Electro- and Magnetobiology

ISSN: 1061-9526 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iebm19

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To cite this article: Andrei G. Pakhomov, H. K. Prol, Satnam P. Mathur, Yahya Akyel & C. B. G. Campbell (1997) Frequency-Specific Effects of Millimeter-Wavelength Electromagnetic Radiation in Isolated Nerve, Electro- and Magnetobiology, 16:1, 43-57, DOI: 10.3109/15368379709016172

To link to this article: https://doi.org/10.3109/15368379709016172

ELECTRO- AND MAGNETOBIOLOGY, 16(1), 43–57 (1997)

FREQUENCY-SPECIFIC EFFECTS OF MILLIMETER-WAVELENGTH ELECTROMAGNETIC RADIATION IN ISOLATED NERVE

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ABSTRACT

Effects of low-intensity millimeter waves (MMW) were studied in isolated frog nerve using a high-rate stimulation (HRS) functional test. Irradiation was performed in 3 frequency bands (41.14–41.54, 45.89–45.93, and 50.8–51.0 GHz), at 5 frequencies in each band. The incident power density was 2.5 mW/cm^2 for the 45.89–45.93 GHz band and 10-fold less for the other two bands. Each nerve underwent a single 38-min MMW or sham exposure accompanied by an HRS train (20 paired stimuli/s for 17 min). The second stimulus in each pair was delivered during the relative refractory period, 9 ms after the first one. HRS caused a temporary and reversible decrease of the amplitude and conduction velocity of compound action potentials. MMW irradiation attenuated these changes; the MMW effect on the conduction velocity could be caused by microwave heating, while the effect on the amplitude apparently was not thermal. The amplitude changed significantly only in the test action potential (the one evoked during the refractory period), thus testifying to an improvement of the nerve refractory properties. This effect depended on MMW frequency rather than intensity and reached

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maximum at 41.34 GHz. A 100-MHz deviation from this frequency (to 41.24 or 41.44 GHz) reduced the effect more than twofold, and a 200-MHz deviation eliminated it. The results provided further evidence for the existence of frequency-specific, resonance-type mechanisms of MMW interaction with biological systems.

INTRODUCTION

Nonthermal, frequency-specific biological effects of millimeter waves (MMW) have been demonstrated in a variety of subjects, from isolated biomolecules to human beings (1–21), but most of the attempts to replicate these effects in an independent laboratory have not been successful (22–28; see Ref. 29 for review). This contention, at least in part, comes from essential differences in exposure facilities and techniques, along with the absence of reliable knowledge on what exposure particulars are critical to evoke a biological response.

In many studies that revealed a bioeffect of low-intensity MMW, the frequency of the radiation was found to be a critical parameter, while the intensity was not. When several isolated MMW frequencies were tested, their ability to produce bioeffects was different (1–3), and even close MMW frequencies could cause opposite reactions (4–6). At the same time, the biological response at an effective frequency was virtually the same for field intensities differing by an order or several orders of magnitude (7,8,16,17).

Still, little is known about the dependence of MMW effects on the frequency of the radiation. Development of response spectra requires stable effects and a lot of experimentation, and at the present time it has been accomplished by only a few investigators.

In noncellular systems, the Mossbauer spectroscopy of lyophilized hemoglobin samples revealed at least 10 resonant frequencies in the 44.5–50.35 GHz band (9). Infrared spectroscopy also demonstrated that the deformational vibrations of NH_3^+ and COO^- groups in amino acids are affected by MMW in a resonant manner (10). More than 20 resonances for alanine and 10 resonances for glycine were observed in the 37–100 GHz band, with the half-width of 60–80 MHz.

Belyaev and co-authors (8,11–13) established that the MMW effect on the anomalous viscosity time dependence of cell lysates (which, supposedly, reflects changes in chromatin conformation) has a bell-shaped dependence on the radiation frequency. The resonance frequencies for *Escherichia coli* K12 cells were 41,324 ± 1 MHz and 51,765 ± 2 MHz. The half-width of the resonance was about 80 MHz at an incident power density of 10^{-3} W/cm^2, and decreased to 3 MHz at 10^{-18} W/cm^2. The resonances shifted to the lower frequencies for lysogenic *E. coli* strains with an increased DNA length. Similar results were obtained in rat thymocytes: the resonance was at 41,610 ± 10 MHz, with the half-width of 20–50 MHz at 0.001 mW/cm^2 (13).

Resonance MMW effects on the growth rate of the yeast *Saccharomyces cerevisiae* have been consistently observed for over 20 years (6,14–17). Depending on the particular frequency within a 41.8–42.0 GHz band, the growth rate of the irradiated yeast cultures either increased by up to 15%, or decreased by up to 29%. The resonant peaks were shown to be as narrow as 8–10 MHz. The authors have recently reported that the resonances become narrower when the field intensity is decreased "by some orders of magnitude" (16,17), thus providing a strong and independent confirmation to the above-mentioned findings with the *E. coli* genome (8).The frequency-specific growth

rate effects were also found in blue-green algae, *Spirulina platensis,* (18), and in yeast, *Candida albicans* (1), but no response spectra were developed.

The potency of different MMW frequencies to alter transmembrane currents was analyzed in *Nitellopsis* alga cells (5). Frequencies in the 38–78 GHz band were tested with a step of 1 GHz, and most of the frequencies were found to have a remarkable effect on the chloride current. However, the response spectrum did not reveal any clear dependence of the effect on the frequency: it appeared almost random, with the frequencies that suppressed the current to zero being next to those which enhanced it 2–4 times. Perhaps, an interval of much less than 1 GHz was needed to evince a resonance dependence.

Comparison of the rate effects of MMW in isolated, *in situ* and *in vivo* frog hearts led the authors to a conclusion that the frequency dependence of the effects becomes smoother with increasing complexity of the physiological control mechanisms involved in the response of the subject (19). The response spectrum for the isolated pacemaker area of the heart was characteristic for each individual preparation and had at least 4 extremes between 54 and 78 GHz. The peaks became less clear for exposures of the open heart *in situ* and disappeared when MMW were applied to peripheral areas that affected the heart rate via a reflex pathway.

Our previous work was intended to reveal possible frequency-specific effects of MMW radiation on isolated nerve function (30,31). The functional indices employed were the compound action potential (CAP) conduction velocity, rise time and amplitude, as well as the nerve refractoriness and ability to tolerate a high-rate electrical stimulation (HRS). The experiments established no MMW-induced changes in CAP conduction and refractoriness except for thermal effects which occurred regardless of the radiation frequency at high enough field levels (2–2.5 mW/cm^2, 0.3–0.4°C heating). Results with the last functional test, the HRS tolerance, were substantially different. MMW attenuated CAP suppression caused by the HRS, but only at certain frequencies of the radiation. Moreover, the extent of this attenuation at 2–2.5 mW/cm^2 (45.9 and 51.4 GHz) was almost the same as at 0.25 mW/cm^2 (41.2 and 50.9 GHz). These findings suggested a microwave-specific, frequency-dependent mechanism of interaction.

The objective of the present study was to analyze the frequency dependence of this effect in more detail, as well as to confirm it in an independent series of experiments. Based on the observations done by other authors, the anticipated width of a frequency resonance, if any, was expected to be more than 3–8 MHz and less than 1 GHz. Therefore, we chose to carry out three sets of experiments, each set to compare the effects of five frequencies separated by 100 MHz, 50 MHz, and 10 MHz (ranges 41.14–41.54 GHz, 50.8–51.0 GHz, and 45.89–45.93 GHz, respectively).

MATERIALS AND METHODS

Nerve Preparation and Data Acquisition

Experiments were performed on an isolated nerve preparation (*n. ischiadicus* + *n. peroneus*) of the frog *Rana berlandieri* or *R. pipiens.**Active adult frogs (males) were

*The use of animals was in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to the principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 85-23. The animal use protocol No. AD4002 has been approved by the Armstrong Laboratory Animal Care and Use Committee.

kept in vivarium conditions (22–25°C, 30–70% relative humidity, 12 h light/12 h dark diurnal light cycle) for at least 1 week prior to experiments. Animals were immobilized by pithing the brain and spinal cord. The sciatic nerve was isolated in a conventional manner, ligated and submerged into chilled Ringer's solution containing: NaCl 102.6; KCl 1.0; $NaHCO_3$ 0.7; $CaCl_2$ 0.9 (mmol/L); pH 7.4–7.6. From this solution, the nerve was transferred into an exposure chamber and covered with mineral oil. A thin layer of oil over the preparation (0.3–0.5 mm) effectively prevented the nerve from drying and was virtually transparent to MMW.

The chamber was equipped with two pairs of artifact-free saline bridge electrodes which contacted the opposite ends of the nerve. CAPs propagated from stimulating to recording electrodes through a 30-mm middle portion of the nerve covered with the oil and exposed to MMW. The saline electrodes were designed so as to provide exactly the same positioning of every nerve preparation in the chamber. To eliminate biphasic CAP signals, the nerve was crushed between the two electrodes connected to the amplifier.

A block diagram of the experimental setup is provided in Figure 1. CAPs were evoked by paired square pulses from a Grass Instruments Stimulator S8800 and recorded on a Tektronics 2430 digital oscilloscope. We used a supramaximal nerve stimulation that was 2–5 V at 0.2 ms pulse width. Stimulating pulses were continually delivered at a rate of either 4 pairs/s (low-rate stimulation) or 20 pairs/s (high-rate stimulation, HRS). The interval between two pulses in a pair was 9 ms, so that the second CAP fell into the relative refractory period after the first one. Automatic on-line averaging of 16 consecutive CAP records was employed in order to improve the signal-to-noise ratio and to deemphasize the incidental CAP variability. The amplitude, latency, and peak latency of conditioning and test CAPs (i. e., the CAPs evoked by the first and the second stimuli in a pair, respectively) were measured every 5 min throughout the experiment.

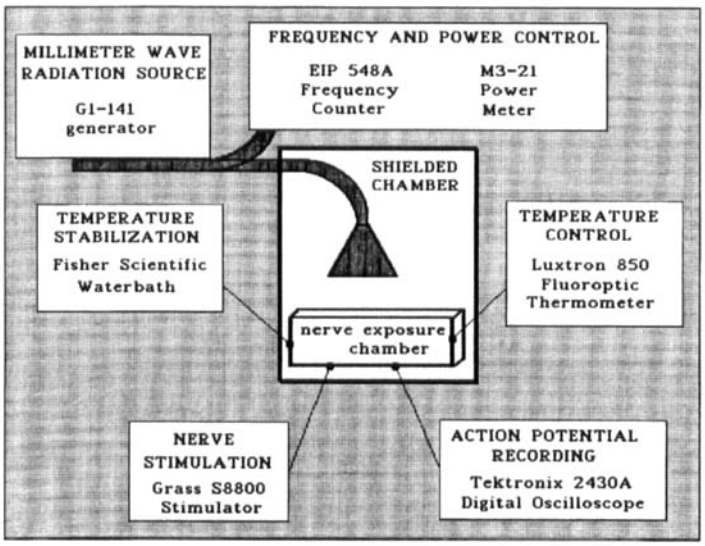

FIGURE 1. Block diagram of the setup employed for studying effects of the millimeter wavelength radiation on isolated nerve function.

The chamber was cooled to 11–12°C by the constant flow of chilled water from a thermostabilized water bath. This attenuated, but did not entirely prevent, nerve heating by the radiation because of slow heat conduction through the mineral oil. The temperature in the chamber was monitored by a Luxtron Instruments model 850 multichannel fluoroptic thermometer. The temperature probe was situated in the mineral oil, 2–3 mm from the middle of the nerve. As measured by this probe, microwave heating at the maximum tested incident power density of 2.5–2.6 mW/cm^2 did not exceed 0.3–0.4°C, and at the lower power densities (0.25–0.27 mW/cm^2) heating was not detectable. One should note that the local MMW heating values along the exposed site of the nerve could be different from the thermometer readings (this issue will be addressed in more detail in Discussion).

Irradiation and Dosimetry

The microwave power generator (model G4–141, Russia) operated in a continuous-wave regime in the frequency range 37–53 GHz. The waveguide line included two attenuators, a 10-dB bidirectional coupler, a slotted section with a broadband probe for standing wave measurements, and terminated in a horn antenna. The horn was situated 52 mm above the isolated nerve in the exposed chamber so that the nerve was aligned with the E-field. Exact radiation frequency and net input power to the horn were monitored via the coupler by an M3-21 wattmeter and Ch3-34/Ch5-16 frequency meter (Russia), or by an EIP model 548A frequency counter (USA).

As a result of the intense absorption of MMW in biological tissues, the incident power density, rather than the specific absorption rate, should be used for characterization of exposure conditions. The incident power density was measured with a broadband electric field probe (Narda Microwave Corp., model #8721). The probe readings in 37–53 GHz range were verified by a comparison with the field values calculated by a free-space standard field method (32). Local field intensity measurements and field mapping were completed using a calibrated miniature flat crystal detector (Narda part #4824) and a computer controlled robotic tri-axial scanner. More details on the methods of field characterization and justification of the calibration procedures will be provided in a separate paper.

Exposures at selected radiation frequencies in the bands 41.14–41.54 GHz and 50.8–51.0 GHz were performed at the incident power density of 0.25–0.27 mW/cm^2 on the axis of the antenna; the field intensity for the 45.89–45.93 GHz band was 10-fold higher. The permissible inaccuracy of adjustment of the MMW frequency was 10 MHz for the first 2 bands and 2 MHz for the third band. MMW exposures at different frequencies randomly alternated with sham exposures, which were performed in exactly the same manner. For a sham exposure, the waveguide attenuators were tuned to maximum (about 80 dB field attenuation), while the MMW generator and all other devices stayed turned on. Each nerve preparation received either a single exposure or sham exposure, each lasting for 38 min.

Experiment Protocol and Data Analysis

The high-rate stimulation (HRS) test is a sensitive tool that is commonly used to study functional changes in excitable tissues, including effects of electromagnetic fields in nerves (33–36).

CAPs evoked by a low-rate stimulation usually are remarkably stable for hours, while the HRS causes gradual decrease of the CAP amplitude and increases its latency. The ability of the nerve to tolerate the high-rate stimulation, which is also often referred to as the preparation "lability" or "vitality"(33,34), can be quantitatively assessed by a tolerance index (TI). For any time point t during the HRS, and for any chosen CAP parameter (amplitude, latency, etc.), TI is the ratio of the value of this parameter in this timepoint (V_t) to its initial value before the HRS (V_i):

$$TI_t = V_t / V_i$$

The stability of CAPs under low-rate stimulation makes it reasonable to take V_i as an average of several CAP records, in order to reduce a TI calculation error that could potentially result from the incidental CAP variability. In this study, V_i was calculated as the average of three CAP records taken with 5-min intervals before the HRS.

The HRS tolerance is characteristic for each individual nerve preparation, but varies substantially from one preparation to another, even for preparations taken from two legs of the same animal. The TI variability over a group of nerve preparations could mask or obscure an effect of electromagnetic radiation. These circumstances made it necessary to measure TI in each individual preparation first during an HRS train without any irradiation (to establish TI level characteristic for this preparation) and then during another HRS train accompanied with either MMW or sham irradiation (to find out whether the characteristic TI level will be changed by the treatment).

Thus, each successful nerve preparation was subjected to the HRS two times, in two identical trials (Fig. 2). The HRS train duration was set short enough (17 min) to

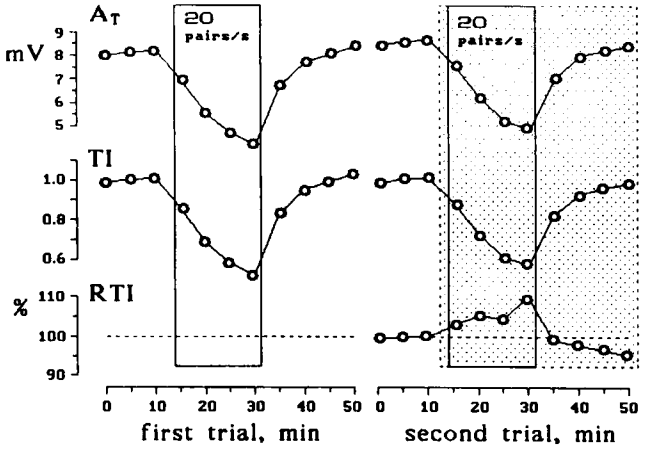

FIGURE 2. Timeline of experimental procedures employed in the study and an illustration of data processing. The top graph represents the original values of the test response amplitude (A_t, mV) measured with 5-min intervals during two consecutive 50-min trials. Both the trials are identical, except for a microwave or sham exposure (dotted area) during the second trial only. Boxes show the intervals of the high-rate stimulation (20 paired pulses/s), otherwise the stimulation rate is 4 paired pulses/s. Lower graphs represent the tolerance index (TI) and relative tolerance index (RTI, %) values calculated from the raw data above; methods of the calculation are described in text.

avoid any irreversible effects on nerve function. The first trial began after sufficient stabilization of the preparation in the exposure chamber, which usually took from 40 to 90 min. Each trial included recording of 3 datapoints before the HRS, 4 datapoints during the 17-min HRS train, and 4 more datapoints during the recovery period after the HRS. The time interval between the trials, as well as between the datapoints, was set at 5 min, which corresponded to a 38-min interval between the HRS trains. The first trial was always done without any exposure and was only needed to establish the individual TI values characteristic for each nerve preparation. In the second trial, MMW or sham exposure began 2 min before the HRS and continued till the end of the experiment.

The ratio of TI's in the matching timepoints t of the second and the first trials (RTI) served as an index of the exposure effect:

$$RTI = ({}^2TI_t / {}^1TI_t)\ 100\% = ({}^2V_t / {}^2V_i) / ({}^1V_t / {}^1V_i)\ 100\%,$$

where the superscripts 1 and 2 correspond to the first and the second trials. Obviously, if the nerve tolerance to HRS has not been changed during the second trial, the RTI will be equal to 100%. One must note, however, that at least two factors other than MMW could cause RTI deviation from 100%: these are the gradual loss of the preparation viability during its maintenance *in vitro*, and incomplete nerve recovery after the first HRS train. Hence, the obtained RTI values were compared not to the 100% level, but to the corresponding RTI values in experiments with sham exposure.

The RTI values were calculated separately for each of six CAP parameters (amplitude, latency, and peak latency of the conditioning and test CAPs) for every time point of the second trial. For convenience, these RTI values will be referred to below as "relative amplitude," "relative latency," and "relative peak latency" of the conditioning and test responses, and the time scales in subsequent figures and text will correspond to the second trial (0 min corresponds to the first timepoint of the second trial, and 50 min is the end of the entire experiment).

A total of 82 experiments with MMW exposures at 15 different frequencies (5 for each band) and 11 experiments with sham irradiation were carried out. The RTI values for different preparations exposed at the same frequency (or at close frequencies within one of the bands) were averaged and compared with the sham exposure data. Statistical comparison employed a χ^2 test and t-test with Dunnet's correction (37) when applicable. The MMW effect shown in the earlier series of experiments (31) was the increase of the test CAP relative amplitude by the end of the HRS. Therefore, one-sided tests instead of two-sided could now be employed for statistical verification of this effect. Other CAP parameters did not previously show statistically significant changes under MMW action, so their changes were still analyzed using two-sided confidence intervals.

RESULTS

The initial data analysis was intended to determine which CAP parameters are sensitive to MMW irradiation, and whether there are any major differences in the effectiveness of the three tested frequency bands. All the data for each frequency band were pooled together and compared with sham exposures; possible differences in the effectiveness of different frequencies within each band were disregarded at this time. This analysis established two statistically significant effects of irradiation, namely a

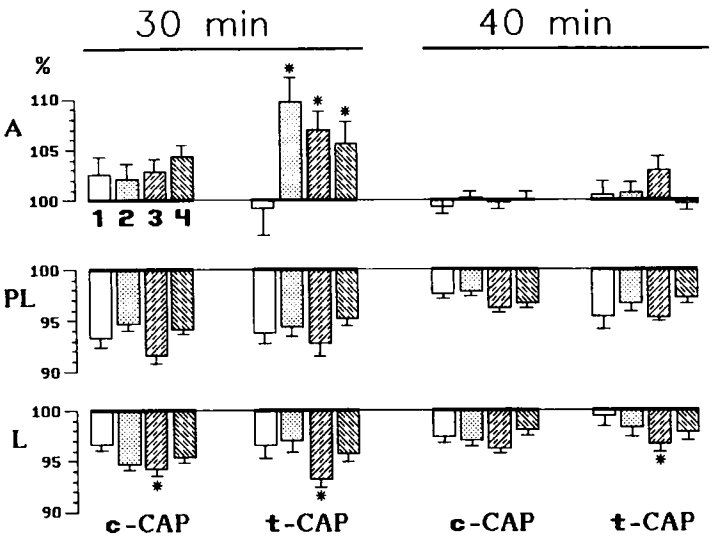

FIGURE 3. Millimeter-wave radiation effect on parameters of the conditioning and test compound action potentials (c-CAP and t-CAP, respectively). Parameters shown are the relative values of amplitude (A), peak latency (PL), and latency (L). Shown are two datapoints that correspond to the end of the high-rate stimulation train (30 min) and recovery after it (40 min). The data for different frequencies of the radiation within each studied band are pooled together: **2**, 41.14–41.54 GHz band (n = 28), **3**, 50.8–51.0 GHz band (n = 25), and **4**, 50.8–51.0 GHz band (n = 30). Vertical bars are the standard error of the mean; asterisks indicate significant differences ($P < .05$) from the sham exposure (**1**, n = 11).

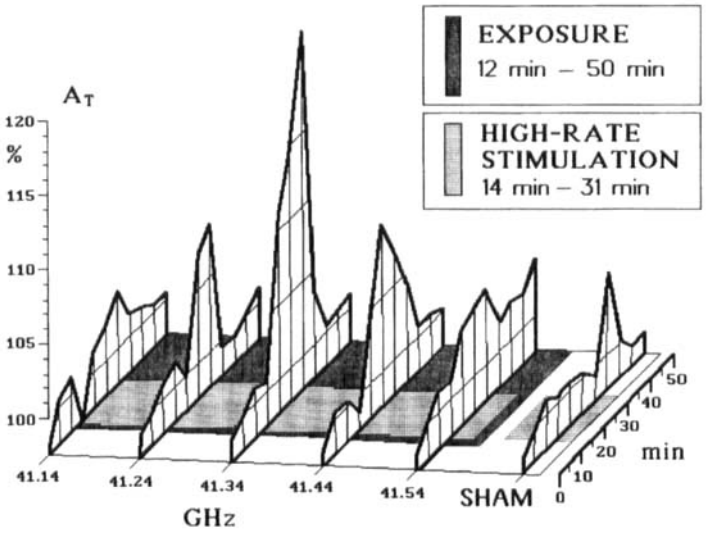

FIGURE 4. Effect of exposure at different frequencies in the 41.14–41.54 GHz band on the relative amplitude of the test compound action potential (A_t). MMW irradiations at 0.25–0.27 mW/cm^2 and sham exposures began at 12 min and continued till the end of the experiment. The high-rate nerve stimulation (20 paired pulses/s) was applied from 14 min to 30 min, otherwise the rate was 4 paired pulses/s. Shown are the average values from 5–7 independent experiments in each MMW-exposed group and 11 experiments in the sham-exposed group.

decrease of CAP relative latency and an increase of the test CAP relative amplitude (Fig. 3).

The CAP latency was decreased only by irradiation at the highest tested field intensity, i.e., at 2.5–2.7 mW/cm^2, which was employed in the 45.89–45.93 GHz band. This effect, although statistically significant, did not exceed 4–6% and showed the same time course and magnitude for all 5 MMW frequencies in this band. Most likely, this effect could be attributed to slight microwave heating of the nerve preparation.

Another MMW effect, the increase of the test-CAP relative amplitude, was characteristic for all 3 bands. This effect reached maximum by the end of the HRS train (25–30 min into the second trial), similar to that found in our previous studies. The data for the recovery period after the HRS train were not different from the sham-treated group. Further analysis has established that this effect is critically dependent upon the radiation frequency (Figs. 4 and 5).

Maximum increase of the test-CAP relative amplitude (22 ± 7% over the sham control) occurred in the 41.14–41.54 GHz band at the median frequency of 41.34 GHz. A 100-MHz deviation from this "resonant" frequency (to 41.24 or 41.44 GHz) reduced the effect more than twofold, and a 200-MHz deviation eliminated it. Within 2 other bands, the CAP increase was significant at 45.89, 50.8, and 50.9 GHz, but no bell-shaped dependence of the effect on the radiation frequency was observed. Perhaps, the chances to reveal a resonance-like frequency dependence could be better if these two bands had the same or greater width than the first one.

Figure 5 shows that at the same field intensity the effect ranges from maximum to zero, depending solely on the MMW frequency. At 41.34 GHz, the effect is more

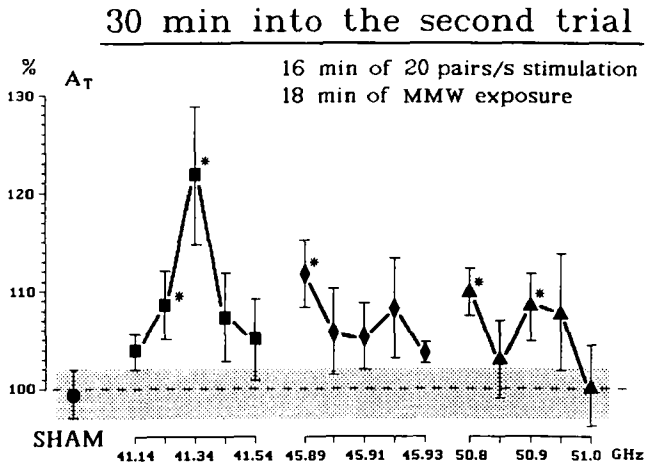

FIGURE 5. Millimeter wave-induced changes in the test response amplitude by the end of the high-rate stimulation train. The incident power density was 0.25–0.27 mW/cm^2 for the 41.14–41.54 GHz and 50.8–51.0 GHz frequency bands, and about 2.5 mW/cm^2 for the 45.89–45.93 GHz band. Each datapoint is the average of 5–7 independent experiments in the MMW exposed groups and 11 experiments in the sham-exposed group. Note different frequency scales: the interval between the neighboring frequencies for the above bands is, respectively, 100 MHz, 50 MHz, and 10 MHz. See Figures 3 and 4 for other explanations.

pronounced than, for example, at 45.90 and 45.93 GHz, despite the 10-fold increase in the incident power density. These results suggest that the mechanism of MMW action was different from microwave heating.

DISCUSSION

The results of this study confirmed that a short-term, low-level MMW irradiation can induce significant biological effects. As in our earlier experiments, MMW exposure at certain frequencies increased the test-CAP relative amplitude, or, in other words, irradiation attenuated the HRS-induced diminution of the test CAP. This effect can be interpreted as an improvement of the nerve refractory properties under the action of MMW.

A key question about any study that demonstrates biological effects of a low-intensity microwave radiation is whether these effects could possibly result from microwave heating of the subject. The Luxtron fluoroptic temperature probe we used in this study was primarily intended to monitor the temperature of the medium to make sure it is stable throughout the experiment. Due to high insertion losses of MMW in biological tissues, the local heating, particularly at the MMW-exposed surface of the subject, might be substantially different from the gross heating measured by the thermometer. The microwave field in the vicinity of the horn antenna is nonuniform, meaning that the local field intensity values can be smaller or greater than the incident power measured on the axis of the horn. This results in the formation of so-called "hot spots," which correspond to the local field maxima. Dimensions, spatial distribution, and temperature of the "hot spots" are highly dependent on the radiation frequency (38), and, hypothetically, this could be a reason for frequency-specific bioeffects of MMW.

In our exposure conditions, the temperature and spatial distribution of the "hot spots" could not be measured directly. Another approach was to analyze the field structure and compare the position and magnitude of local field maxima at different frequencies. This was accomplished by a detailed field mapping at all MMW frequencies used in this study. The maps were taken with 1-mm steps over a 40 × 40 mm area at the distance of 52 mm from the horn antenna. A sample field map for the frequency of 41.34 GHz is presented in Figure 6. The incident power density steeply decreased in the H-field direction from the geometrical center, but stayed fairly stable in the E-field direction. The actual area of interest is a narrow stripe in the middle of the map (shaded area), which corresponds to the position of the nerve preparation under the horn.

Figure 7 shows the "center slices" of the field maps through this shaded area, i.e., they show the frequency-dependent field variations over the exposed length of the nerve. The maximum field variation within this 41.14–41.54 GHz band did not exceed 1 dB, and the field variation for the other two bands was even less (apparently, because the interval between the tested frequencies in these two bands was also less). The difference in the on-axis incident power employed for the 41.14–41.54 GHz and 45.89–45.93 GHz bands was about 10 dB; hence, any local field maximum for any first band frequency was well below any local field minimum for any second band frequency. MMW absorption in biological tissues for these two bands is almost the same (39), so the MMW heating at, for example, 45.93 GHz was greater than at 41.34 GHz in any portion of the nerve. If the mechanism of the MMW effect were thermal, then the

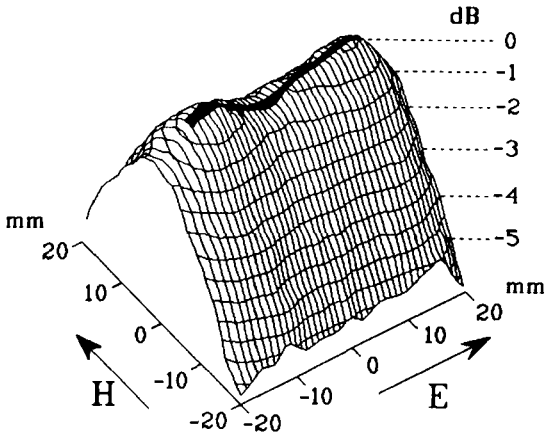

FIGURE 6. Sample field map developed for the frequency of 41.34 GHz at 52 mm from the edge of the horn irradiator. The vertical scale is the ratio of local and maximum incident power density values, in dB. The horizontal scales are the distance from the axis of the horn (in mm) in the E-field and H-field directions. To develop this map, the field intensity was measured in a 40 × 40 mm square (1600 points with 1-mm intervals).The shaded stripe corresponds to the position of the nerve preparation.

frequency of 45.93 GHz at 2.5 mW/cm2 would be more effective than 41.34 GHz at 0.25 mW/cm^2. However, the dependence established in our experiments was quite the opposite, so a thermal mechanism for the MMW effect on CAP relative amplitude can be ruled out.

Another argument that the mechanism of the MMW action was not merely thermal comes from a comparison of the two observed biological effects: The MMW effects on the CAP relative latency and amplitude had different dependencies on the radiation frequency and intensity. The latency was affected by all the radiation frequencies

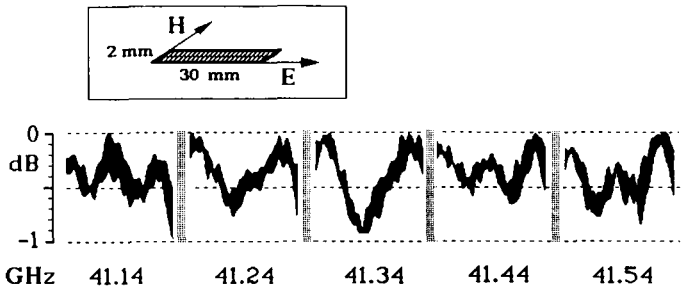

FIGURE 7. Nonuniformity of the incident power density over the nerve preparation for five radiation frequencies in the 41.14–41.54 GHz band. The field intensity values are obtained as "center slices" through the shaded area of the three-dimensional field maps (see Fig. 6); each "slice" is 2 mm thick and 30 mm long, and corresponds to the position of the nerve preparation. The vertical axis is the ratio of local and maximum incident power density values, in dB.

applied at 2.5 mW/cm^2, but not by other frequencies applied at 0.25 mW/cm^2; the amplitude could be affected or not at either field intensity, with a complicated dependence upon the frequency. If both the effects were thermal, then both should appear in the same irradiation regimes which produced more heat in the preparation, but this was not the case. Hence, at least one of the effects was not produced by microwave heating.

The half-width of the frequency resonance observed at 41.34 GHz can be estimated as being 150–350 MHz, which is close to what has been reported by other investigators. Surprisingly, the discovered effective frequency of 41.34 GHz was virtually the same as found in *E. coli* experiments (12). These authors demonstrated that the likely intracellular target for MMW is DNA, and that the resonance frequency depends on the haploid genome length. The isolated peripheral nerve does not contain the cell's genome; however, DNA still can be found in mitochondria, and the structure of mitochondrial DNA is similar to that of prokaryotic organisms. It is too early to speculate whether mitochondrial DNA could really play any role in MMW effects on isolated nerve function, but this possibility can be considered for future investigations.

Another possible mechanism of the effect may be related to MMW effects on Ca^{2+}-activated K^+ channels in cell membranes (40,41). As demonstrated by the patch voltage-clamp method, a 20-min exposure at 42.25 GHz, 0.1 mW/cm^2 altered the Ca^{2+} binding (Hill coefficient) and the open state probability of the channel. The same effect could be evoked using a MMW-exposed solution, without irradiation of the cell membrane. Irradiated solutions retained their ability to affect channel function for at least 10–20 min after cessation of the exposure. Long-term changes in the state of water after exposure to low-intensity MMW could be demonstrated by physical methods directly (42). These results supposed that water could play a "universal receptor role" and mediate various biological effects of MMW.

ACKNOWLEDGMENTS

The work was performed while A. G. Pakhomov held a National Research Council-AMRMC Research Associateship and was supported by the US Army Medical Research and Materiel Command under contract DAMD17–94-C–4069 awarded to McKesson BioServices. The views, opinions, and findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision. The authors would like to thank J. C. Lee for his efforts in setting up the field mapping.

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