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RESEARCH ARTICLE

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Characterising core body temperature response of free-moving C57BL/6 mice to 1.95 GHz whole-body radiofrequency-electromagnetic fields

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Abstract

The present study investigated the core body temperature (CBT) response of free-moving adult male and female C57BL/6 mice, during and following a 2-h exposure to 1.95 GHz RF-EMF within custom-built reverberation chambers, using temperature capsules implanted within the intraperitoneal cavity and data continuously logged and transmitted via radiotelemetry postexposure. Comparing RF-EMF exposures (WBA-SAR of 1.25, 2.5, 3.75, and 5 W/kg) to the sham-exposed condition, we identified a peak in CBT within the first 16 min of RF-EMF exposure (+0.15, +0.31, +0.24, +0.37°C at 1.25, 2.5, 3.75, and 5 W/kg respectively; statistically significant at WBA-SAR ≥ 2.5 W/kg only), which largely dissipated for the remainder of the exposure period. Immediately before the end of exposure, only the CBT of the 5 W/kg condition was statistically differentiable from sham. Based on our findings, it is apparent that mice are able to effectively compensate for the increased thermal load at RF-EMF strengths up to 5 W/kg. In addition, the elevated CBT at the end of the exposure period in the 5 W/kg condition was statistically significantly reduced compared to the sham condition immediately after RF-EMF exposure ceased. This would indicate that measures of CBT following the end of an RF-EMF exposure period may not reflect the actual change in the CBT of mice caused by RF-EMF exposure in mice.

KEYWORDS

body temperature, mouse, radiofrequency, radiotelemetry

1 | INTRODUCTION

Radiofrequency electromagnetic fields (RF-EMF) refer to naturally occurring or man-made electromagnetic fields within the frequency range 100 kHz–300 GHz. Many important technologies, like mobile phones, operate within the RF-EMF spectrum. As it stands, the primary established biological effect of RF-EMF is via the deposition of thermal energy into tissue (Ziegelberger et al., 2020). Such biological effects of RF-EMF exposure have been investigated in rodent models for many years (Kim et al., 2020, 2022; Lu et al., 1987; Ohtani et al., 2016;

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Yamaguchi et al., 2003). Despite this, the effects of RF-EMF exposure on the core body temperature (CBT) of rodents is not well understood. The current standard practice for rodent CBT assessment in the context of RF-EMF exposure is via rectal thermometry immediately before and after a period of RF-EMF exposure. Not only does this fail to provide any information on CBT during exposure, but the procedure itself can elicit a substantial rise in CBT, which can take up to an hour to return to baseline (Poole & Stephenson, 1977; Van Der Heyden et al., 1997; Zethof et al., 1994). That is, temperature measurements obtained via this procedure postexposure to RF-EMF would likely be contaminated by stress-mediated and handling-induced temperature rise due to the measurement procedure. It is also not practical to routinely assess rectal temperature during an exposure interval given that it is invasive and interferes with the exposure itself. Less invasive methods of CBT measurement, such as the scanning of a subcutaneously implanted microchip, may reduce contamination from stress but are not true measures of CBT. That is, as the microchips are implanted subcutaneously rather than in the animal's core, it only provides data regarding superficial temperature, which can fluctuate rapidly due to a range of factors such as ambient air temperature (Meyer et al., 2017).

The use of intraperitoneally implanted telemetric capsules enables the continuous assessment of CBT without interaction with or manipulation of the rodents, thereby avoiding the influence of handling or procedural stress. Additionally, continuous data logging enables the evaluation of CBT during RF-EMF exposure, providing a far greater insight as to the CBT during exposure, as well as any thermoregulatory responses of rodents during that exposure. Evaluation of CBT during RF-EMF exposure using telemetry in free-moving rats has been previously reported (Kim et al., 2022; Ohtani et al., 2016). The most recent of which did not obtain CBT measurements past the end of RF-EMF exposure, nor did they investigate the effect of different RF-EMF exposure levels, which may have provided valuable insight. In addition, only male rats were studied, which have been reported to differ in thermal tolerance to RF-EMF exposures compared to female rats (Wyde et al., 2018). However, rats also differ in terms of thermoregulation and tolerance to RF-EMF exposures compared to mice, with the rat's smaller surface area-to-mass ratio leading to greater CBT rise (Wyde et al., 2018). The CBT of free-moving mice during a high RF-EMF exposure level has not been reported. The present study investigated the CBT response of free-moving adult male and female C57BL/6 mice, before, during, and following a 2-h exposure to 1.95 GHz RF-EMF of different intensities within custom-built reverberation chambers, using temperature capsules implanted within the intraperitoneal cavity and data transmitted postexposure via radiotelemetry.

Highlights

- Exposure of 1.95 GHz electromagnetic fields at 5 W/kg whole-body average specific absorption rate increases core body temperature by 0.4°C.
- The increased thermal energy at 3.75 W/kg is effectively managed by thermoregulation (max increase = 0.24°C).
- An extended habituation period prior to RF-EMF exposure is necessary to account for the large effect of handling on mice core body temperature.

2 | METHODS

2.1 | Animals and housing

Twelve adult male and 12 adult female C57BL/6 mice with a C3H background (wild-type mice from a B6C3-Tg (APPswe, PSEN1dE9)85Dbo/JAusb breeding colony) were obtained from Animal BioResources. Males were housed individually to avoid aggression between cage-mates after surgery, while females were housed in groups of three per cage in Tecniplast GM500 individually ventilated cage housing units lined with corn cob bedding. The housing environment was maintained at a room temperature of 22 ± 0.2°C and operated on a 12-h dark and light cycle (0700–1900 h light, 1900–0700 h dark). Mice were allowed ad libitum access to water and standard laboratory chow. Environmental enrichment including house domes, a chew block, a tunnel and nesting materials were present in all cages. During the RF-EMF exposures, mice were transferred to a different "exposure cage," where food and water was not available. The exposure cage apparatus consisted of an H-temp polysulfone (PSU) cage base and PSU H-temp frame, with a polyester filter sheet (Tecniplast). Half a paper tissue was added to each exposure cage (to absorb urine). Female mice were exposed in groups of three while male mice were exposed individually. The age of mice ranged from 34 to 43 weeks of age. The weight of the mice is given in Table 1.

TABLE 1 Demographics for the male and female cohort of mice.

	Male	Female
n	12	12
Weight (g)	36.3 ± 0.93[a]	27.4 ± 0.43
Age (weeks)	38.9 ± 0.99	38.9 ± 0.99

[a]The weight of the male cohort was higher than the female cohort weight as determined with an independent *t*-test ($t_{(15.47)} = 8.69$, $p < 0.001$). Data are presented as mean ± standard error of the mean.

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All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (AE21/11) and adhered to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

2.2 | AniPill implantation

The AniPill (www.animals-monitoring.com) is a proprietary telemetric capsule of size 8.9 × 17.7 mm (diameter × length), weight 1.7 g, with a stated accuracy of ±0.2°C (over the temperature range 25–45°C) that is implanted into the intraperitoneal cavity of mice to provide measures of CBT which are continuously logged throughout the course of the experiment and downloaded postexposure (or other data acquisition period) using radiotelemetry. The methods for implantation of the AniPill were similar to that described by Chappell et al. (2011). In brief, mice were first inducted with isoflourane (5%) and injected with subcutaneous fluids and buprenorphine. The intraperitoneal cavity was accessed via a 1 cm incision in the right flank region of the lateral abdominal muscle. A bupivicaine line block was injected into the incision site. The AniPill was then placed into the intraperitoneal space, the muscle and skin were sutured and the skin was closed with flexible skin glue. Mice were recovered in oxygen and warmed in a clean home cage before returning to the holding room. All surgical procedures were performed by trained technical staff in a sterile environment.

During recovery, the incision site was monitored regularly to ensure there was no bleeding, infection or signs of pain. One female mouse exhibited signs of abdominal pain at 24 h postsurgery and was given an additional subcutaneous injection of meloxicam (5 mg/kg). Additionally, two male mice required a brief period of anaesthetisation after surgery to re-close the skin (within 24 h postsurgery). All mice had a minimum of twelve days recovery prior to the first RF-EMF exposure to allow adequate time for healing of the surgical site, as well as the return of normal activity levels and circadian body temperatures (Chappell et al., 2011; Helwig et al., 2012; Leon et al., 2004). One AniPill ceased communication shortly after implantation, therefore all CBT data presented is from 12 male mice and 11 female mice. Despite the communication failure, the female mouse was exposed to the RF-EMF with its cage mates.

2.3 | Exposure system and dosimetry

RF-EMF exposure was provided by a reverberation chamber (RC) in which the mice were placed. The RC delivered 1.95 GHz RF-EMF at five exposure levels, corresponding to Whole-Body Average–Specific Absorption Rate (WBA-SAR) levels of 0 (sham), 1.25, 2.5, 3.75 and 5 W/kg.

Detailed information on the dosimetry of the RC is provided in Iskra et al. (2024). In brief, the RC was 1.45 m × 1.45 m × 1.45 m in internal chamber dimensions (manufactured by Compliance Engineering). Mode stirring was achieved using a combination of six antennas within the RC (placed centrally on each of the six sides) and three small metallic stirrers (with blades of around 30 cm length and 6 cm width) placed in corners of the floor of the RC. Statistical field uniformity in the central working volume was achieved by directing power to each antenna sequentially (10 s per antenna), and with the stirrers operating at 0.5 turns per second. Based on measurements in the central working volume, the standard uncertainty in the field uniformity was 1.39 dB. The RF-EMF into the RC was provided by a signal generator, a digital step attenuator, an RF amplifier, and a six-way coaxial switch feeding the signal into the antennas. Control software enabled the precise amount of power to be directed to each antenna. The forward RF power at the output of the amplifier was continuously measured and adjusted to maintain WBA-SAR at the predetermined level. The E-field strength in the chamber was monitored to verify that power was being delivered into the chamber. A fan, attached with a rubber mat base to a fine metallic mesh panel on the top of the RC, provided suitable airflow for the mice (with the air in the RC being fully refreshed every 2 min), with the air input through a second mesh panel inserted into one of the sides.

The determination of the input power to achieve the desired WBA-SAR levels was performed through measurement of the RF-EMF within the RC using phantom mice (cylindrical tubes filled with 23.6 g of tissue simulant liquid with dielectric properties of electrical conductivity $\sigma = 1.4$ S/m, and relative permittivity $\varepsilon_r = 40$), and through comparison with computational analysis of the exposure using the finite-difference time-domain (FDTD) technique including voxel models of the phantoms. Voxel models of the mice (IT'IS Foundation, 2023; McIntosh et al., 2010) were then used to extrapolate the settings for the phantoms to mice. The WBA-SAR sensitivity was 291.5 μW/kg/(V/m^2). Validation of the WBA-SAR levels was performed through a total of six thermal measurements using fluoroptic probes placed in two of the phantoms and using the "lumped-heat-capacity" method (Holman 1997). To satisfy the assumption of uniform temperature, as required in the lumped-heat-capacity method, each of the two phantoms were suspended by nonmetallic strings and moved mechanically around every 5 s to gently stir the tissue simulant liquid. Across all six measurements the average ratio of the measured to computed WBA-SAR was 0.63 dB and the maximum ratio was 1.1 dB, providing confidence that the computational estimates of WBA-SAR are well matched to exposures in the RC.

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The total expanded uncertainty ($k = 2$) in exposure, combining measurement and computational sources of uncertainty, was estimated to be 3.89 dB (95th percentile). The size of this variation is consistent with findings in the study by De Santis et al. (2023).

2.4 | Validation of AniPill measurements

Since temperature measurements via rectal thermometry are the most commonly used method of obtaining CBT in rodents, we wanted to assess whether CBT as measured by the AniPill was similar to the CBT measured via rectal thermometry. To do this, mice were taken from their cage using their tunnel, gently restrained by the base of the tail, and a mouse rectal probe (World Precision Instruments) lubricated with Vaseline was inserted 2 cm into the rectum. The probe was left in place for approximately 5 s before being removed and the mouse returned to its home cage. The rectal probe had a stated accuracy and resolution of ±0.3 and 0.1°C respectively, and a time constant of 0.5 s. This procedure was timed so that the AniPill measure was recorded concurrent to the rectal measurement.

The impact of RF-EMF exposure on the performance of the AniPill was explored as part of pre-trial investigations. Investigations were performed with 4 mice and 18 phantoms (one phantom with an AniPill placed inside) in the RC and a second, external AniPill, in a free space sitting on a shelf in proximity to the mouse and phantom. The input power to the chamber was set to achieve an average WBA-SAR level of 3.75 W/kg in the mice/phantom, however the estimated mass in the chamber was lower than initially calculated, resulting in an average WBA-SAR level of approximately 4.4 W/kg. The exposure period was 2 h to allow for the temperature in the phantom to reach its maximum level. Three exposure runs were performed. The temperature profiles for both AniPills were smooth and continuous with an average maximum temperature increase of 2.96°C in the phantoms and the external AniPill temperature rising by ~0.1°C over a 2 h continuous exposure. It should be noted, however, that the temperature rise recorded by the external AniPill may not be related to a direct impact of the field on the capsule. It may have been due to a small increase in local air temperature generated by heat from nearby mice and phantom, and from the shelf on which it was placed.

2.5 | Experiment design

All RF-EMF exposures were performed during the light phase. Six females were exposed together in two cages of three mice, and six males were exposed together, one mouse per cage. Each group was exposed for two consecutive hours per day, at each of the five exposure levels (WBA-SAR of 0, 1.25, 2.5, 3.75, and 5 W/kg). These were spread over a 5-day interval (pseudorandom with constraints; see below), with exposure occurring in the morning (9:30–11:30 AM) for the female group and in the afternoon (1:30–3:30 PM) for the male group. Following a 2-day interval with no testing, each group repeated this testing, differing only in that the female and male groups were tested in the afternoon and morning respectively. This was then repeated with another cohort of six female and six male mice.

The general exposure protocol was as follows: Mice were transported from the holding room to the exposure room where they were placed into the exposure cage (14 × 31 × 17 cm) in which they were able to freely move. Exposure cages were placed in the RC with the lights, stirrers and fans operating (but no RF-EMF) for a 1-h habituation period. Following habituation, mice were exposed to RF-EMF at one of the above five designated exposure levels for 2 h. After exposure cessation, mice remained in the chamber for an additional hour. CBT was recorded automatically once every 2 min throughout this procedure.

AniPills were also placed into phantom mice to record temperature changes during the exposures that were not influenced by thermoregulation. The specific heat capacity of the liquid was stated to be 3390 J/(kg °C), similar to that of typical animal tissues.

In total, each loading of the RC consisted of the six mice and four phantoms, all with AniPills. Nine additional phantoms, without AniPills, were also placed in the RC so that the total mass of lossy matter within the RC was in the range at which the WBA-SAR levels had been verified. The four phantoms containing AniPills were arranged within the chamber to capture all locations for each RF-EMF exposure level: the RC had two shelves in which exposure cages were placed, each of which had space for four exposure cages. There were two designated AniPill locations for each cage position resulting in a total of 16 AniPill locations that were recorded for each RF-EMF exposure. For positions within the chamber where a mouse exposure cage was placed, the phantom was positioned on the cage lid to avoid heat transfer between the mice and phantoms. In all other cases, phantoms were placed on shelves at the same level as the exposure cages.

To remove potential confounds due to experimenter interaction with the rodents and transferring from the holding room to the testing room, the above 1-h habituation period was employed to ensure that any effects of handling on CBT had sufficient time to abate before exposures commenced.

2.6 | Randomisation and blinding

Given that female and male mice were exposed as (separate) groups, there were not enough conditions to enable full counterbalancing. To account for this, a

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pseudorandomised order was used for the morning exposures, and the reverse order used for the afternoon exposures, with these orders kept constant across the two, 5-day testing intervals. Further, the pseudorandomised order was constrained such that the same exposure level could not be repeated, and the exposure level on any given day needed to differ from that of the preceding day by at least 2.5 W/kg. The male and female groups thus had identical exposure orders (when averaged over the two 5-day testing intervals).

No blinding occurred within this study. To remove any experimenter-induced effects on CBT, sufficient time was given pre-exposure to allow the initial CBT rise due to handling to abate and to obtain a stable CBT prior to exposure. Also, all measurements were automated (did not involve "any" experimenter intervention).

2.7 | Data and statistical analysis

2.7.1 | Primary

Temperature profiles across the five exposure conditions were first averaged to create a single "temperature by time" profile, for males and females separately (Figure 1). Since the free movement of the mice caused variations in baseline CBT, we identified from this the most stable pre-exposure interval to define as the baseline, and the interval that best represented the maximum temperature rise following exposure onset. Accordingly, for each male and female data set, the baseline was defined as the average 6-min period immediately prior to the beginning of exposure, and the maximum temperature rise ("Max") as the average of the first 16 min following exposure onset (CBT fell after this interval). Additionally, as it is typically assumed that temperature will be expected to continue to rise if thermoregulation is not sufficient to compensate for the RF-induced temperature rise in the mice, we also computed the average of the last 16 min of exposure ("End"). For each mouse and exposure condition, the baseline temperature was subtracted from each of "Max" and "End," to produce the CBT change scores used for statistical analysis (i.e., MaxΔT and EndΔT respectively). To determine CBT change following RF-EMF exposure, we computed the average of the first 16 min following exposure cessation ("Post") and subtracted the End average from this to produce "PostΔT." MaxΔT, EndΔT, and PostΔT data were analysed separately using mixed-design analysis of variance (ANOVA) with planned contrasts, comparing each of the (repeated measures) 1.25, 2.5, 3.75, and 5 W/kg WBA-SAR to sham (0 W/kg WBA-SAR), as well as the interaction of these comparisons with sex (between subjects). It should be noted that the factor "sex" is confounded by the number of mice in the exposure cages, that is, male mice were exposed individually while female mice were exposed in groups of three. Results concerning this factor cannot be solely attributed to sex. Temperature data from the mouse phantoms was treated in the same way as data from real mice except sex was not a factor.

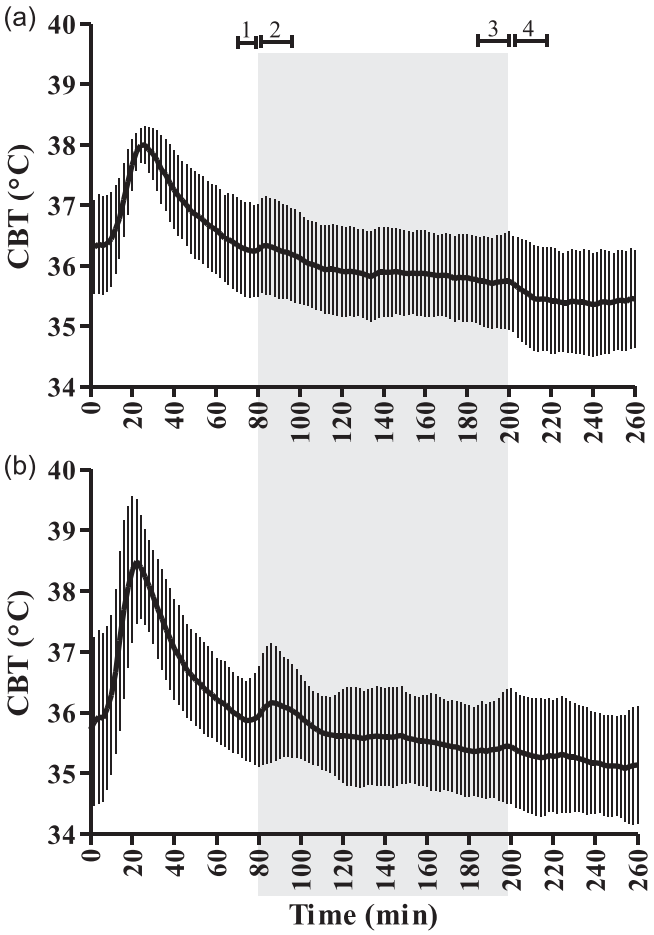


FIGURE 1 Core body temperature (CBT) of female ($n = 11$) (a) and male ($n = 12$) (b) mice over the duration of the exposure protocol (all WBA-SAR CBT profiles averaged to produce a single CBT profile over time). Period 1 was used to calculate baseline CBT; period 2 is first 16 min after RF-EMF exposure began which was used to calculate the MaxΔT; period 3 is the last 16 min of RF-EMF exposure which was used to calculate EndΔT and period 4 indicates the first 16 min postexposure which was used to calculate PostΔT.

CBT data obtained from the concurrent AniPill and rectal thermometer measurements were organised into a Bland–Altman plot to assess the agreement between the two methods. Additionally, a paired *t*-test was performed to determine whether the mean CBT from each method was different.

2.7.2 | Exploratory

A hypothesis generating analysis was also conducted to assess CBT differences in the exposure conditions compared to sham throughout the entire duration of exposure. All CBT measures throughout the exposure were averaged into 30-min time blocks, to produce a

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single CBT value for the 0–30, 32–60, 62–90, and 92–120 min time-intervals. Each of these intervals was analysed separately using mixed-design ANOVA with planned contrasts, comparing each of the (repeated measures) 1.25, 2.5, 3.75, and 5 W/kg WBA-SAR to sham (0 W/kg WBA-SAR), as well as the interaction of these comparisons with sex (between subjects).

All statistical analyses were performed using SPSS (version 28; IBM). All data were assessed for normal distribution using a Shapiro–Wilk test. Some data did not meet all assumptions of the mixed-design ANOVA. Sham PostΔT, MaxΔT, and EndΔT of the 1.25 W/kg condition and MaxΔT and PostΔT of the 5 W/kg condition data were not normally distributed. In these cases, equivalent Wilcoxon signed-rank tests were also performed to determine whether *p*-values from parametric tests were importantly influenced by the failure to meet normality assumptions. The Wilcoxon signed-rank test and mixed-design ANOVA produced equivalent results, thus the outliers were not responsible for the results.

3 | RESULTS

3.1 | Validation of experimental protocols

3.1.1 | CBT profile during the RF-EMF exposure protocol

The average CBT of male and female mice is presented in Figure 1. The protocol to expose mice to RF-EMF, including transport to the exposure room, transfer from their home cage to the exposure cage and placement into the RC caused a large increase in the CBT of both male and female mice (Figure 2). Mice required approximately 1 h for this rise in CBT to return to baseline.

3.1.2 | RC ambient temperature during RF-EMF exposure

The ambient temperature within the RC increased slightly over the course of the RF-EMF exposures (compared to sham). Averaging the temperature measurements from the free AniPill sitting on the shelf during all 5 W/kg exposures, the temperature within the chamber increased 0.05 ± 0.03°C within the first 16 min after exposure began to 0.20 ± 0.06°C in the 16 min before the end of the RF-EMF exposure.

3.1.3 | Comparison of AniPill and rectal CBT measurements

The Bland–Altman plot (Figure 3) revealed good agreement between the AniPill and rectal temperature measurements made without concurrent RF-EMF exposure, with only one measure lying outside the limits of agreement (95% confidence interval, 1.16 to −0.89). Additionally, the bias was close to zero, suggesting little systematic difference between the two methods of measurement. A paired *t*-test between the CBT values measured by the AniPill (mean = 36.17, SD = 1.10) and by rectal thermometry (mean = 36.03, SD = 1.21) showed no evidence that the two methods returned statistically significant different CBT measurements ($t_{(22)} = 1.297$, $p = 0.208$).

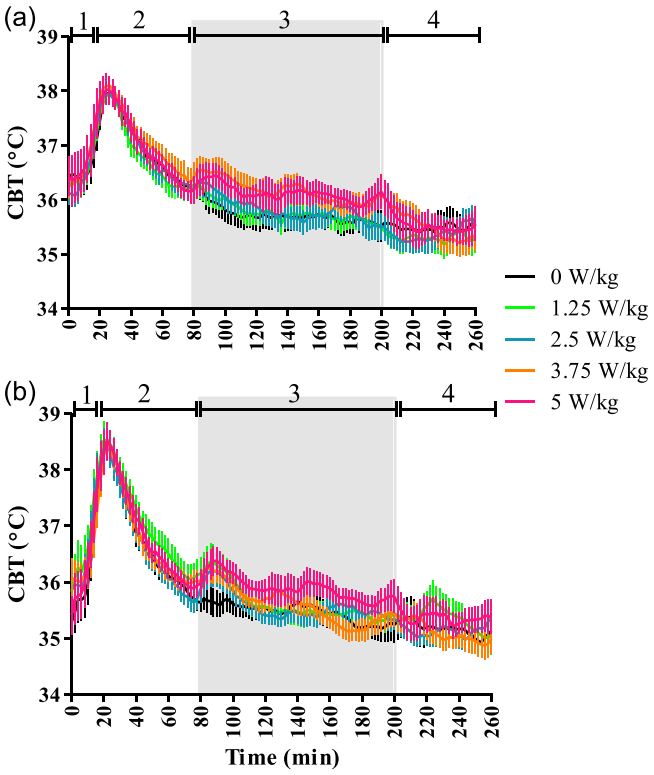


FIGURE 2 Core body temperature (CBT) of female ($n = 11$) (a) and male ($n = 12$) (b) mice over the duration of the exposure protocol. Period 1 (0–20 min) shows the transport of mice from the holding room and placement into the RC; period 2 (20–80 min) shows the 1 h habituation; period 3 (80–200 min) shows the 2 h RF-EMF (or sham) exposure; period 4 (200–260 min) shows the 1 h postexposure period. Error bars represent ± standard error of the mean.

3.1.4 | Increasing WBA-SAR caused a relative rise in the temperature of mouse phantom

While the phantom temperature appears stable over the course of the sham exposure, it increased over the duration of each RF-EMF exposure (Figure 4): EndΔT = 0, 0.71, 1.41, 2.12, and 2.85°C for sham, 1.25, 2.5, 3.75, and 5 W/kg WBA-SAR, respectively. Compared to sham, both MaxΔT and EndΔT were elevated at all WBA-SAR ($F_{1,7} \geq 226.3$, $p \leq 0.001$), and PostΔT was

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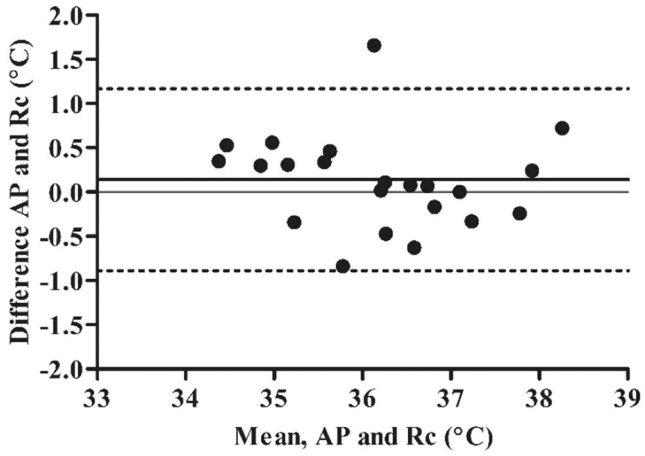


FIGURE 3 Agreement between rectal measurement (Rc) and AniPill (AP) measurement of core body temperature in the mice ($n = 23$). The upper and lower limits of agreement represent a 95% confidence interval. Each dot represents data from one mouse.

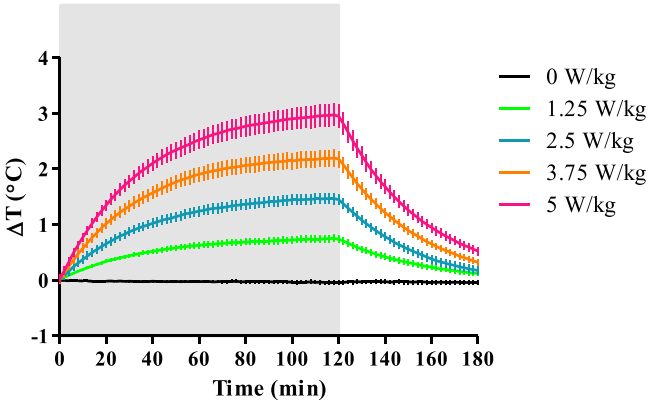


FIGURE 4 Change in temperature (relative to baseline) in mouse phantoms ($n = 8$) during and following exposure cessation from 2 h exposure to 1.95 GHz RF-EMF at a WBA-SAR of 0 W/kg (sham), 1.25, 2.5, 3.75, and 5 W/kg. RF-EMF exposure starts at 0 min; shaded area indicates RF-EMF exposure is ON; error bars represent ± standard error of the mean. Relative to sham, each of the RF-EMF exposures resulted in statistically significant temperature rises at the first and last 16-min interval of the exposure, as well as a statistically significant decrease at the 16-min interval immediately after exposure cessation.

reduced at all WBA-SAR ($F_{1,7} \geq 289.2$, $p \leq 0.001$). MaxΔT, EndΔT, and PostΔT values are given in Table 2.

3.2 | RF-EMF exposures > 1.25 W/kg induce a statistically significant increase in the CBT of mice compared to sham exposure

3.2.1 | MaxΔT

Compared to sham, a WBA-SAR of 2.5 W/kg ($F_{1,21} = 20.242$, $p < 0.001$), 3.75 W/kg ($F_{1,21} = 9.429$, $p = 0.006$) and 5 W/kg ($F_{1,21} = 24.303$, $p < 0.001$) increased CBT, with no statistically significant change observed for 1.25 W/kg ($F_{1,21} = 2.601$, $p = 0.122$) (Figure 5 and Tables 2 and 3). These RF-induced increases were larger for males than females, but only at 2.5 W/kg: $F_{1,21} = 5.667$, $p = 0.027$ (1.25 W/kg: $F_{1,21} = 0.655$, $p = 0.427$; 3.75 W/kg: $F_{1,21} = 0.279$, $p = 0.603$; 5 W/kg: $F_{1,21} = 0.106$, $p = 0.748$) (Tables 2 and 3).

3.2.2 | EndΔT

Compared to sham, there were no statistically significant changes observed for WBA-SAR of 1.25 W/kg ($F_{1,21} = 0.699$, $p = 0.423$), 2.5 W/kg ($F_{1,21} = 0.391$, $p = 0.538$) or 3.75 W/kg ($F_{1,21} = 0.020$, $p = 0.888$), whereas there was a statistically significant increase in the 5 W/kg condition ($F_{1,21} = 5.024$, $p = 0.036$). No interaction between sex and RF-EMF exposure level was found ($F_{1,21} \leq 1.826$, $p \geq 0.131$) (Tables 2 and 3).

3.2.3 | PostΔT

There were no statistically significant changes observed for the 1.25, 2.5, or 3.75 W/kg conditions compared to sham ($F_{1,21} \leq 2.927$, $p \geq 0.102$), whereas there was a statistically significant CBT reduction after the 5 W/kg exposure ($F_{1,21} = 4.66$, $p = 0.047$). No interaction between sex and RF-EMF exposure level was found ($F_{1,21} \leq 2.377$, $p \geq 0.138$) (Tables 2 and 3).

3.3 | CBT elevation in mice is sustained throughout RF-EMF exposure at 5 W/kg

At all exposure strengths >1.25 W/kg, the CBT of mice was higher than during the sham condition within the first 30-min average (Figure 5; 2.5 W/kg: $F_{1,21} = 11.998$, $p = 0.002$; 3.75 W/kg: $F_{1,21} = 7.197$, $p = 0.014$; 5 W/kg: $F_{1,21} = 29.333$, $p < 0.001$). At this time point, there was no interaction between power and sex. For the remainder of RF-EMF exposure, CBT was higher than sham for only the 5 W/kg exposure (60 min: $F_{1,21} = 8.076$, $p = 0.01$; 90 min: $F_{1,21} = 7.514$, $p = 0.012$; 120 min: $F_{1,21} = 6.173$, $p = 0.021$). There was a significant interaction between power and sex at the 32–60 min ($F_{1,21} = 6.932$, $p = 0.016$) and 62–90 min ($F_{1,21} = 7.073$, $p = 0.015$) average time blocks in the 3.75 W/kg exposed condition compared to sham.

4 | DISCUSSION

This study aimed to characterise the CBT changes of free moving mice due to 1.95 GHz RF-EMF exposure compared to sham exposure. We found that the

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TABLE 2 MaxΔT, EndΔT, and PostΔT values (°C) at a WBA-SAR of 0, 1.25, 2.5, 3.75, and 5 W/kg for phantom ($n = 8$), male mice ($n = 12$), female mice ($n = 11$) and the average of male and female data ($n = 23$).

		0 W/kg (sham)	1.25 W/kg	2.5 W/kg	3.75 W/kg	5 W/kg
MaxΔT	Phantom	−0.02 (0.00)	0.24 (0.01)	0.46 (0.03)	0.71 (0.05)	0.94 (0.02)
	M + F	−0.09 (0.06)	0.06 (0.08)	0.22 (0.07)	0.15 (0.06)	0.27 (0.07)
	Male	−0.05 (0.11)	0.17 (0.13)	0.43 (0.09)	0.15 (0.10)	0.34 (0.13)
	Female	−0.14 (0.06)	−0.07 (0.06)	0.00 (0.06)	0.14 (0.05)	0.20 (0.06)
EndΔT	Phantom	−0.03 (0.01)	0.71 (0.03)	1.41 (0.04)	2.12 (0.10)	2.85 (0.06)
	M + F	−0.56 (0.10)	−0.70 (0.11)	−0.46 (0.10)	−0.55 (0.11)	−0.22 (0.12)
	Male	−0.48 (0.15)	−0.76 (0.15)	−0.26 (0.13)	−0.66 (0.17)	−0.24 (0.16)
	Female	−0.65 (0.15)	−0.64 (0.16)	−0.68 (0.13)	−0.42 (0.16)	−0.20 (0.18)
PostΔT	Phantom	0.00 (0.00)	−0.13 (0.01)	−0.27 (0.01)	−0.37 (0.02)	−0.49 (0.02)
	M + F	0.01 (0.08)	−0.11 (0.07)	−0.23 (0.09)	−0.02 (0.09)	−0.20 (0.10)
	Male	0.10 (0.14)	0.04 (0.09)	−0.23 (0.14)	0.02 (0.11)	−0.26 (0.13)
	Female	−0.08 (0.06)	−0.27 (0.07)	−0.22 (0.10)	−0.06 (0.15)	−0.14 (0.15)

Note: Data are presented as mean (standard error of the mean). M + F refers to averaged male and female data.

maximum RF-induced temperature rise of the mice occurred within approximately the first 16 min of exposure, and was statistically significant for a WBA-SAR of 2.5, 3.75, and 5 W/kg (but not 1.25 W/kg) exposures. This temperature rise had largely dissipated by the end of the 2-h exposure period; indeed immediately before cessation of the 2-h exposure, 5 W/kg was the only exposure that was statistically differentiable from the sham-exposed condition.

Importantly in this study, we employed a 1-h habituation period prior to the start of RF-EMF exposure to account for the CBT rise induced by the transport and handling of these mice. It has been well documented these procedures can increase the CBT of mice considerably. In particular, the use of rectal thermometry to obtain a baseline measure prior to the start of exposure is likely to elevate CBT. When we consider the small magnitude of the change in CBT induced by RF-EMF exposure (<0.4°C) compared to the CBT rise of almost 3°C that can be induced by the transport and handling of rodents (Van Bogaert et al., 2006), not allowing adequate time for CBT to return to baseline is likely to confound any initial CBT changes caused by RF-EMF exposure at the RF-EMF exposure levels investigated here.

Only one other study has examined CBT change of mice during RF-EMF exposure at similar exposure levels (Ebert et al., 2005). That study fixed rectal probes to mouse tails to provide a continuous measure of CBT and attempted to account for the stress-related thermal rise induced by this procedure by waiting 10–20 min for the thermal rise to abate prior to applying RF-EMF. Several mice exposed to a WBA-SAR of 5 W/kg were determined to be within the thermal regulatory region, that is, the regulatory system of the mouse can compensate for the heat load induced by RF-EMF exposure, while all mice exposed to a WBA-SAR of 2 W/kg were determined to be below this region, that is, there was no discernible CBT change upon RF-EMF exposure. Ebert et al. reported two patterns of CBT change within the thermal regulatory region. One, where exposure initially increased CBT, before plateauing until termination of their 2-h exposure, which was followed by a reduction in CBT postexposure. The other, where there was no noticeable change during RF-EMF exposure, but a reduction in CBT was evident postexposure. For WBA-SAR up to 2.5 W/kg in our study, we observed a small peak in CBT compared to sham within the first 16 min of exposure (although not statistically significant at 1.25 W/kg), followed by a reduction in CBT towards sham for the remainder of the exposure duration. This is opposed to the absence of a discernible CBT change at a WBA-SAR of 2 W/kg reported by Ebert et al. (2005). This difference is likely due to differences in the experimental protocols: First, Ebert et al.'s mice were constrained within tubes, which would not have allowed either behavioural or peripheral thermoregulatory mechanisms to operate efficiently. Second, the stress hyperthermia caused by rectal measurement can inflate CBT for at least an hour after the procedure (Poole & Stephenson, 1977; Van Der Heyden et al., 1997; Zethof et al., 1994), which, as Ebert et al. only allowed 10–20 min to account for this effect, would again have inflated temperature due to factors unrelated to RF-EMF. Both of these factors would have masked the initial EMF-induced CBT peak that was observed in the present study. At a higher WBA-SAR of 5 W/kg, we observed a rise in CBT that remained

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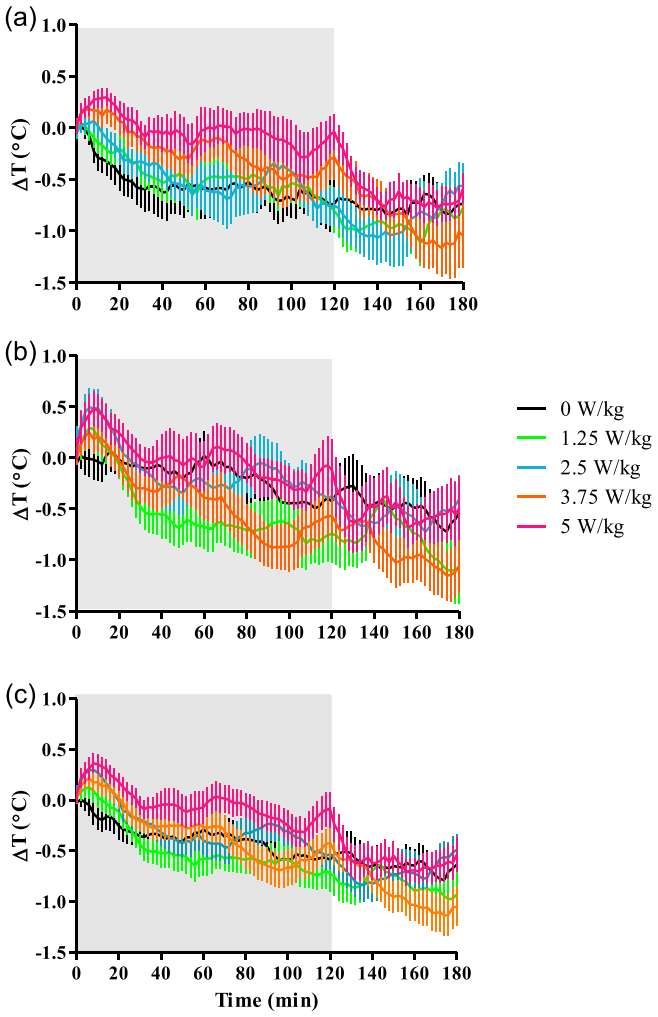


FIGURE 5 Change in temperature from baseline in mice ([a] female mice, n = 11; [b] male mice, $n = 12$; [c] male and female averaged, $n = 23$) during 2 h exposure to 1.95 GHz RF-EMF at a WBA-SAR of 0 W/kg (sham), 1.25, 2.5, 3.75, and 5 W/kg. Shaded area indicates RF-EMF exposure is ON; error bars represent ± standard error of the mean. Relative to sham, all RF-EMF exposures >1.25 W/kg increased core body temperature within the first 16-min interval of exposure (statistically significant), while only the 5 W/kg exposure caused a statistically significant increase compared to sham within the last 16-min interval.

sustained above sham CBT for the duration of the RF-EMF exposure, similar to the thermal regulatory profile described by Ebert et al. In female mice, this sustained increase in CBT was also apparent at a WBA-SAR of 3.75 W/kg. In another study, Jeong et al. (2015) reported RF-induced CBT changes of mice within an RC. This would be particularly useful as it was not affected by the limitations of using constraint within a tube (as it used an RC), but as CBT was only measured before and after exposure, and as it used manually administered rectal thermometry, we are unable to meaningfully compare our results with it.

Despite the strong statistical significance of the RF-EMF induced increase in CBT, the highest exposure level elicited an average CBT increase of only 0.4°C. It is thus evident in this cohort of mice, that RF-EMF of up to 5 W/kg was not a potent thermal stressor. There are several factors which may have contributed to this. The first is that the mice's thermoregulatory system was able to effectively compensate for the increased thermal energy (i.e., the temperature rise in mice was <10% of that found in the phantoms, which do not have active thermoregulation). Owing to their large surface area-to-mass ratio, mice primarily offload heat via dry heat loss—vasodilation of blood vessels at the paws and tail to enable heat transfer to the environment (Gordon, 2012; Škop et al., 2020). Behaviours like the reduction of physical activity and the spreading of saliva (evaporative heat loss) may have also countered the increased thermal load. It would appear that these thermoregulatory mechanisms effectively compensated for the increased thermal load from RF-EMF such that CBT did not rise after the initial peak (and were not statistically distinguishable from sham for WBA-SAR lower than 5 W/kg). This is supported by comparison to the pattern of temperature rise observed within the phantom mice (no thermoregulation). In the phantoms, the highest strength of exposure induced a 0.9°C increase within the first 16 min of exposure. Additionally, there was a 2.85°C elevation in phantom temperature at EndΔT

TABLE 3 MaxΔT, EndΔT, and PostΔT values (°C) at a WBA-SAR of 1.25, 2.5, 3.75, and 5 W/kg relative to sham.

		1.25 W/kg	2.5 W/kg	3.75 W/kg	5 W/kg
MaxΔT	M + F	0.15 (0.09)	0.32 (0.08)	0.24 (0.08)	0.37 (0.08)
	Male	0.22 (0.17)	0.47 (0.12)	0.20 (0.14)	0.39 (0.13)
	Female	0.07 (0.05)	0.15 (0.07)	0.29 (0.07)	0.34 (0.09)
EndΔT	M + F	−0.14 (0.16)	0.10 (0.15)	0.01 (0.15)	0.34 (0.14)
	Male	−0.28 (0.25)	0.22 (0.23)	−0.19 (0.20)	0.24 (0.12)
	Female	0.01 (0.21)	−0.03 (0.19)	0.23 (0.23)	0.45 (0.27)
PostΔT	M + F	−0.13 (0.11)	−0.24 (0.14)	−0.03 (0.11)	−0.22 (0.11)
	Male	−0.07 (0.19)	−0.33 (0.23)	−0.08 (0.16)	−0.37 (0.17)
	Female	−0.19 (0.09)	−0.14 (0.13)	0.02 (0.14)	−0.06 (0.14)

Note: Data are presented as mean (standard error of the mean). M + F refers to averaged male and female data.

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during the 5 W/kg exposures compared to sham, while in mice only a minor increase in CBT (+0.4°C) was observed under the same conditions. Without thermoregulation, as seen in the phantoms, heat continues to accumulate within tissues causing CBT to increase throughout the duration of the exposure period. With thermoregulation, as seen in the mice, there is a minor increase in CBT but this is quickly controlled.

The ambient temperature at which mice were exposed to RF-EMF may be another factor contributing to the minimal rise in CBT. The temperature within the RC was maintained at 22°C throughout the exposure period, which is typical of the housing practices of laboratory rodents (Gordon, 2004). However, this is substantially lower than the thermoneutral zone (TNZ) reported for mice, that is, the range of ambient temperatures at which CBT is maintained without the need to generate heat above the resting metabolic rate, which is approximately 29–31°C (Ganeshan & Chawla, 2017). Thus at colder ambient temperatures, like that at which mice are typically housed, a considerable amount of heat needs to be generated to maintain thermal homeostasis, via activation of non-shivering thermogenesis and increased metabolic rate (Ganeshan & Chawla, 2017; Škop et al., 2020). Furthermore, mice employ several behavioural strategies including huddling and nest building, reducing heat loss to the environment (Ganeshan & Chawla, 2017; Gordon, 2009). For our RF-EMF exposure protocol, the mice were exposed in cages fitted with one half of a paper tissue. Such limited nesting material is unlikely to have contributed to the thermal comfort of the mice. Additionally, males were housed and exposed individually so huddling was not possible. Considering these points, the thermal energy from RF-EMF may have been utilised by the mice to maintain thermal homeostasis (Kuhne et al., 2020). In this case it is likely a greater increase in CBT would have been observed in mice if they were exposed at temperatures closer to the TNZ. However, studies that investigate the effect of RF-EMF exposure on the CBT of rodents are not typically performed within the TNZ, making the present results applicable to the environmental conditions employed within the research domain only.

Considering the CBT change of male and female mice separately, there appeared to be a sustained increase of CBT above sham for the duration of RF-EMF exposure at WBA-SAR ≥3.75 W/kg in female mice, with our exploratory analyses revealing a significant interaction between exposure strength and sex at 3.75 W/kg. As mentioned previously, female mice were exposed with cage mates, while male mice were individually exposed. Since huddling is an important behavioural thermoregulatory strategy in mice (Ganeshan & Chawla, 2017), this behaviour may have contributed to the higher CBT throughout RF-EMF exposure observed in the female mice. Alternatively, these findings may suggest a thermoregulatory limit of female mice at WBA-SAR ≥ 3.75 W/kg, that is, thermoregulation was not sufficient to maintain CBT at the same level as sham. Supporting this, a rise in CBT can be seen in the last 10 min of RF-EMF exposure at a WBA-SAR ≥ 3.75 W/kg. Given there was no experimenter presence in the exposure room around this time, these peaks in CBT are unlikely to be a result of external disruption to the mice and might indicate a limit in thermoregulatory capacity. Another possibility is that mice may have simply learned that the exposure was about to end (from previous trials) due to the consistent exposure duration. A new study investigating CBT for a longer duration of exposure would need to be performed to determine whether this is the case. Additional studies are also required to determine the extent that these findings can be attributed to sex. Ideally male and female mice would be housed and exposed in identical group sizes, however it was not possible in the present study which makes any sex differences difficult to interpret.

It is important to note that there are some limitations to the present study. Despite the implementation of a habituation period prior to RF-EMF exposure, we still observed variability in the CBT of the undisturbed mice both prior to and throughout the exposure period. The CBT of mice is known to be quite dynamic, with changes of up to 4°C within a 30-min period reported in undisturbed mice (Gordon, 2009). Given our mice were free moving (to avoid the influence of restraint stress or anaesthesia on CBT of mice), this variability is expected and cannot be avoided, even with a habituation period. As such, it is difficult to obtain a true, stable baseline CBT prior to RF-EMF exposure in mice. Regardless, efforts should be made to control for some of this variability so that the effects of RF-EMF exposure on CBT are not ambiguous. In the female cohort, CBT in the sham exposed condition appears to decrease for a further 30 min after the habituation period, suggesting a 1-h habituation period may not be sufficient for these mice. Extending the habituation period in female mice to 1.5 h may provide a clearer baseline prior to RF-EMF exposure. It is important to consider both the physiological consequences and animal welfare if implementing a long habituation period, particularly when food and water is not available to the mice.

5 | CONCLUSION

We found a small increase in the CBT of mice exposed to whole-body average SAR levels >1.25 W/kg RF-EMF for 2 h, relative to a sham exposed condition. This increase was mostly evident within the first 16 min of exposure, after which it was largely abated, indicating that mice were able to efficiently compensate for the increased thermal energy provided by RF-EMF

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exposure. At the highest WBA-SAR, CBT appeared to remain higher than the sham condition throughout the duration of exposure, suggesting mice may not be able to fully compensate for the increased thermal energy at 5 W/kg. Previous studies have not accounted for the effect of handling on the CBT of rodents prior to RF-EMF exposure or have utilised rectal thermometry immediately prior to and after an exposure to determine CBT change, which challenges the accuracy of their results. To obtain informative data on the CBT of mice exposed to RF-EMF, researchers need to pay particular attention to the method of CBT measurement and also include an appropriate habituation period prior to the start of RF-EMF exposure.

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CONFLICTS OF INTEREST STATEMENT

Robert McIntosh is a current employee of a telecommunications company; Raymond McKenzie is a former employee of a federal national telecommunications industry association; and Steve Iskra, Raymond McKenzie, and John Frankland are former employees of a telecommunications company. All authors read and approved the paper for publication.

ETHICS STATEMENT

All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (AE21/11), and adhered to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

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