, variations in the rectal temperature of the retained rats were measured using the fiber-optic thermometer. The rectal temperature rapidly increased by 0.4°C for 6 min, and it mostly corresponded to the intraperitoneal temperature, demonstrating that the loggers provided accurate core temperature data in real time.

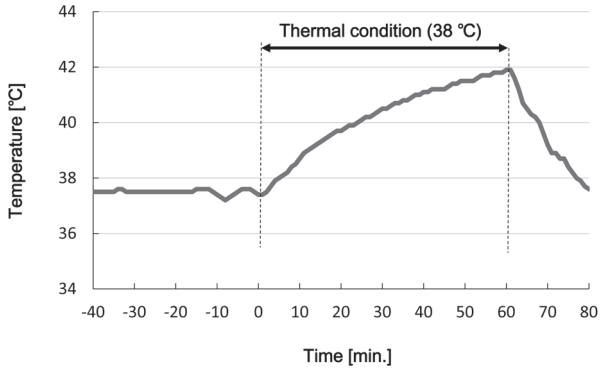


**Fig. 4.** Variation in core body temperature under thermal condition. The core temperature under thermal condition was measured using the iButton data loggers. The rats were placed at 38°C ± 0.5°C for 1 hr. After dissection, the loggers were removed from the rat's body.

## Behavioral observation after RF-EMF exposure

The rats' movements were observed each time immediately after the three consecutive RF-EMF exposures. On the 1st day, all four rats slept or were motionless for

a while, were obviously sick, and regained normal movement 30 min later. In contrast, on the 2nd day, the rats moved immediately, but less frequently after the exposure, and then remained still for a while. On the 3rd day, the rats moved around the cage and appeared to be normal. The reproducibility of these behavioral changes could be confirmed because the same tendency was observed in a preliminary experiment. To explain this interesting behavioral change, further analyses should be conducted in future.

### Gene expression analyses of the *Hsp* family, *Hsf* family, and inflammatory cytokines

The relative gene expression in the RF-EMF-exposed group was compared with that in the control group. Tables 3 and 4 show the relative gene expression levels in the cerebral cortex and cerebellum, respectively. No significant changes in the gene expression of *Hsp* family, *Hsf* family, and inflammatory cytokines were noted in both the cerebral cortex and cerebellum when the exposure duration was 3 hr/day at WBA-SAR of 4 W/kg. However, some genes were significantly upregulated following RF-EMF exposure for 6 hr/day. In case of "1-day" exposure, three genes (*Hsp90aa1*, *Hsp90ab1*, and *Hsf2*) were upregulated in the cerebral cortex, whereas in the case of 3 days of exposure, seven genes (*Hsp40*, *Hsp60*, *Hsp70*, *Hsp90aa1*, *Hsf1*, *Hsf2*, and *Hsf4*) were upregulated in the cerebellum. With regard to *Hsp70* upregulation, there were no statistically significant differences in the cerebral cortex despite a high relative ratio of 4.33. These findings indicated that the number of upregulated genes in the cerebellum increased with the exposure time. In contrast, no significant differences in the expression of inflammatory cytokines were observed under any conditions, implicating that RF-EMF exposure did not cause significant inflammation in the brain.

When the rats were exposed to severe thermal condition (38°C), most of the genes of the *Hsp* and *Hsf* families (*Hsp27*, *Hsp40*, *Hsp60*, *Hsp90aa1*, and *Hsf2* in the cerebral cortex and *Hsp27*, *Hsp40*, *Hsp60*, *Hsp90aa1*, *Hsp90ab1*, *Hsp110*, and *Hsf4* in the cerebellum) were significantly upregulated. However, there were no statistically significant differences in the expression of *Hsp70* in the cerebral cortex and cerebellum because of the extremely high ratio in one subject. Nevertheless, the mean relative ratio of expression of *Hsp70* in all the subjects was obviously high compared with that noted in the RF-EMF-exposed group, indicating that the thermal effects of RF-EMF were obviously lower than those of severe thermal condition. Moreover, at WBA-SAR of 0.4 W/kg, there were no significant changes in the gene expressions of the *Hsp* family, *Hsf* family, and inflammatory cytokines in the cerebral cortex and cerebellum.

## DISCUSSION

Here to identify the possible thermal effects of RF-EMF exposure, we analyzed variations in rats' core temperature during RF-EMF exposure and the gene expression of several stress markers in the brain following RF-EMF exposure. The core temperature increased by approximately 1.5°C, and some genes belonging to the *Hsp* and *Hsf* families were upregulated. These upregulations were particularly observed in the cerebellum and were dependent on the exposure duration (over 3 hr/day at WBA-SAR of 4 W/kg, which is the threshold of behavioral disruption following whole-body exposure). The findings of the present study provide a new insight into the threshold level of real-time core-temperature variation during whole-body RF-EMF exposure and the association between thermal effects of RF-EMF and expression of some stress markers.

We designed the procedure for real-time measurement of core temperature during RF-EMF exposure. Using iButton (DS1922L) data loggers, which have been used for the measurement of core temperature in dormice (Langer and Fietz, 2014), we were able to obtain accurate core temperature data of rats. The logger was efficient in detecting thermal variability in real time and was not affected by RF-EMF when directly placed into the chamber and exposed to RF-EMF. Thus, recorded data were not influenced by RF-EMF exposure. Furthermore, to verify the accuracy of the logger, we compared the intraperitoneal temperature obtained using the logger with the rectal temperature recorded with the fiber-optic thermometer under similar conditions and found that both of them were similar. These prior investigations helped in effective detection of the variation in core temperature resulting from RF-EMF exposure.

At WBA-SAR of 0.4 W/kg, there were no (or little) changes in the core temperature and expression of stress markers following RF-EMF exposure, reconfirming the results reported in many previous studies that RF-EMF exposure below WBA-SAR of 0.4 W/kg does not influence the function of living organisms. In contrast, at WBA-SAR of 4 W/kg under nonanesthetic condition, the core temperature showed three variations, namely, an increase of 1.0-1.5°C after turning on the exposure system, a plateau of approximately 38.5°C during the exposure, and a decrease till baseline after turning off the exposure system. We presumed that the increases in core temperature did not depend on autonomic, but pas-

**Table 2.** Average of core temperature of RF-EMF- and sham-exposed groups.

| | | #E-1 | | | #E-2 | | | #E-3 | | | #E-4 | | | #S-1 | | | #S-2 | | | #S-3 | | | #S-4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT | ave. | ±S.D. | ΔT |
| 1st day | pre 1 hr | 37.6 | 0.04 | | 37.7 | 0.33 | | 37.6 | 0.2 | | 37.8 | 0.09 | | 37.7 | 0.16 | | 37.7 | 0.24 | | 37.5 | 0.21 | | 37.7 | 0.05 | |
| | 1-2 hr | 38.2 | 0.18 | 0.6 | 38.3 | 0.08 | 0.6 | 38.3 | 0.05 | 0.7 | 38.4 | 0.08 | 0.6 | 37.8 | 0.25 | 0.1 | 37.8 | 0.15 | 0.1 | 37.1 | 0.1 | -0.4 | 37.2 | 0.13 | -0.4 |
| | 3-4 hr | 38.2 | 0.08 | 0.6 | 38.3 | 0.13 | 0.6 | 38.3 | 0.1 | 0.7 | 38.4 | 0.1 | 0.6 | 37.8 | 0.28 | 0.1 | 37.8 | 0.12 | 0.1 | 37.1 | 0.16 | -0.4 | 37.2 | 0.19 | -0.5 |
| | 5-6 hr | 38.3 | 0.15 | 0.7 | 38.7 | 0.18 | 0.9 | 38.5 | 0.12 | 0.9 | 38.5 | 0.22 | 0.7 | 37.7 | 0.11 | 0 | 37.8 | 0.08 | 0.1 | 37.8 | 0.16 | 0.3 | 37.2 | 0.33 | -0.4 |
| 2nd day | pre 1 hr | 37.5 | 0.1 | | 37.5 | 0.16 | | 37.6 | 0.12 | | 37.6 | 0.1 | | 37.6 | 0.12 | | 37.4 | 0.15 | | 37.7 | 0.14 | | 37.3 | 0.13 | |
| | 1-2 hr | 38.1 | 0.12 | 0.6 | 38.5 | 0.1 | 1 | 38.1 | 0.08 | 0.6 | 38.3 | 0.13 | 0.7 | 38 | 0.1 | 0.4 | 37.4 | 0.23 | 0 | 37.2 | 0.08 | -0.5 | 37.4 | 0.18 | 0.1 |
| | 3-4 hr | 38.4 | 0.19 | 0.9 | 38.5 | 0.17 | 1.1 | 38.5 | 0.14 | 0.9 | 38.5 | 0.19 | 0.9 | 37.8 | 0.15 | 0.2 | 37.7 | 0.32 | 0.3 | 37.1 | 0.09 | -0.6 | 37.5 | 0.19 | 0.1 |
| | 5-6 hr | 38.6 | 0.15 | 1.1 | 38.9 | 0.21 | 1.4 | 38.6 | 0.05 | 1 | 38.7 | 0.08 | 1.2 | 37.7 | 0.2 | 0.1 | 37.8 | 0.23 | 0.4 | 37.6 | 0.12 | -0.1 | 37.6 | 0.38 | 0.3 |
| 3rd day | pre 1 hr | 37.6 | 0.05 | | 37.8 | 0.24 | | 37.2 | 0.15 | | 37.5 | 0.15 | | 37.8 | 0.18 | | 37.3 | 0.18 | | 37.5 | 0.06 | | 37.7 | 0.33 | |
| | 1-2 hr | 38.1 | 0.15 | 0.5 | 38.4 | 0.19 | 0.6 | 38.3 | 0.14 | 1 | 38.4 | 0.22 | 0.9 | 37.8 | 0.26 | 0 | 37.8 | 0.15 | 0.5 | 37.2 | 0.2 | -0.3 | 37.4 | 0.15 | -0.3 |
| | 3-4 hr | 38.5 | 0.05 | 0.9 | 38.8 | 0.16 | 1.1 | 38.6 | 0.08 | 1.4 | 38.7 | 0.21 | 1.2 | 37.6 | 0.23 | -0.2 | 37.6 | 0.13 | 0.3 | 37.5 | 0.14 | 0 | 37.4 | 0.05 | -0.3 |
| | 5-6 hr | 38.5 | 0.05 | 0.9 | 39 | 0.23 | 1.2 | 38.6 | 0.05 | 1.3 | 38.6 | 0.04 | 1.1 | 37.8 | 0.25 | 0.1 | 37.6 | 0.34 | 0.3 | 37.5 | 0.1 | 0 | 37.3 | 0.04 | -0.4 |

[°C]

An “ave” showed the average of six data of core temperatures picked out every 10 min from -1 to 0 hr (pre 1 hr, baseline), 1-2 hr, 3-4 hr, and 5-6 hr after the onset of RF-EMF exposure. A “ΔT” showed differences between the baseline temperature and the temperature during exposure.

**Table 3.** Relative mRNA expression levels in the cerebral cortex of RF-EMF- and sham-exposed groups.

| | | 6 hr/day (1 day) | | | 3 hr/day (3 days) | | | 6 hr/day (3 days) | | | | | | Heat (38°C, 1 hr) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 W/kg | | | 4 W/kg | | | 0.4 W/kg | | | 4 W/kg | | | | | |
| | | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio |
| *Hsp27* | sham | 0.85 | 0.13 | 1.2 | 0.86 | 0.15 | 1.08 | 1.04 | 0.12 | 0.84 | 1.17 | 0.11 | 1.54 | 1.01 | 0.17 | 17.42 |
| | expose | 1.02 | 0.12 | | 0.93 | 0.12 | | 0.88 | 0.07 | | 1.79 | 0.81 | | 17.53 | 7.39 | |
| *Hsp40* | sham | 0.63 | 0.23 | 0.59 | 0.94 | 0.12 | 0.98 | 0.89 | 0.1 | 0.84 | 1.15 | 0.15 | 1.1 | 0.84 | 0.15 | 2.84 |
| | expose | 0.37 | 0.05 | | 0.93 | 0.13 | | 0.75 | 0.11 | | 1.27 | 0.5 | | 2.37 | 0.69 | |
| *Hsp60* | sham | 0.67 | 0.24 | 1.05 | 0.92 | 0.05 | 1.02 | 0.94 | 0.07 | 0.87 | 1.05 | 0.16 | 1.25 | 1 | 0.22 | 1.56 |
| | expose | 0.7 | 0.05 | | 0.94 | 0.04 | | 0.82 | 0.08 | | 1.31 | 0.55 | | 1.56 | 0.29 | |
| *Hsp70* | sham | 0.8 | 0.25 | 1.71 | 0.88 | 0.15 | 1.18 | 1.29 | 0.18 | 0.82 | 1.06 | 0.26 | 4.33 | 0.64 | 0.45 | 680.3 |
| | expose | 1.37 | 0.34 | | 1.04 | 0.17 | | 1.06 | 0.17 | | 4.6 | 6.47 | | 434.05 | 449.69 | |
| *Hsp90aa1* | sham | 0.82 | 0.13 | 1.61 | 0.79 | 0.14 | 0.81 | 0.93 | 0.05 | 0.89 | 0.93 | 0.08 | 1.33 | 1.06 | 0.06 | 1.35 |
| | expose | 1.32 | 0.09 | | 0.64 | 0.14 | | 0.82 | 0.09 | | 1.24 | 0.4 | | 1.42 | 0.06 | |
| *Hsp90ab1* | sham | 0.91 | 0.11 | 1.27 | 0.93 | 0.06 | 1.02 | 0.93 | 0.06 | 1.06 | 0.87 | 0.26 | 0.82 | 1.01 | 0.03 | 1.06 |
| | expose | 1.16 | 0.07 | | 0.95 | 0.07 | | 0.99 | 0.18 | | 0.71 | 0.06 | | 1.07 | 0.11 | |
| *Hsp110* | sham | 0.89 | 0.15 | 1.17 | 0.98 | 0.05 | 1.11 | 0.97 | 0.07 | 0.97 | 1.08 | 0.09 | 1.02 | 0.86 | 0.16 | 1 |
| | expose | 1.05 | 0.09 | | 1.08 | 0.11 | | 0.94 | 0.12 | | 1.1 | 0.18 | | 0.86 | 0.08 | |
| *Hsf1* | sham | 1.19 | 0.21 | 1.31 | 0.9 | 0.08 | 1.09 | 0.96 | 0.06 | 1.07 | 1.29 | 0.43 | 1 | 1.3 | 0.34 | 0.99 |
| | expose | 1.57 | 0.2 | | 0.97 | 0.08 | | 1.03 | 0.1 | | 1.29 | 0.32 | | 1.29 | 0.11 | |
| *Hsf2* | sham | 0.96 | 0.06 | 1.77 | 1 | 0.18 | 1.42 | 1.05 | 0.05 | 1.08 | 1.06 | 0.06 | 1.27 | 1.26 | 0.16 | 1.31 |
| | expose | 1.69 | 0.16 | | 1.42 | 0.69 | | 1.14 | 0.05 | | 1.34 | 0.61 | | 1.65 | 0.18 | |
| *Hsf4* | sham | 0.73 | 0.21 | 0.89 | 0.92 | 0.12 | 0.96 | 1.21 | 0.13 | 0.82 | 1.23 | 0.2 | 1.19 | 0.88 | 0.2 | 0.84 |
| | expose | 0.66 | 0.07 | | 0.88 | 0.1 | | 0.99 | 0.11 | | 1.47 | 0.61 | | 0.73 | 0.04 | |
| *Tnfa* | sham | 0.76 | 0.15 | 1.09 | 0.83 | 0.15 | 1.36 | 1.08 | 0.09 | 0.94 | 0.91 | 0.1 | 1.69 | 0.83 | 0.11 | 1.93 |
| | expose | 0.83 | 0.09 | | 1.13 | 0.27 | | 1.01 | 0.14 | | 1.54 | 0.81 | | 1.6 | 0.66 | |
| *Il6* | sham | 0.99 | 0.43 | 0.87 | 0.75 | 0.2 | 1.65 | 1.76 | 0.77 | 1.08 | 1.21 | 0.46 | 1.74 | 1.09 | 0.47 | 1.03 |
| | expose | 0.87 | 0.3 | | 1.23 | 0.59 | | 1.89 | 0.51 | | 2.11 | 0.7 | | 1.12 | 0.52 | |
| *Il1a* | sham | 0.71 | 0.18 | 1.53 | 0.69 | 0.26 | 0.83 | 0.64 | 0.22 | 1.08 | 1.69 | 0.62 | 1.11 | 0.88 | 0.16 | 10.09 |
| | expose | 1.08 | 0.43 | | 0.57 | 0.45 | | 0.69 | 0.15 | | 1.89 | 0.7 | | 8.89 | 12.02 | |
| *Il1b* | sham | 1.01 | 0.35 | 1.54 | 1.4 | 0.27 | 0.92 | 0.94 | 0.21 | 0.89 | 2.04 | 0.63 | 1.83 | 1.27 | 0.22 | 6.28 |
| | expose | 1.57 | 0.79 | | 1.28 | 0.49 | | 0.84 | 0.1 | | 3.73 | 3.69 | | 7.95 | 10.72 | |

Significant changes in the gene expressions in the RF-EMF-exposed rats are shown by the relative ratio; light gray: $P < 0.05$ and dark gray: $P < 0.01$.

**Table 4.** Relative mRNA expression levels in the cerebellum of RF-EMF- and sham-exposed groups.

| | | 6 hr/day (1 day) | | | 3 hr/day (3 days) | | | 6 hr/day (3 days) | | | | | | Heat (38°C, 1 hr) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 W/kg | | | 4 W/kg | | | 0.4 W/kg | | | 4 W/kg | | | | | |
| | | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio | average | ±S.D. | relative ratio |
| *Hsp27* | sham | 1 | 0.11 | 0.9 | 1 | 0.61 | 1.41 | 0.97 | 0.28 | 0.79 | 1.04 | 0.08 | 1.15 | 0.97 | 0.07 | 22.05 |
| | expose | 0.89 | 0.14 | | 1.42 | 0.51 | | 0.76 | 0.22 | | 1.2 | 0.24 | | 21.45 | 10.59 | |
| *Hsp40* | sham | 0.96 | 0.1 | 0.94 | 1.13 | 0.2 | 1 | 0.69 | 0.24 | 0.55 | 1.15 | 0.1 | 2.14 | 0.85 | 0.11 | 9.92 |
| | expose | 0.9 | 0.17 | | 1.12 | 0.23 | | 0.38 | 0.08 | | 2.45 | 0.36 | | 8.43 | 2.43 | |
| *Hsp60* | sham | 0.86 | 0.14 | 0.74 | 0.97 | 0.09 | 0.84 | 0.59 | 0.26 | 0.71 | 1.15 | 0.11 | 2.04 | 0.91 | 0.09 | 2.72 |
| | expose | 0.64 | 0.07 | | 0.82 | 0.35 | | 0.42 | 0.08 | | 2.33 | 0.36 | | 2.49 | 0.56 | |
| *Hsp70* | sham | 1.07 | 0.47 | 1.47 | 1.15 | 0.21 | 1.05 | 0.41 | 0.36 | 0.3 | 1.62 | 0.44 | 5.99 | 0.84 | 0.22 | 8941.6 |
| | expose | 1.57 | 0.34 | | 1.21 | 0.34 | | 0.12 | 0.07 | | 9.73 | 2.7 | | 7513.25 | 6772.61 | |
| *Hsp90aa1* | sham | 1.19 | 0.12 | 0.94 | 0.7 | 0.21 | 0.93 | 1.14 | 0.17 | 0.87 | 1.09 | 0.07 | 1.35 | 0.98 | 0.07 | 2.13 |
| | expose | 1.12 | 0.09 | | 0.65 | 0.26 | | 0.99 | 0.17 | | 1.47 | 0.23 | | 2.09 | 0.4 | |
| *Hsp90ab1* | sham | 0.97 | 0.03 | 0.95 | 1.12 | 0.13 | 1 | 1.17 | 0.11 | 0.92 | 1.23 | 0.19 | 1.15 | 0.89 | 0.07 | 1.93 |
| | expose | 0.92 | 0.11 | | 1.12 | 0.12 | | 1.08 | 0.14 | | 1.42 | 0.22 | | 1.73 | 0.3 | |
| *Hsp110* | sham | 1.06 | 0.12 | 0.7 | 0.88 | 0.12 | 1.02 | 1.06 | 0.04 | 1.09 | 0.99 | 0.06 | 1 | 0.94 | 0.05 | 1.31 |
| | expose | 0.74 | 0.06 | | 0.9 | 0.09 | | 1.16 | 0.07 | | 0.99 | 0.16 | | 1.22 | 0.16 | |
| *Hsf1* | sham | 1.07 | 0.06 | 1.05 | 1.02 | 0.13 | 0.97 | 0.59 | 0.26 | 0.64 | 1.06 | 0.08 | 2.13 | 0.93 | 0.04 | 1.26 |
| | expose | 1.11 | 0.12 | | 0.99 | 0.15 | | 0.38 | 0.07 | | 2.27 | 0.49 | | 1.18 | 0.19 | |
| *Hsf2* | sham | 0.87 | 0.17 | 0.81 | 0.77 | 0.26 | 1.09 | 0.57 | 0.27 | 0.73 | 0.9 | 0.07 | 2.06 | 0.97 | 0.07 | 1.08 |
| | expose | 0.7 | 0.12 | | 0.83 | 0.09 | | 0.41 | 0.07 | | 1.86 | 0.35 | | 1.04 | 0.07 | |
| *Hsf4* | sham | 0.91 | 0.09 | 0.96 | 0.81 | 0.15 | 0.88 | 1.14 | 0.23 | 0.76 | 1.02 | 0.04 | 1.95 | 0.97 | 0.08 | 1.53 |
| | expose | 0.87 | 0.09 | | 0.71 | 0.14 | | 0.86 | 0.19 | | 1.99 | 0.22 | | 1.49 | 0.11 | |
| *Tnfa* | sham | 0.8 | 0.23 | 1.22 | 0.74 | 0.29 | 1.01 | 1.17 | 0.17 | 1.03 | 1.01 | 0.19 | 1.72 | 0.79 | 0.23 | 1.48 |
| | expose | 0.98 | 0.14 | | 0.75 | 0.21 | | 1.2 | 0.19 | | 1.75 | 0.58 | | 1.17 | 0.4 | |
| *Il6* | sham | 1.23 | 0.14 | 1.03 | 0.75 | 0.24 | 1.12 | 2.27 | 1.19 | 1.76 | 0.69 | 0.22 | 1.73 | 0.89 | 0.3 | 1.18 |
| | expose | 1.26 | 0.14 | | 0.84 | 0.39 | | 4.01 | 1.06 | | 1.19 | 0.55 | | 1.05 | 0.43 | |
| *Il1a* | sham | 0.79 | 0.29 | 2.16 | 0.35 | 0.38 | 0.72 | 0.69 | 0.2 | 1.64 | 0.69 | 0.19 | 0.64 | 0.51 | 0.29 | 7.16 |
| | expose | 1.72 | 0.79 | | 0.26 | 0.18 | | 1.13 | 0.52 | | 0.45 | 0.25 | | 3.67 | 3.4 | |
| *Il1b* | sham | 1.25 | 0.57 | 1.43 | 0.89 | 0.23 | 0.85 | 0.58 | 0.25 | 0.86 | 0.95 | 0.19 | 0.83 | 1.11 | 0.31 | 4.36 |
| | expose | 1.78 | 0.89 | | 0.76 | 0.25 | | 0.5 | 0.04 | | 0.79 | 0.36 | | 4.86 | 4.31 | |

Significant changes in the gene expressions in the RF-EMF-exposed rats are shown by the relative ratio; light gray: $P < 0.05$ and dark gray: $P < 0.01$.

sive heat production derived from RF-EMF exposure. In most of the cases, the core temperatures reached a peak or plateau in 10 min after turning on the exposure system, indicating that the RF energy was absorbed by the rat's body and induced increase in core temperature. These observed increases mostly corresponded with the simulated values. Furthermore, the plateau of core temperature was assumed to obey a heat balance equation (Adair and Black, 2003). Heat balance is one of the prominent factors that maintain plateau, taking into account the absorbed RF energy, metabolic process, heat storage in the body, and heat dissipation from the body. Under the exposure condition employed in the present study, when the ambient temperature in the reverberation chamber was 23°C, heat balance remained at approximately 38.5°C. In a human study using MRI measurement, RF-EMF exposure induced core temperature rise by 0.15°C in warm (31°C) ambient temperature but did not induce the same in two lower (24°C or 28°C) ambient temperatures (Adair *et al*. 2001). Here the core temperature linearly increased from 37.5°C to 42°C in 1 hr when the ambient temperature was set at 38°C (Fig. 4). Thus, ambient temperature plays a significant role in the regulation of increasing core temperature induced by RF-EMF exposure. The decrease in the core temperature showing high set point was caused by heat dissipation after turning off the exposure system, and approximately 30 min was required for the core temperature to reach the baseline. This result suggested that the core temperature could be decreased in rats through normal regulation, such as cooling with blood flow, heat transfer to the bedding or acrylic cage, insensible perspiration, and saliva spreading (Hainsworth, 1967).

In contrast, if the increase in core temperature depends on autonomic heat production, we should take into account other upregulation factors, such as basal metabolism, food intake, shivering thermogenesis, physical activity, and stresses. Therefore, we performed analyses during sleep stage (daylight) to reduce the effect of basal metabolism, food intake, and circadian variation as much as possible. However, the effects of physical activities cannot be completely removed, and the small variations in the core temperature throughout the experiment in all rats, except those under anesthesia, might have been induced by physical activities (data not shown). In addition, the core temperature may be temporarily downregulated if rats overlapped over one another in the cage during exposure because under such condition, the rat's body might fail to absorb the RF energy.

Subsequently, we assessed whether RF-EMF exposure produced any stresses on the rats. At WBA-SAR of 4 W/kg, several *Hsp* and *Hsf* genes were significantly upregulated in the cerebral cortex and cerebellum following RF-EMF exposure, which may be because of the increases in body temperature. Although no upregulated genes were observed when the exposure duration was 3 hr//day, statistically significant upregulation of four *Hsp* (*Hsp40*, *Hsp60*, *Hsp70*, and *Hsp90aa1*) and three *Hsf* (*Hsf1*, *Hsf2*, and *Hsf4*) genes were noted in the cerebellum when the exposure duration was 6 hr/day. These findings implicated that short-term exposure, such as less than 3 hr/day at WBA-SAR of 4 W/kg, had little thermal effects on the transcriptional changes of *Hsp* and *Hsf* genes, whereas the thermal effects were significantly high, particularly in the cerebellum, following long-term exposure, such as more than 6 hr/day. Moreover, the thermal effects were more evident in the cerebellum than in the cerebral cortex, which may be because of the variation in thermal regulation in each part of the brain during RF-EMF exposure. However, this variation in the thermal regulation makes investigation of the distribution and regulation of body temperature induced by RF-EMF exposure difficult. Therefore, further analyses must be performed by taking into account the numerical dosimetry of RF-EMF (Hirata and Fujiwara, 2012) and association between cerebral blood flow and temperature (Masuda *et al*., 2011).

The results of gene expression of *Hsp70* are almost similar to those reported in previous studies. Using in-situ hybridization analysis, Fritze *et al*. (1997) showed that *Hsp70* mRNA was slightly induced in the cerebellum after RF-EMF exposure for 4 hr at WBA-SAR of 4.2 W/kg. Furthermore, Yang *et al*. (2012) reported the upregulation of *Hsp70* after 20 min of RF-EMF exposure at WBA-SAR of 6 W/kg. These findings implicate that transcriptional upregulation of *Hsp70* is more significant following RF-EMF exposure for more than 3 hr/day at WBA-SAR of over 4 W/kg. One of the main physiological functions of *Hsp70* is acquired thermotolerance (Kregel, 2002). Similarly, *Hsp40* also plays a significant role in heat resistance along with *Hsp70* (Hattori *et al*., 1993). Therefore, it can be presumed that the upregulation of both *Hsp40* and *Hsp70* might be caused by the thermal effects of RF-EMF exposure in the cerebellum. In contrast, upregulation of *Hsp60* or *Hsp90* reflects the secondary effects derived from thermal effects. Both *Hsp90* and *Hsp60* are not necessarily upregulated by thermal stress and have various functions to maintain homeostasis. *Hsp90* not only helps in repairing proteins by folding and unfolding but also possesses many homeostatic functions as a client protein, such as cell cycle, cell growth, cell survival, apoptosis, oncogenesis, steroid hormone, and stress tolerance and has been applied to therapies, such as anticancer drugs (Miyata, 2003; Bagatell

and Whitesell, 2004). *Hsp60*, located in the mitochondria or cytosol, has a role in the regulation of apoptotic cell death induced by ischemia or hypoxia (Lin *et al*., 2001; Gupta and Knowlton, 2002). To understand the cause of these secondary effects, upstream or downstream signal transductions associated with the expression of *Hsp* and *Hsf* must be investigated using molecular and biochemical analyses.

The maximum relative ratio of *Hsp70* in the RF-EMF-exposed group (WBA-SAR = 4 W/kg) was 5.99 in the cerebellum, which was obviously lower than that in the thermal exposure group. In addition, no significant differences in the inflammatory cytokines were observed under any conditions. These results implicated that RF-EMF exposure does not induce organic disorders and prominent inflammation. In contrast, significant upregulation of *Hsf1*, which regulates heat-shock response (Pirkkala *et al*., 2001), was noted in the cerebellum. This finding, along with the behavioral change noted after RF-EMF exposure, indicated that the rats were under stress when exposed to RF-EMF for more than 6 hr/day.

Therefore, the present study provides new insight into the real-time variation in core body temperature during RF-EMF exposure and the changes in the expression of *Hsp* and *Hsf* families after RF-EMF exposure at a frequency of 2.14 GHz and WBA-SAR of 4 W/kg. The core temperature increased by 1.0-1.5°C compared with the baseline and remained as a plateau at approximately 38.5°C, depending on the ambient temperature (23°C) during RF-EMF exposure. Transcriptional analyses revealed significant upregulation of some *Hsp* and *Hsf* genes, particularly in the cerebellum. It was presumed that RF-EMF exposure for less than 3 hr/day, even at WBA-SAR of 4 W/kg, produced negligible effects on the upregulation *Hsp* and *Hsf* families in the brain. In addition, our data of low-intensity RF-EMF exposure at WBA-SAR of 0.4 W/kg reinforced that RF-EMF exposure within the limit for general public and occupational exposure does not produce any biological effect.

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**Conflict of interest----** The authors declare that there is no conflict of interest.

## REFERENCES

ICNIRP (1998): Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). International Commission on Non-Ionizing Radiation Protection. Health Phys., **74**, 494-522.

Adair, E.R. and Black, D.R. (2003): Thermoregulatory responses to RF energy absorption. Bioelectromagnetics, **Suppl. 6**, S17-38.

Adair, E.R., Mylacraine, K.S. and Allen, S.J. (2003): Thermophysiological consequences of whole body resonant RF exposure (100 MHz) in human volunteers. Bioelectromagnetics, **24**, 489-501.

Adair, E.R., Mylacraine, K.S. and Cobb, B.L. (2001): Human exposure to 2450 MHz CW energy at levels outside the IEEE C95.1 standard does not increase core temperature. Bioelectromagnetics, **22**, 429-439.

Bagatell, R. and Whitesell, L. (2004): Altered Hsp90 function in cancer: a unique therapeutic opportunity. Mol. Cancer Ther., **3**, 1021-1030.

Chakarothai, J., Wang, J., Fujiwara, O., Wake, K. and Watanabe, S. (2013): Dosimetry of a reverberation chamber for whole-body exposure of small animals. IEEE Trans. Microw. Theory Techn., **61**, 3435-3445.

Foster, K.R. and Morrissey, J.J. (2011): Thermal aspects of exposure to radiofrequency energy: report of a workshop. Int. J. Hyperthermia, **27**, 307-319.

Fritze, K., Wiessner, C., Kuster, N., Sommer, C., Gass, P., Hermann, D.M., Kiessling, M. and Hossmann, K.A. (1997): Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. Neuroscience, **81**, 627-639.

Gupta, S. and Knowlton, A.A. (2002): Cytosolic heat shock protein 60, hypoxia, and apoptosis. Circulation, **106**, 2727-2733.

Hainsworth, F.R. (1967): Saliva spreading, activity, and body temperature regulation in the rat. Am. J. Physiol., **212**, 1288-1292.

Hattori, H., Kaneda, T., Lokeshwar, B., Laszlo, A. and Ohtsuka, K. (1993): A stress-inducible 40 kDa protein (hsp40): purification by modified two-dimensional gel electrophoresis and co-localization with hsc70(p73) in heat-shocked HeLa cells. J. Cell. Sci., **104 (Pt 3)**, 629-638.

Hirata, A. and Fujiwara, O. (2012): Health effects of electromagnetic fields on humans. J. Plasma Fusion Res., **88**, 420-424.

Jauchem, J.R. and Frei, M.R. (1997): Body heating induced by subresonant (350 MHz) microwave irradiation: cardiovascular and respiratory responses in anesthetized rats. Bioelectromagnetics, **18**, 335-338.

Kregel, K.C. (2002): Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol., **92**, 2177-2186.

Langer, F. and Fietz, J. (2014): Ways to measure body temperature in the field. J. Therm. Biol., **42**, 46-51.

Lin, K.M., Lin, B., Lian, I.Y., Mestril, R., Scheffler, I.E. and Dillmann, W.H. (2001): Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation, **103**, 1787-1792.

Masuda, H., Hirata, A., Kawai, H., Wake, K., Watanabe, S.,

Arima, T., Poulletier de Gannes, F., Lagroye, I. and Veyret, B. (2011): Local exposure of the rat cortex to radiofrequency electromagnetic fields increases local cerebral blood flow along with temperature. J. Appl. Physiol., **110**, 142-148.

Miyata, Y. (2003): [Molecular chaperone HSP90 as a novel target for cancer chemotherapy]. Nihon Yakurigaku Zasshi, **121**, 33-42.

Ohtani, S., Ushiyama, A., Maeda, M., Ogasawara, Y., Wang, J., Kunugita, N. and Ishii, K. (2015): The effects of radio-frequency electromagnetic fields on T cell function during development. J. Radiat. Res., **56**, 467-474.

Paparini, A., Rossi, P., Gianfranceschi, G., Brugaletta, V., Falsaperla, R., De Luca, P. and Romano Spica, V. (2008): No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics, **29**, 312-323.

Pirkkala, L., Nykänen, P. and Sistonen, L. (2001): Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J., **15**, 1118-1131.

Sanchez, S., Masuda, H., Ruffié, G., Poulletier de Gannes, F., Billaudel, B., Haro, E., Lévêque, P., Lagroye, I. and Veyret, B. (2008): Effect of GSM-900 and -1800 signals on the skin of hairless rats. III: Expression of heat shock proteins. Int. J. Radiat. Biol., **84**, 61-68.

Shi, J., Chakarothai, J., Wang, J., Wake, K., Watanabe, S. and Fujiwara, O. (2014): Quantification and Verification of Whole-Body-Average SARs in Small Animals Exposed to Electromagnetic Fields inside Reverberation Chamber. IEICE Trans. Commun., **97**, 2184-2191.

Takahashi, S., Imai, N., Nabae, K., Wake, K., Kawai, H., Wang, J., Watanabe, S., Kawabe, M., Fujiwara, O., Ogawa, K., Tamano, S. and Shirai, T. (2010): Lack of adverse effects of whole-body exposure to a mobile telecommunication electromagnetic field on the rat fetus. Radiat. Res., **173**, 362-372.

Watilliaux, A., Edeline, J.M., Lévêque, P., Jay, T.M. and Mallat, M. (2011): Effect of exposure to 1,800 MHz electromagnetic fields on heat shock proteins and glial cells in the brain of developing rats. Neurotox. Res., **20**, 109-119.

Yang, X.S., He, G.L., Hao, Y.T., Xiao, Y., Chen, C.H., Zhang, G.B. and Yu, Z.P. (2012): Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. Brain Res. Bull., **88**, 371-378.