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Impact of specific electromagnetic radiation on wakefulness in mice

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Electromagnetic radiation (EMR) in the environment, particularly in the microwave range, may constitute a public health concern. Exposure to 2.4 GHz EMR modulated by 100 Hz square pulses was recently reported to markedly increase wakefulness in mice. Here, we demonstrate that a similar wakefulness increase can be induced by the modulation frequency of 1,000 Hz, but not 10 Hz. In contrast to the carrier frequency of 2.4 GHz, 935 MHz EMR of the same power density has little impact on wakefulness irrespective of modulation frequency. Notably, the replacement of the 100 Hz square-pulsed modulation by sinusoidal-pulsed modulation of 2.4 GHz EMR still allows a marked increase of wakefulness. In contrast, continuous sinusoidal amplitude modulation of 100 Hz with the same time-averaged power output fails to trigger any detectable change of wakefulness. Therefore, alteration of sleep behavior by EMR depends upon not just carrier frequency but also frequency and mode of the modulation. These results implicate biological sensing mechanisms for specific EMR in animals.

electromagnetic radiation (EMR) | sleep behavior | modulation frequency | carrier frequency | modulation mode

Significance

Increased wakefulness in mice was previously found to be a direct result of prolonged exposure to 2.4 GHz electromagnetic radiation (EMR) with 100 Hz square-pulsed modulation at 1/8 duty cycle. Several key issues remain unaddressed. Does the frequency of the square-pulsed modulation matter? Are the sharp edges of the square pulses a major contributor to sleep/wakefulness alteration? Can carrier frequencies other than 2.4 GHz induce sleep/wakefulness alteration? Does the duty cycle matter? In this study, we answer these questions by demonstrating the dependency of sleep/wakefulness alteration on EMR modulation frequency, carrier frequency, and modulation mode.

Electromagnetic radiation (EMR) in the environment, particularly the wireless signal, causes serious public concern over its potential negative impact on health. Our previous study identified alteration of sleep architecture in mice as a specific physiological response to prolonged wireless-range EMR exposure (1). EMR-induced biological/physiological consequences may be influenced by multiple parameters, including carrier frequency of the electromagnetic field, peak and average power density of EMR, duty cycle (percentage time EMR is applied), and frequency and mode of the modulation. In our previous study, mice exposed to 2.4 GHz EMR with a peak power of 64 W and a time-averaged power density of 8 W (1/8 duty cycle), modulated by 100 Hz square pulses, showed markedly increased wakefulness (1).

The carrier frequency and power density of EMR are two principal parameters that may determine the biological consequence. Exposure to 900 MHz and 2 GHz EMR, both widely used for global telecommunication, is thought to result in different sleep-disturbing effects in humans (2, 3). The key questions are whether such behavioral change is associated with specific carrier frequencies, and if yes, what physical basis is responsible for perceiving EMR and mediating its effect. Unfortunately, few studies directly compare the effects of different EMR carrier frequencies on sleep architecture. Most published studies focused on the sleep effect of 900 MHz carrier frequency (3), with only a few studies on 2.4 GHz (4). At present, it is unclear whether EMR carrier frequencies other than 2.4 GHz may also trigger increased wakefulness in mice (1).

Modulation frequency and duty cycle may also contribute to biological impact. For example, exposure to 900 MHz EMR pulse-modulated by 14 Hz, but not 217 Hz, led to increased EEG power in the 12.75 to 13.25 Hz range during NREM sleep (5). Exposure to 450 MHz EMR with select modulation frequencies between 7 Hz and 1,000 Hz caused increasingly stronger EEG energy levels and higher EEG rhythms at higher frequencies (6–8). Calcium efflux from the frog heart was affected only by 240 MHz EMR with 16-Hz modulation, but not by continuous-wave or 0.5 Hz modulation (9). The interbeat interval of aggregated cardiac cells from chicken embryos increases during continuous EMR exposure but decreases after pulse-modulated EMR exposure (10). Modulation frequencies may impact neural rhythms formed by coupling neuronal population, bringing potential changes to brain function and sleep behavior. It remains unknown whether modulation frequencies other than 100 Hz can alter sleep behaviors in mice (1).

Last but not least, the mode of modulation, which in this study specifically refers to the temporal appearance of the modulated electromagnetic field, might also play a role. Specific modulation modes, exemplified by square and sinusoidal pulses, are speculated to trigger distinct effects on neural function due to their different modulation patterns on the carrier EMR. However, exposure to 591 MHz EMR modulated by three different

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modes (continuous, sinusoidal amplitude, and square-pulsed) led to a similarly decreased level of ATP and creatine phosphate concentrations in rats (11). It remains to be investigated whether and to what extent the mode of modulation affects sleep alteration (1).

In our previous study, we established an experimental system for the investigation of EMR impact on mice (1). In this study, we use this system to address the above-listed questions concerning EMR consequence on sleep in mice. Our experimental evidence unequivocally demonstrates that the change of sleep behavior depends on EMR modulation frequency, modulation mode, and the carrier frequency.

Results

Impact of Modulation Frequency on Wakefulness. In our previous study, mice that showed increased wakefulness were exposed to 2.4 GHz EMR modulated by 100 Hz square pulses (1). In this study, we investigated the impact of modulation frequencies of 1,000 Hz and 10 Hz, with all other experimental conditions identical as before (1) (Fig. 1*A*). The surgery of planting intracranial electrodes was conducted 2 wk prior to data recording to allow mice recovery and habitation. Two sets of the sleep polysomnography data were collected: on day −1 before radiation serving as the baseline (referred to as "Pre") and on day 9 after radiation (referred to as "Pos9"). In each case, the 2.4 GHz carrier electromagnetic field has a duty cycle of 1/8 and a maximal output of 64 W, referred to as Pulse64W (Fig. 1*B*).

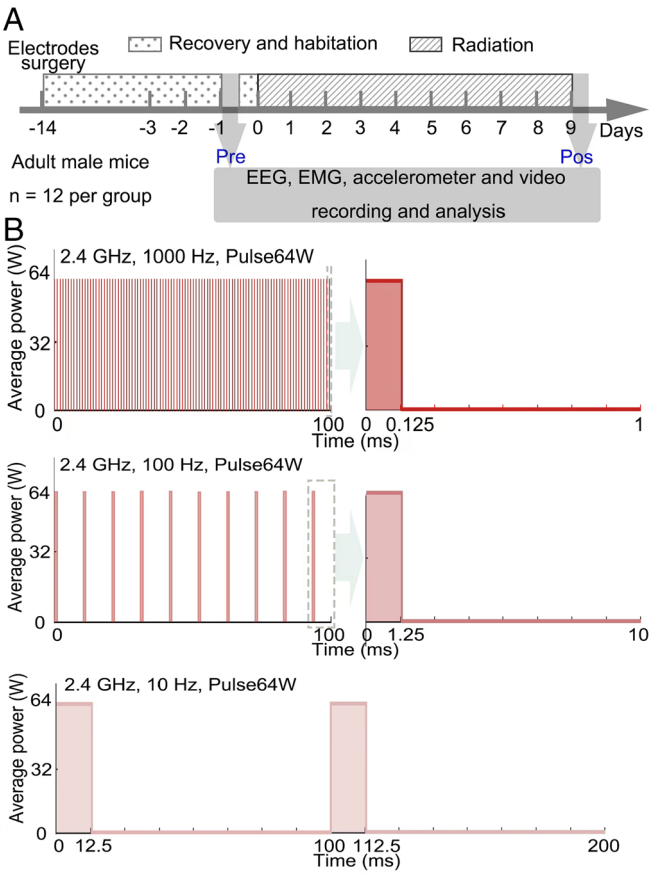

Fig. 1. Experimental design and EMR dosage. (*A*) Animal treatment and data recording. Electrodes are implanted in mice on day −14. Continuous EMR is given on days 1–9. Polysomnography is recorded for 12 h during the light phase of day −1 (Pre) and after day 9 (Pos9). (*B*) Schematic diagrams of 2.4 GHz EMR with three modulation frequencies. The EMR regimen 2.4 GHz Pulse64W has a peak power output of 64 W with 1/8 duty cycle. The carrier EMR 2.4 GHz is modulated by square pulses of three distinct frequencies. 1,000 Hz: 1-millisecond (ms) repeats, each with 0.125 ms exposure to EMR (*Top*). 100 Hz: 10-millisecond (ms) repeats, each with 1.25 ms exposure to EMR (*Middle*). 10 Hz: 100-millisecond (ms) repeats, each with 12.5 ms exposure to EMR (*Bottom*).

We first analyzed the data of 1,000 Hz modulation (Fig. 2*A*). During the 12-h sleep period, the average time of wakefulness at Pre is similar between the Control and the 1,000 Hz group, being 226.1 ± 7.9 and 216.5 ± 5.4 min, respectively (Fig. 2 *A*, *Left* panel). In contrast, the average time of wakefulness at Pos9 is increased to 255.6 ± 8.3 min for the 1,000 Hz group, which is 12.6% higher than that for the Control (226.9 ± 6.8 min), with a *P* value of 0.016. Thus, there is a statistically significant increase of wakefulness at Pos9 for the 1,000 Hz modulation. Notably, however, both the net increase and the increased percentage of wakefulness associated with 1,000 Hz modulation are less than those for 100 Hz modulation (1) (Fig. 2 *A*, *Right* panel). The average time of wakefulness at Pos9 is 286.7 ± 13.1 min for 100 Hz modulation, 21.0% higher than that for the Control (236.9 ± 5.0 min).

We then assessed the impact of 10 Hz modulation (Fig. 2*B*). There are few differences between the Control and the 10 Hz group or between Pre and Pos9. The average time of wakefulness at Pos9 is 219.4 ± 6.7 and 224.5 ± 7.5 min for the Control and the 10 Hz group, respectively (Fig. 2*B*). Therefore, in sharp contrast to 1,000 Hz or 100 Hz, the modulation frequency of 10 Hz has little impact on wakefulness at Pos9 in mice.

Next, we used the radiation effect index at Pos9 (REI_{Pos9}) (1) to evaluate wakefulness change (Fig. 2*C*). An REI value of 0 indicates no change compared to Pre, and a value of 0.1 means 10% increase of wakefulness. The REI_{Pos9} value for 1,000 Hz modulation is 0.186 ± 0.035. The raincloud plot suggests a statistically significant increase of wakefulness for the 1,000 Hz group at Pos9, with a *P* value of less than 0.001 (Fig. 2*C*). The REI_{Pos9} value for 100 Hz modulation is 0.338 ± 0.090, with a *P* value of 0.003. In contrast, REI_{Pos9} for 10 Hz modulation is only 0.035 ± 0.031 with a *P* value of greater than 0.05. A scatter plot of the total time of wakefulness for individual mouse confirms the increasing trend from Pre to Pos9 for the modulation frequencies of 1,000 Hz and 100 Hz, but not 10 Hz (*SI Appendix*, Fig. S1 *A*–*C*).

We also compared the wakefulness REI_{Pos9} values among the three modulation frequencies. As anticipated, the REI_{Pos9} value of 10 Hz modulation is significantly different from that of 1,000 Hz ($P = 0.009$) or 100 Hz ($P = 0.019$) (*SI Appendix*, Fig. S1*D*). There is no significant difference between 100 Hz and 1,000 Hz ($P > 0.05$). Together, our experimental results suggest that the effect of 2.4 GHz EMR on wakefulness depends on specific modulation frequencies.

Impact of Modulation Frequency on NREM and REM Sleep. To further examine the impact of modulation frequency on sleep behavior, we analyzed the data on NREM sleep and REM sleep. Based on the scatter plots of the 1,000 Hz and 100 Hz groups, both NREM sleep and REM sleep display a clearly decreasing trend from Pos9 to Pre, each with a *P* value of smaller than 0.01 (*SI Appendix*, Fig. S2 *A* and *B*). For the 10 Hz group, however, there is no statistically significant change at Pos9 compared to Pre (*SI Appendix*, Fig. S2*C*).

This conclusion is confirmed by the REI analysis of NREM and REM sleep data (*SI Appendix*, Fig. S3 *A* and *B*). The REI_{Pos9} value of the 10 Hz group is significantly different from that of the 1,000 Hz group ($P = 0.04$ for NREM and $P = 0.014$ for REM) or the 100 Hz group ($P = 0.001$ for NREM and $P = 0.004$ for REM) (*SI Appendix*, Fig. S3*C*). In contrast, no statistically significant difference was found between the 100 Hz group and 1,000 Hz group ($P > 0.05$).

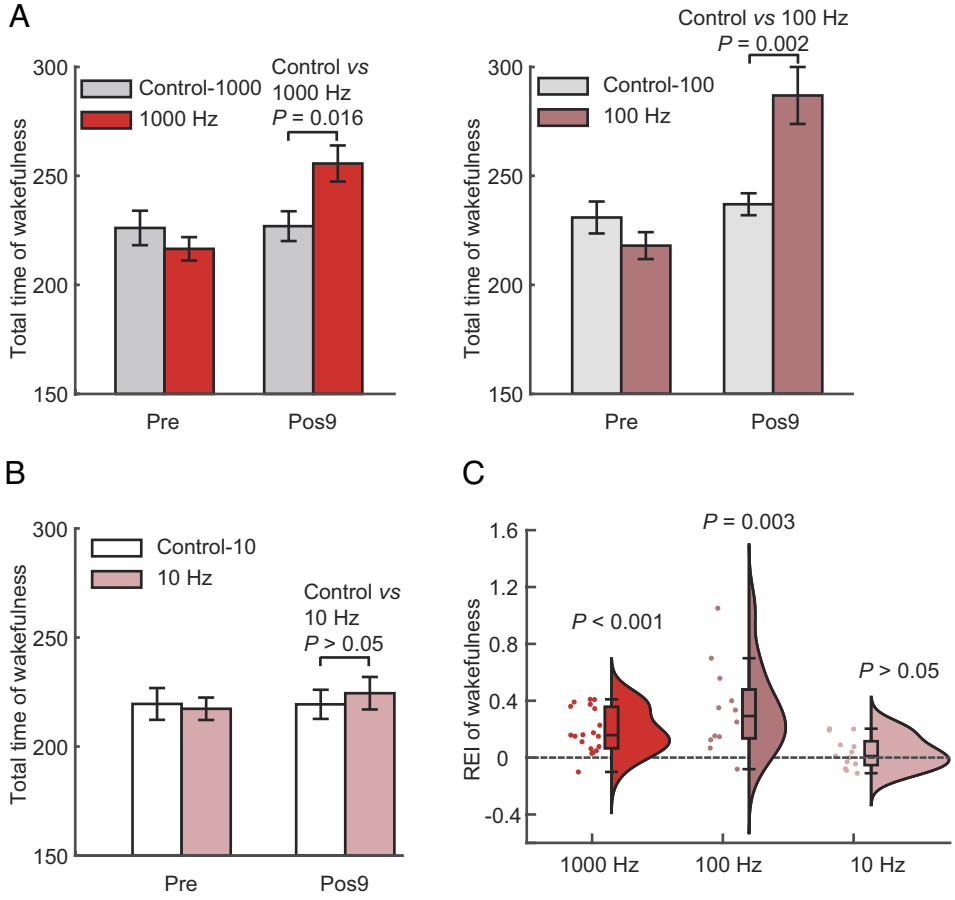

Fig. 2. The square-pulsed modulation frequencies of 1,000 Hz and 100 Hz, but not 10 Hz, for the 2.4 GHz Pulse64W regimen result in increased wakefulness. (*A*) The modulation frequencies of 1,000 Hz and 100 Hz for the 2.4 GHz Pulse64W regimen lead to increased wakefulness time at Pos9. In each case, the average time of wakefulness for the Control and the radiation group at Pre and Pos9 are shown. For the 1,000 Hz experiments, $n = 14$ for the Control; $n = 19$ for the radiation group (*Left* panel). The error bars throughout the manuscript are SEM. The published results for 100 Hz ($n = 12$ for the Control; $n = 12$ for the radiation group) are shown here for comparison (*Right* panel). (*B*) The 10 Hz modulation frequency exhibits no obvious impact on the wakefulness. Shown here is the average time of wakefulness for the Control and the radiation group. $n = 11$ for the Control; $n = 13$ for the radiation group. (*C*) Evaluation of wakefulness changes through REI analysis for the 1,000 Hz, 100 Hz, and 10 Hz groups. The REI_{Pos9} values from each group are presented as a raincloud plot. Each REI_{Pos9} value is shown as a dot. The median value is represented by the boxplots defined by 25th/75th percentiles and bracketed by the minimum/maximum REI_{Pos9} values of 95% confidence. The probability density function of the REI_{Pos9} data is shown in the distribution plots.

Impact of Modulation Mode. The square pulses allow full power of EMR during the 1/8 duty cycle but zero power at all other times. Such all-or-none feature of EMR may contribute to biological consequence such as alteration of sleep architecture. In contrast to square pulses, sinusoidal pulses allow smooth changes of the EMR power density. To examine the role of modulation mode, we investigated the impact of sinusoidal pulse modulation (SPM) on sleep architecture. The SPM has the same time-averaged radiation power as the square pulse modulation. The SPM mode was applied to 2.4 GHz carrier frequency with 100 Hz modulation (Fig. 3*A*).

The average time of wakefulness at Pos9 is 269.7 ± 7.9 min for the SPM group, which is 15.5% more than that for the Control (233.5 ± 7.1 min), with a *P* value of 0.002 (Fig. 3*B*). A scatter plot of the total time of wakefulness for individual mouse confirms the increase from Pre to Pos9 within the SPM group (Fig. 3*C*). Compared to Pre, the average REI_{Pos9} value for wakefulness of the SPM group is 0.173 ± 0.033 (Fig. 3*D*). The raincloud plot confirms a statistically significant increase of wakefulness for the SPM group at Pos9, with a *P* value of less than 0.001.

In contrast to the Control that maintained a relatively steady average time of NREM and REM sleep, the SPM group at Pos9 exhibits a 6.6% decrease of NREM sleep and a 13.1% decrease of REM sleep, each with a *P* value smaller than 0.05 (Fig. 3 *E* and *F*). Consistently, the REI_{Pos9} values of the NREM sleep and REM sleep are −0.073 ± 0.013 and −0.092 ± 0.052, respectively (Fig. 3*D*). Based on REI analysis, the decrease of the NREM sleep (with a *P* value smaller than 0.001), but not of the REM sleep (with a *P* value greater than 0.05), is statistically significant.

Impact of Continuous Modulation on Sleep. Unlike the SPM mode, which restricts EMR at 1/8 duty cycle, sinusoidal amplitude modulation (SAM) allows continuous exposure to EMR. We assessed the impact on sleep architecture by 2.4 GHz EMR with SAM (Fig. 4*A*), which has the same time-averaged power density as that of the SPM treatment. Notably, however, the average time of wakefulness at Pos9 is very similar between the Control (230.3 ± 7.5 min) and the SAM group (232.4 ± 10.3 min) (Fig. 4*B*). The scatter plot and the REI analysis show no statistically significant change of wakefulness (Fig. 4 *C* and *D*). Compared with the Control, the 2.4 GHz SAM group exhibits no statistically significant change in NREM sleep (Fig. 4*E*) or REM sleep (Fig. 4*F*). These results were confirmed by the REI analysis (Fig. 4*D*)

Impact of 935 MHz Carrier Frequency on Sleep. The carrier frequency of 935 MHz is frequently used in 2G telecommunication. We investigated the impact on sleep architecture by 935 MHz Pulse64W EMR modulated by 1,000 Hz, 100 Hz, and 10 Hz

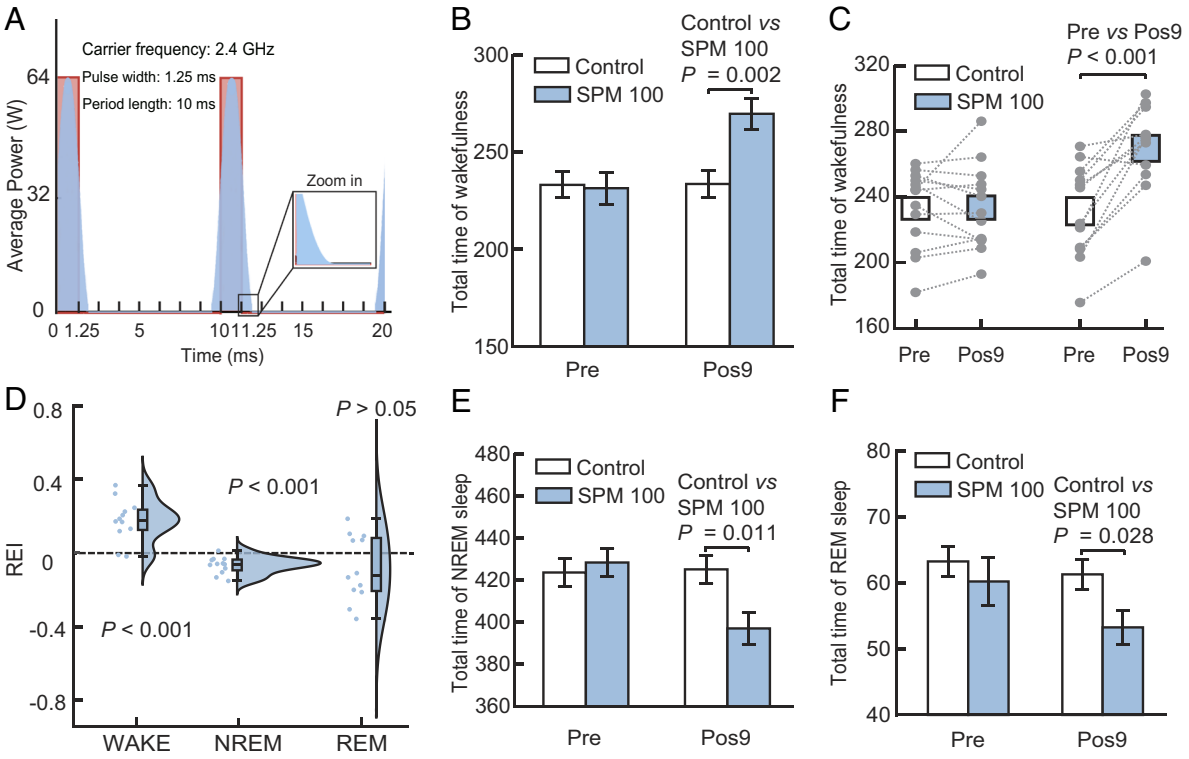

Fig. 3. The SPM frequency of 100 Hz for the 2.4 GHz Pulse64W regimen results in increased wakefulness during sleep. (*A*) A schematic diagram of the 100 Hz SPM. The 2.4 GHz Pulse64W regimen has a peak power output of 64 W with 1/8 duty cycle. 100 Hz SPM: 10 ms repeats, each with 1.25 ms sinusoidal pulsed exposure to EMR. (*B*) The 100 Hz SPM for the 2.4 GHz Pulse64W regimen markedly increases the wakefulness time at Pos9 in mice. Shown here is the average time of wakefulness for the Control and the radiation group at Pre and Pos9. n = 13 for the Control; n = 12 for the radiation group. (*C*) A scatter plot of the wakefulness for the Control and the radiation group. Each dot represents the total time of wakefulness for one mouse. Each dotted line connects the data for the same mouse. (*D*) Evaluation of the changes of sleep architecture through REI analysis. Shown here are the REI_{Pos9} values of wakefulness, NREM sleep, and REM sleep for the 100 Hz SPM group. (*E*) The 100 Hz SPM results in decreased NREM sleep at Pos9 in mice. Shown here is the average time of NREM sleep for the Control and the radiation group. (*F*) The 100 Hz SPM decreases the REM sleep at Pos9 in mice. Shown here is the average time of REM sleep for the Control and the radiation group.

(*SI Appendix*, Fig. S4). The average power density remains the same as that of the 2.4 GHz Pulze64W regimen.

The average time of wakefulness at Pos9 is 266.6 ± 11.1 min for the 1,000 Hz group or 264.7 ± 7.2 min for the 100 Hz group, which is 8.1% or 7.0% more than that of its respective Control (246.5 ± 7.2 min or 247.3 ± 6.7 min) (Fig. 5 *A* and *B*). In both cases, the *P* values are greater than 0.05. For 10 Hz modulation, the average time of wakefulness at Pos9 is very similar between the Control (238.8 ± 5.9 min) and the radiation group (242.8 ± 7.7 min) (Fig. 5*C*). Therefore, compared to 2.4 GHz, the impact of 935 MHz EMR on sleep is greatly diminished, with a statistically insignificant increase of wakefulness for 1,000 Hz or 100 Hz but no change for 10 Hz modulation.

This conclusion is confirmed by the scatter plot of wakefulness time at Pos9, where no statistically significant change is seen between the Control and the radiation group for each of the three modulation frequencies (*SI Appendix*, Fig. S5*A*). Consistently, the REI_{Pos9} value is 0.055 ± 0.045 for 1,000 Hz, 0.051 ± 0.031 for 100 Hz, and −0.014 ± 0.042 for 10 Hz modulation, each with a *P* value of greater than 0.05 (Fig. 5*D*).

Next, we performed a similar analysis on the NREM and REM data. Compared to Pre, none of the modulation frequencies with 935 MHz EMR has a statistically significant impact on NREM sleep at Pos9 (*SI Appendix*, Fig. S5*B*). This conclusion is supported by the REI analysis (*SI Appendix*, Fig. S5*C*). Notably, however, the scatter plots of the REM data identify a statistically significant decrease at Pos9 over Pre for each of the three modulations, with a *P* value of smaller than 0.05 (*SI Appendix*, Fig. S5*D*). This conclusion is confirmed by the REI analysis (*SI Appendix*, Fig. S5*E*).

Impact of Modulation Mode for 935 MHz EMR. Finally, we investigated the impact of 935 MHz EMR with two distinct modulation modes: SPM and SAM. Similar to that for 2.4 GHz EMR (Fig. 3*A*), SPM of the same time-averaged power density was applied to 935 MHz EMR with 100 Hz modulation. The change of sleep architecture for the SPM group is relatively mild. The average time of wakefulness at Pos9 for the SPM group is 259.7 ± 5.5 min, 8.30% more than that for the Control, with a *P* value of 0.019 (*SI Appendix*, Fig. S6*A*). However, the scatter plot and REI analysis both reveal no statistical significance for the wakefulness change, each with a *P* value of greater than 0.05 (*SI Appendix*, Fig. S6 *B* and *C*). The average NREM sleep and REM sleep at Pos9 for the SPM group is 3.79% and 6.45%, respectively, less than those of the Control groups, each with a *P* value of greater than 0.05 (*SI Appendix*, Fig. S6 *D* and *E*). This conclusion is corroborated by the REI analysis (*SI Appendix*, Fig. S6*C*).

Next, we analyzed the impact of 935 MHz EMR modulated by 100 Hz continuous SAM. The average time of wakefulness at Pos9 for the SAM group is 291.5 ± 16.1 min, 13.3% more than that for the Control, with a *P* value of greater than 0.05 (*SI Appendix*, Fig. S7*A*). The scatter plot and REI analysis confirm the wakefulness increase, each with a *P* value of smaller than 0.05 (*SI Appendix*, Fig. S7 *B* and *C*). There is a modest decrease of NREM sleep at Pos9 but with no statistical significance (*SI Appendix*, Fig. S7*D*); this conclusion is confirmed by the REI analysis (*SI Appendix*, Fig. S7*C*). In contrast, there is a statistically significant decrease of REM sleep at Pos9 (*SI Appendix*, Fig. S7*E*), confirmed by the REI analysis (*SI Appendix*, Fig. S7*C*).

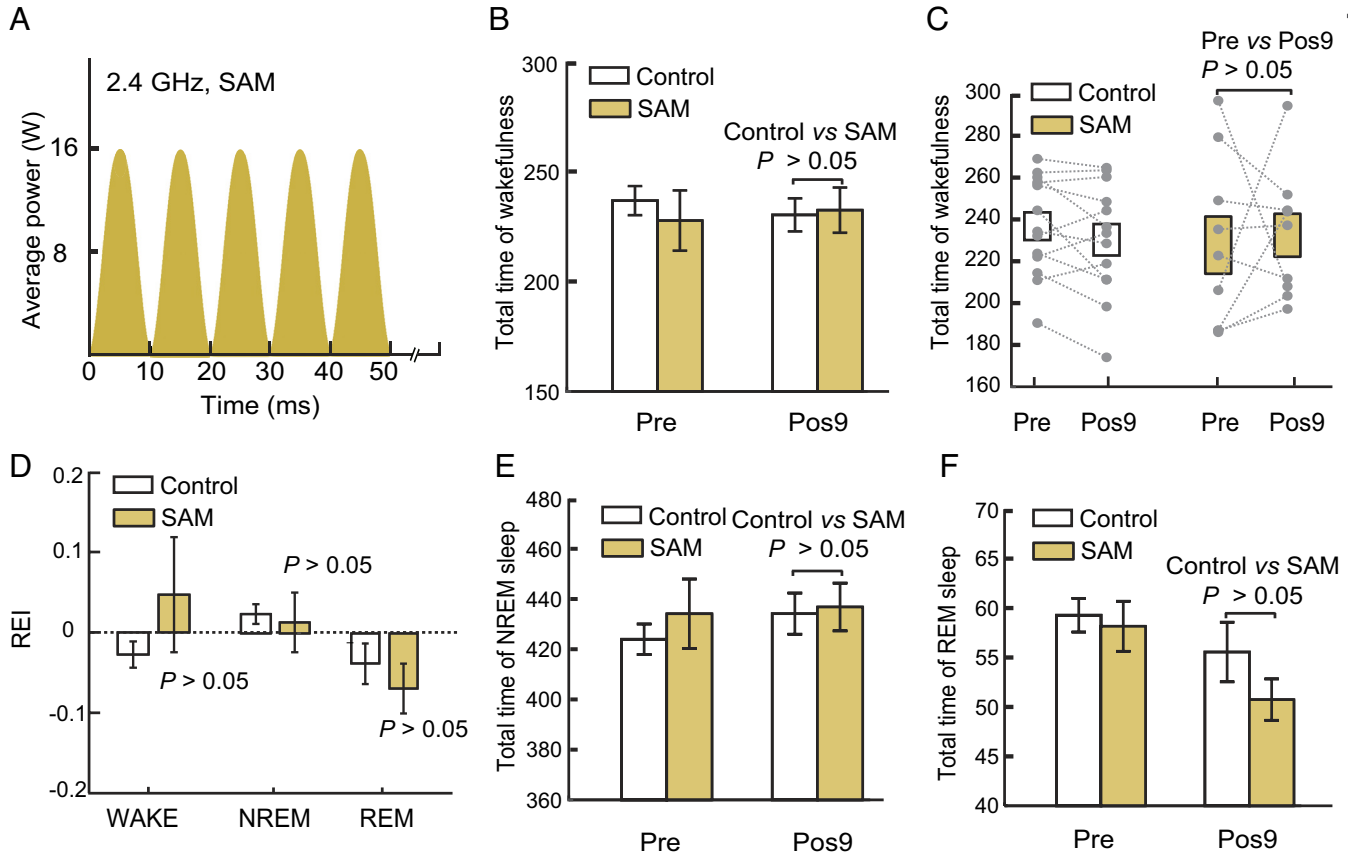

Fig. 4. The modulation mode of SAM for the 2.4 GHz regimen has little impact on sleep. (*A*) A schematic diagram of SAM for the carrier frequencies of 2.4 GHz. The carrier frequency is modulated by 100 Hz SAM with an averaged output power of 8 W. This regimen, named SAM8W, has the same time-averaged power density as the Pulse64W regimen. (*B*) The 2.4 GHz SAM8W regimen results in little change of the wakefulness time at Pos9. Shown here is the average time of wakefulness for the Control and the radiation group at Pre and Pos9. $n = 13$ for the Control; $n = 9$ for the radiation group. (*C*) A scatter plot of the wakefulness for the Control and the radiation group. (*D*) Evaluation of the change of sleep architecture through REI analysis. Shown here are the bar plots of REI_{Pos9} values of wakefulness, NREM sleep, and REM sleep. (*E*) The 2.4 GHz SAM8W regimen has little impact on the NREM sleep at Pos9. Shown here is the average time of NREM sleep for the Control and the radiation group. (*F*) The 2.4 GHz SAM8W regimen results in a statistically insignificant decrease of the REM sleep at Pos9. Shown here is the average time of REM sleep for the Control and the radiation group.

Discussion

Although animals have long been known to respond to low-energy electromagnetic field, there are few studies that systematically address the specificity of such responses (12, 13). Relying on a carefully designed experimental system, we previously showed that prolonged exposure of mice to 2.4 GHz EMR modulated by 100 Hz square pulses at 1/8 duty cycle induces a marked increase of wakefulness during sleep accompanied by commensurate decreases of the NREM sleep and REM sleep (1). Following this finding, numerous important questions have surfaced. The crux of these questions is whether such response is specific. In this study, we investigate the potential influences by modulation frequency, mode of modulation, duty cycle, and the EMR carrier frequency. To ensure valid conclusion, we only alter one single parameter among modulation frequency, modulation mode, and carrier frequency and keep the rest unchanged for each investigation.

Statistically significant increases of wakefulness are associated with the modulation frequencies of 1,000 and 100 Hz, but not 10 Hz (Fig. 2). The decrease of NREM and REM sleep follows a similar pattern (*SI Appendix*, Fig. S2). Intriguingly, the increase of wakefulness at 100 Hz appears to be larger than that at 1,000 Hz. Fine-tuning is required to find the optimal modulation frequency at which maximum increase of wakefulness is achieved. In case of a single optimal frequency over the entire range of EMR, it is likely to be smaller than 1,000 Hz. Consistent with our conclusion, different modulation frequencies appear to trigger distinct human brain activities (14). For 450 MHz EMR, modulation frequencies of 7/14/21 Hz, but not 40/70/217/1,000 Hz, led to increased EEG energy of the alpha band in humans (7, 14).

In contrast to the carrier frequency of 2.4 GHz EMR, 935 MHz has little effect on wakefulness or NREM sleep. Intriguingly, the REM sleep shows a significant decrease at Pos9 (*SI Appendix*, Fig. S5). Our result is consistent with the observation that decreased REM sleep in human is associated with exposure to pulse-modulated 900 MHz EMR for three successive nights or even less (15, 16).

Somewhat to our surprise, the modulation modes of squared and sinusoidal pulses both induce a similar level of increased wakefulness in mice (Fig. 4). This finding suggests an unimportant role for the sharp edges of square pulses in triggering sleep alteration. The duty cycle clearly matters. In contrast to SPM, the continuous SAM for 2.4 GHz has little impact on mouse sleep architecture (Fig. 5). It should be mentioned, however, due to the full duty cycle of SAM, the peak power density of EMR for SAM is only 1/4 of that for SPM.

Modulation frequency and mode of EMR have been reported to influence sleep and other aspects of human health and behavior (17). Pulse-modulated 900 MHz EMR may change sleep architecture (5, 18) and different modulation frequencies have

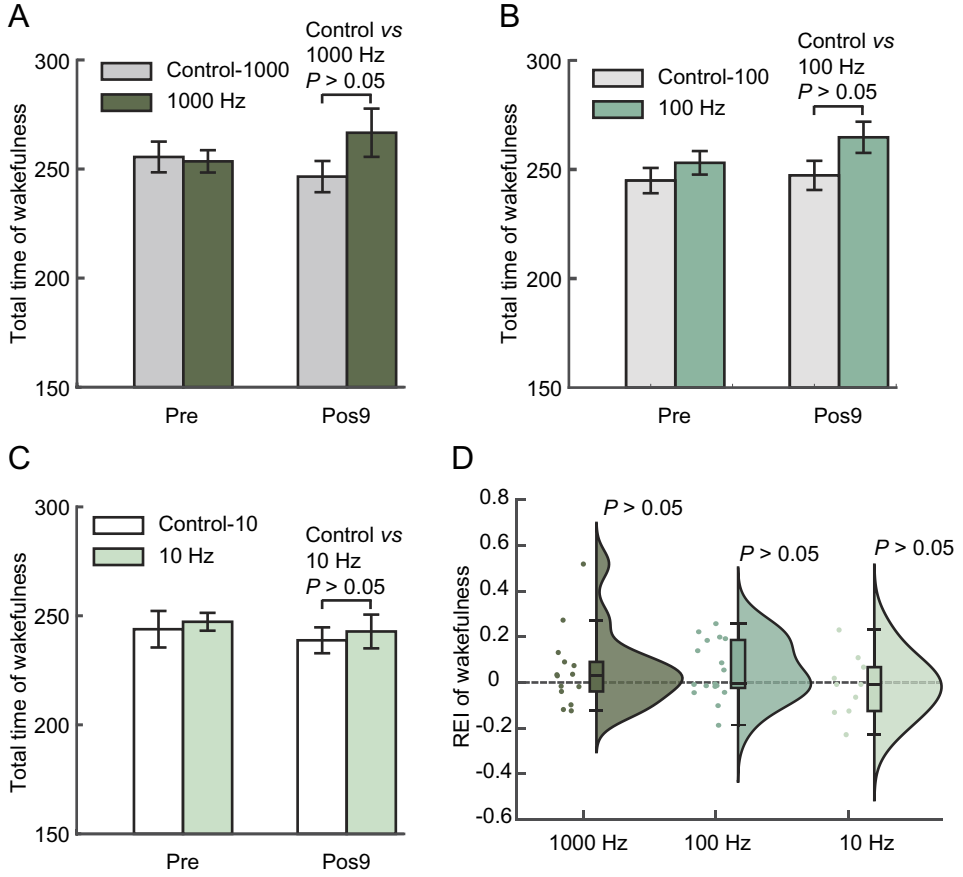

Fig. 5. The 935 MHz Pulse64W regimen results in statistically insignificant changes of wakefulness at the square-pulsed modulation frequencies of 1,000 Hz, 100 Hz, and 10 Hz. (*A*) The 935 MHz Pulse64W regimen with 1,000 Hz square-pulsed modulation results in a statistically insignificant increase of the wakefulness time at Pos9. Shown here is the average time of wakefulness for the Control and the radiation group at Pre and Pos9. *n* = 12 for the Control; *n* = 14 for the radiation group. (*B*) The 935 MHz Pulse64W regimen with 100 Hz square-pulsed modulation results in a statistically insignificant increase of the wakefulness time at Pos9. *n* = 15 for Control; *n* = 17 for radiation. (*C*) The 935 MHz Pulse64W regimen with 10 Hz square-pulsed modulation has no obvious impact on the wakefulness at Pos9. *n* = 10 for Control; *n* = 10 for radiation. (*D*) Evaluation of wakefulness changes through REI analysis for the 1,000 Hz, 100 Hz, and 10 Hz groups.

contrasting consequences on the onset of sleep (19). In addition, calcium efflux from frog heart could be trigged by exposure to EMR of the intermittent sinusoidal mode, but not the continuous mode (9). Exposure to 9.4 GHz EMR with sinusoidal modulation at discrete frequencies between 14 and 41 MHz resulted in increased antibody response in mice (20). Notably, however, due to a serious lack of systematic studies on modulation frequency and mode, their roles in human health and behavior are scarcely described in the guidelines of International Commission for Non-ionizing Radiation Protection (ICNIRP).

During EMR propagation, it may be transmitted, reflected, and absorbed by the medium. Different from the power density of the EMR, the time-averaged specific absorption rate (SAR) describes the absorption of EMR by the medium, mice in our study. In most reported cases, the time-averaged SAR is simulated and calculated in a steady-state continuous mode EMR. The pulse mode of EMR makes simulation of the time-averaged SAR calculation quite challenging. Nonetheless, the time-averaged SAR should be linearly proportional to the time-averaged power density of EMR.

Depending on the power density, EMR may elicit thermal and nonthermal effects on cells, tissues, and living organisms. In our study, all radiation doses are well below those stated in the guidelines of ICNIRP. Modeling and experimental assessment of human exposure to 935 MHz or 2.14 GHz EMR at a dose level of 3.6 W/kg local SAR revealed a maximal skin temperature increase of 0.31 °C and a brain temperature elevation of <0.1 °C (21). In our case, the maximum local SAR value measured with the cSAR3D testing system is 3.6 W/kg and the averaged SAR is 2.81 ± 0.15 W/kg. Hence, the observed EMR impact on sleep in our study is most likely nonthermal.

Additional experiments are needed to examine the timing and location of EMR-induced biological/physiological effect in neural system of the mouse. Given the specific biological effect, what physical basis underlies detection of specific EMR? For the carrier EMR 935 MHz and 2.4 GHz, the wavelengths are 32.1 cm and 12.5 cm, respectively. The energy carried by each photon at 2.4 GHz is about five orders of magnitude lower than that of the visible light. Sensing of such low-energy EMR may require some specialized structure in living organisms. The resonance energy of such structure is likely to be close to the photon energy of 2.4 GHz, but not 935 MHz, because the former, but not the latter, induces strong responses. In addition, because 1,000 Hz or 100 Hz, but not 10 Hz, modulation induces sleep alteration, such structure is likely to have a group property that resonates with specific modulation frequencies. These speculations may only represent an aspect of the possibilities of a much larger scope. How does the sensing of EMR by such structure cause behavior changes? These questions, in their most primitive forms, are poorly defined at the present time. Answering these questions may expand the horizon of human knowledge.

In conclusion, our study reveals distinct specificity of EMR. Prolonged exposure to the carrier frequency 2.4 GHz EMR with square pulse modulation of different frequencies induces varying changes of wakefulness in mice. In contrast to 2.4 GHz, the carrier frequency 935 MHz has little impact on wakefulness or NREM

sleep. Then, 2.4 GHz EMR with sinusoidal pulse modulation, but not continuous sinusoidal modulation of 100 Hz, results in an increase in wakefulness. These findings link specific biological responses to specific parameters of EMR, namely carrier frequency, modulation frequency, and modulation mode. The underlying mechanisms for these observations remain to be unveiled.

Materials and Methods

The methods are detailed in *SI Appendix* and briefly described here.

Radiation Equipment Setup. First, 2.4 GHz and 935 MHz EMRs are generated by a MXG Vector signal generator (Agilent, N5181A) modulated by a function generator (Rflight, NTWPA-1025100). The three modulation frequencies are 1,000 Hz, 100 Hz, and 10 Hz, each of which is presented in squared or sinusoidal mode. Specific customized power amplifier was used to amplify different signal carriers of 2.4 GHz or 935 MHz to a maximal power output of 64 W.

Electrode Implantation. The cranial electrodes are integrated to simultaneously record EEG, EMG, and local field potential signals. Four stainless steel 304 screw were secured to the skull. The EMG electrode wires were embedded under the trapezius muscles in the neck of mouse. All electrodes and screws were attached to the skull with dental cement.

Polysomnographic Recording and Analysis. The EEG and EMG signals were collected via a digital head stage attached to the cranial electrodes. The data acquisition system receives digital signals with a sampling rate of 1,000 Hz followed by signal filtration and amplification. All video signals were monitored. Relying on sleep analysis software, we analyzed the filtered EEG data (band-pass 0.5 to 100 Hz) using fast Fourier transformation (FFT). Using spectral features of EEG, EMG, and acceleration signals, brain states were classified as wakefulness, NREM sleep, and REM sleep for every 4-s epoch (22).

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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