Search for Frequency-Specific Effects of Millimeter-Wave Radiation on Isolated Nerve Function

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Effects of a short-term exposure to millimeter waves (CW, 40–52 GHz, 0.24–3.0 mW/cm^2) on the compound action potential (CAP) conduction were studied in an isolated frog sciatic nerve preparation. CAPs were evoked by either a low-rate or a high-rate electrical stimulation of the nerve (4 and 20 paired pulses/s, respectively). The low-rate stimulation did not alter the functional state of the nerve, and the amplitude, latency, and peak latency of CAPs could stay virtually stable for hours. Microwave irradiation for 10–60 min at 0.24–1.5 mW/cm^2, either at various constant frequencies or with a stepwise frequency change (0.1 or 0.01 GHz/min), did not cause any detectable changes in CAP conduction or nerve refractoriness. The effect observed under irradiation at a higher field intensity of 2–3 mW/cm^2 was a subtle and transient reduction of CAP latency and peak latency along with a rise of the test CAP amplitude. These changes could be evoked by any tested frequency of the radiation; they reversed shortly after cessation of exposure and were both qualitatively and quantitatively similar to the effect of conventional heating of 0.3–0.4 °C. The high-rate electrical stimulation caused gradual and reversible decrease of the amplitude of conditioning and test CAPs and increased their latencies and peak latencies. These changes were essentially the same with and without irradiation (2.0–2.7 or 0.24–0.28 mW/cm^2), except for attenuation of the decrease of the test CAP amplitude. This effect was observed at both field intensities, but was statistically significant only for certain frequencies of the radiation. Within the studied limits, this effect appeared to be dependent on the frequency rather than on the intensity of the radiation, but this observation requires additional experimental confirmation. *Bioelectromagnetics 18:324–334, 1997.*

Key words: nerve conduction; compound action potential; frequency-specific bioeffects

INTRODUCTION

One of the most intriguing problems in modern electromagnetobiology is the existence and physiological significance of frequency-specific, resonance-type biological effects of millimeter waves [MMW, see for example Grundler et al., 1977; Keilmann, 1985; Grundler and Kaiser, 1992; Kataev et al., 1993; Belyaev et al., 1992, 1994]. Many investigators are rather skeptical about such effects and regard them as experimental artifacts [Gandhi et al., 1980; Bush et al., 1981; Furia et al., 1986]. At the same time, in the former Soviet Union the effects of low-level MMW have not only been demonstrated in a multitude of studies, but have been used for years in clinical practice. Diseases reported to be successfully treated by MMW range from peptic ulcers to cardiomyopathy, stenocardia, hypertension, wound infections, etc. Experience claimed with more than 100 000 clinical cases [Sit'ko et al., 1992; Betzky, 1992] should be considered and may provide evidence for the reality of specific or nonthermal biological effects of MMW electromagnetic radiation.

Of particular interest are recent experimental data for MMW effects on excitable tissue and cell membrane function. Burachas and Mascoliunas [1989] have demonstrated a pronounced MMW-induced suppression of compound action potentials (CAPs) in isolated frog sciatic nerves. After 10–20 min of irradiation at 77.7 GHz, 10 mW/cm^2, CAP amplitude decreased ex-

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ponentially and fell 10-fold within the next 40–90 min. The CAP decrease due to the second and the subsequent exposures became increasingly steeper, taking only 10–15 min. In addition to this ''slow'' response, switching the field on increased CAP amplitude instantaneously by 5–7%, and switching the field off caused the opposite short-term reaction.

Chernyakov et al. [1989] carried out a complex study of effects induced by MMW in various excitable structures. Exposure of an isolated frog tailor's muscle (*m. sartorius*) at 0.1–0.15 mW/cm^2 in the range of 53–78 GHz decelerated the natural loss of transmembrane potential in myocytes and reduced the overshoot voltage, diminishing CAP amplitude by 7–25% and conduction velocity by 10–20%. Exposure of isolated frog sciatic nerves for 20–40 min altered the late CAP components or caused an abrupt ''rearrangement'' of CAPs: position, magnitude, and even polarity of CAP peaks (the initial CAP was polyphasic) suddenly changed in an unforeseeable manner. Sinoatrial area pacemakers changed their firing rate after just a few seconds of irradiation at 1 mW/cm^2, when microwave heating of the preparation did not exceed 0.005 °C.

Sazonov and Rizshkova [1995] reported that exposure at 42.19 ± 0.15 GHz facilitates isolated frog nerve recovery after a 1-kHz electrical stimulation train. The time needed for CAP restoration in exposed preparations decreased to 60–80% of the control group values.

Kataev et al. [1993] used voltage-clamp techniques to study MMW effects on membrane currents in the alga *Nitellopsis obtusa* (*Characea*). Irradiation for 30–60 min at 41, 50, and 71 GHz (5 mW/cm^2) suppressed the chloride current to zero with no recovery for 10–14 hr. The greater part of other tested frequencies in the range 38–78 GHz enhanced the chloride current, in some cases up to 200–400% (49, 70, 76 GHz). This activation was reversible, and recovery to the initial value took 30–40 min after the treatment. Neither activating nor inhibitory effects could be reproduced or explained by MMW heating.

In contrast to the above findings, Kazarinov et al. [1984] observed only thermal changes in the isolated frog skin potential under exposure to 35- to 41.6 GHz radiation. Khramov et al. [1991] demonstrated that all types of alterations in electrical activity of slow-adapting neurons of crayfish stretch receptor caused by 34–78 GHz MMW resulted from microwave heating. Motzkin and Feinstein [1989] did not find any effects of 5 mW/cm^2, 51.72 or 51.81 GHz radiation on miniature end plate potentials in rat neuromuscular junctions.

The purpose of the present study was to verify the existence of specific effects of MMW on excitable tissues under precisely controlled experimental conditions. High MMW sensitivity of CAP conduction in isolated frog sciatic nerve, as reported by other authors, motivated us to choose this preparation for our investigation. We used an approach which is standard for analysis of functional changes in excitable preparations, which is an electrical stimulation by paired pulses at either a low or a high pulse repetition rate. These techniques allow one to reveal changes in CAP conduction, nerve refractory properties, and to evaluate its ability to sustain a high-rate stimulation.

MATERIALS AND METHODS

Nerve Preparation and Data Acquisition

All experiments were performed on isolated nerve preparation (*n. ischiadicus* + *n. peroneus*) of the frog *Rana berlandieri.* Active adult frogs (males) were kept in vivarium conditions (22–25 °C, 30–70% relative humidity, 12-hr light/12-hr dark diurnal light cycle) for at least 1 week before experiments. After animal immobilization by pithing the brain and the spinal cord, sciatic nerves from both legs were 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. Then the two nerves were transferred into individual exposure and control chambers made of polyvinylchloride. These chambers had identical design and dimensions (145 mm length, 40 mm width, 58 mm height) with a 13 mm wide, 10 mm deep slot at the top. The nerve preparation was laid in this slot, open to MMW irradiation from the top.

The research described in this report was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to the principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 85–23.

The depth of MMW penetration into water ranges from 0.1 to 0.5 mm [Ryakovskaya and Shtemler, 1983], and it is even less in saline. Therefore, we exposed nerves under a thin layer of mineral oil (0.3–0.5 mm), which is almost transparent to MMW and effectively prevents nerve drying. Artifact-free nerve stimulation and CAP recording were accomplished via two pairs of saline bridge electrodes that contacted the opposite ends of the nerve and also limited the MMW-reachable area. These electrodes were made of 12 mm long pieces of silicone tubing (2.5 mm inner diameter, 1.5 mm wall thickness). They were permanently mounted on the bottom of the slot and filled with Ringer's solution. Thus, CAPs propagated from stimulating to recording

electrodes through a 30-mm middle portion of the nerve covered with oil and exposed to MMW. The saline electrodes were designed so as to provide exactly the same positioning of every nerve preparation, about 7 mm above the bottom of the slot. To eliminate biphasic CAP signals, the nerve was crushed between two recording electrodes connected to the amplifier.

CAPs were evoked by paired square pulses from a Grass Instruments Stimulator S8800 and recorded on a Tektronics 2430 digital oscilloscope. Usually, we used a supramaximal nerve stimulation (0.2-ms pulse width, 2–3 V amplitude). The relative refractory period lasted for 9.5–12 ms, and the interpulse interval was chosen to be 9 ms. Stimulating pulses were continually delivered at a rate of either 4 pairs/s (low-rate stimulation, LRS) or 20 pairs/s (high-rate stimulation, HRS). Automatic averaging of 16 consecutive CAP records was used in most of the experiments, 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 (*A*, *L*, *PL*, and A_t, PL_t, see Fig. 1) were measured every 2 or 5 minutes throughout the experiment.

Both the chambers were cooled to 11–12 °C by constant flow of chilled water from a thermostabilized water bath. This attenuated but did not prevent nerve heating by the radiation because of slow heat conduction through mineral oil. Actual temperature in the chambers was monitored by a Luxtron Instruments model 850 multichannel fluoroptic thermometer; the fluoroptic probe was situated in the oil 1.5–2 mm from the middle of the nerve. This probe was primarily intended to measure conventional heating of the nerve. To quantify MMW heating of the preparation, we used an original ''biological'' technique, which will be described in detail in the ''Results'' section.

Most experimental reports about microwave bioeffects are based on a comparison of the data with either a control, or a sham-exposed population. A parallel control can be performed synchronously with the exposure, but has to be in a different physical location; sham exposures can be performed in exactly the same physical location as microwave exposures, but only at a different time. Though neither of the two approaches can entirely exclude the possible impact of uncontrolled factors other than irradiation, use of both sham and parallel controls in the same series of experiments can make a strong case that a difference (if observed) was in fact a result of irradiation. To make the experimental conditions as strict as possible, this ''duplicative control'' protocol was used in all experiments with HRS.

Control and exposed nerve chambers were situated in the same Faraday cage, but the control chamber

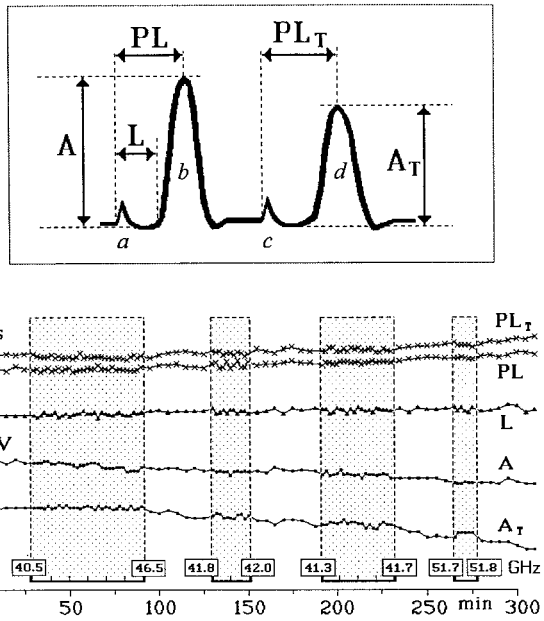

Fig. 1. Isolated nerve performance with a low-rate stimulation (4 paired pulses/s) and repetitive millimeter wave exposure. Periods of irradiation are shown by dotted areas. The indicated radiation frequencies are those at the beginning and at the end of each exposure; during irradiation, the frequency is increased stepwise at either 0.01 or 0.1 GHz/min; the incident power density for all the exposures is 2.2–2.8 mW/cm^2 (see Table 1 for details). The inset at the top shows the compound action potentials (CAPs) as evoked by paired pulses (*a*, *c*) with a 9-ms interval and defines the CAP parameters: *A*, *PL* and *L* are the amplitude, peak latency, and latency of the conditioning CAP (*b*), A_t and PL_t are the amplitude and peak latency of the test CAP (*d*).

was shielded from the radiation. Nerves in the exposed chamber could be either MMW-irradiated or sham-irradiated. To provide the sham exposure conditions, we tuned the waveguide attenuators to maximum, leaving all other devices and the microwave generator turned on. The incident power density still reaching the preparation during sham exposures was calculated to be about 10^{-8} mW/cm^2. The parallel control nerves were never exposed, but underwent all the same stimulation routines simultaneously with exposed and sham-exposed preparations. Statistical comparisons were done between exposed and sham-exposed data sets and then between their respective parallel controls. Any significant difference in performance of exposed and sham-exposed preparations should only be taken into account if it was not accompanied by a similar difference between their parallel controls.

Irradiation and Dosimetry

The microwave power generator (model G4-141, made in Russia) operated in a CW regime in the fre-

quency range 37–53 GHz. This generator uses a backward-wave oscillator and has the half-power bandwidth of less than 5 MHz. The waveguide line included two attenuators, a 10-dB bidirectional coupler, a slotted section with a broadband probe for standing wave measurements, and a horn antenna at its end. 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 (EIP Microwave Inc., USA). These devices were used to tune the MMW generator to the desired output power and frequency before each irradiation. If needed, these parameters could be adjusted during irradiation, but usually their drift was not considerable.

As a result of the intense absorption of MMW at the surface of biological tissues, the incident power density (IPD), rather than the specific absorption rate, should be used for characterization of exposure conditions. However, no IPD meters calibrated for the 37–53 GHz range could be found on the market. The most appropriate device proved to be a broadband electric field probe (Narda Microwave Corp., model 8623), which is calibrated from 300 kHz to 38 GHz and also at 94 GHz, but not between 38 and 94 GHz. It was the opinion of the probe manufacturer, that the design of the probe is suitable for the entire range, and no substantial variations of its sensitivity should be expected. To verify this, we calibrated the probe against IPD values calculated by a free-space standard field method (see Appendix for details). Theoretical IPD per unit of the net input power to the horn irradiator was calculated for frequencies from 37 to 78 GHz and for various far field distances from the horn. Theoretical and measured IPD differed by no more than ± 1.5 dB throughout the entire frequency range. Most of this difference could be attributed to the combined error of the theoretical model and the net input power measurements, so the probe was concluded to be sufficiently accurate. Close IPD values were obtained later by another probe rated up to 45.5 GHz (Narda model 8721).

Both of these probes, although accurate, were much too big (53 mm diameter) for near-field dosimetry or field mapping. For these purposes we used a miniature flat crystal detector (Narda part 4824). A custom-made holder for the detector minimized field distortion and enabled precise movements in any direction over the irradiated area. The detector was calibrated in the far field against the other probe (8623) individually at every frequency of interest. The detector's sensitivity markedly varied within the frequency range, but always remained perfectly linear, at least in the studied interval from 0.1 to 8 mW/cm^2. More details on the performance of this crystal detector and field mapping are to be presented in a separate paper.

IPD values measured by the broadband probe and the crystal detector were normalized per unit of the net input power to the horn, and plotted against the distance from the horn. These plots proved to be rather similar for different frequencies, even in the near field. For the 52-mm distance from the horn, the normalized IPD measured from 45 to 65 (μW/cm^2)/mW, which did not exceed the presumed inaccuracy of the meters. Therefore, these variations were ignored; the mean coefficient of 55 (μW/cm^2)/mW was used to calculate IPD from the measured net input power values. For individual frequencies from the 38 to 53 GHz range, the field non-uniformity along the exposed site of the nerve was found to be no more than ± 2 dB.

RESULTS

Low-Rate Stimulation (LRS) Experiments

The effectiveness of various regimens of irradiation, which are summarized in Table 1, was tested in a total of 49 experiments. Six regimens of exposure were focused on possible immediate frequency-specific effects of MMW; the radiation frequency during an exposure was constantly increased by steps of either 0.01 or 0.1 GHz/min. Most of the preparations were exposed several times with 20- to 60-min intervals between exposures. LRS continued throughout the experiment without interruption, and CAPs were measured every 2 min during irradiation and every 5 min between exposures. Other regimens used various constant frequencies chosen from three supposedly effective frequency bands. As measured by the fluoroptic probe, the maximum temperature rise under irradiation at 2.2–2.8 mW/cm^2 was 0.3–0.5 °C, and at lower IPD it was negligible.

LRS by itself did not affect the functional state of the nerve, even if continued for hours. The nerve performance and parameters of conditioning and test CAPs remained essentially stable regardless of repeated MMW exposures (Fig. 1). The only noticeable and repeatable reaction to the irradiation was a subtle and transient decrease of the test CAP peak latency (PL_t) and increase of its amplitude (A_t). Although these changes did not exceed a few percent, they could be statistically significant ($P < .05$) in comparison with both the control preparations and with the preexposure values (Figure 2A and B).

This effect occurred only at the highest tested IPD, apparently without any dependence on the radiation frequency, thus suggesting that it could be caused

TABLE 1. Millimeter-Wave Irradiation Experiments with the Low-Rate Stimulation Protocol

Frequency band (GHz)	Stepping rate (GHz/min)	Exposure time (min)	Intensity (mW/cm^2)	No. of exper./ No. of nerves	Summary of findings[d]
40.50–46.50[a]	0.1	60	2.2–2.6	4/4	(+)
41.30–41.70[a]	0.01	40	2.2–2.5	6/6	(+)
41.80–42.00[a]	0.01	20	2.3–2.5	5/5	(+)
41.80–42.00[a]	0.01	20	0.23–0.25	3/3	(−)
49.50–53.50[a]	0.1	40	2.4–3.0	4/4	(+−)
51.70–51.80[a]	0.01	10	2.7–2.8	6/6	(+)
41.15–41.30[b]	—	30	0.26; 1.0; 2.6	14/10	(−); (−); (+)
41.70–42.10[b]	—	30	2.3; 2.6	3/3	(−); (+−)
51.60–51.70[b]	—	30	0.28; 2.8	4/3	(−); (+−)
			Total:	49/24[c]	

[a]Radiation frequency was increased stepwise to cover the entire band during the exposure period.
[b]Irradiation was performed at various constant frequencies chosen from the specified band. Only one frequency was used per experiment.
[c]One nerve could be exposed several times in different regimens, so that the total (24) is less than the arithmetic sum of the upper rows.
[d](+) refers to a consistent thermal effect (gradual simultaneous decrease in the test CAP peak latency and increase in its amplitude, such as shown in Fig. 2); (+−) indicates changes analogous to this thermal effect, but lacking statistical significance; (−) shows absence of any considerable MMW effects.

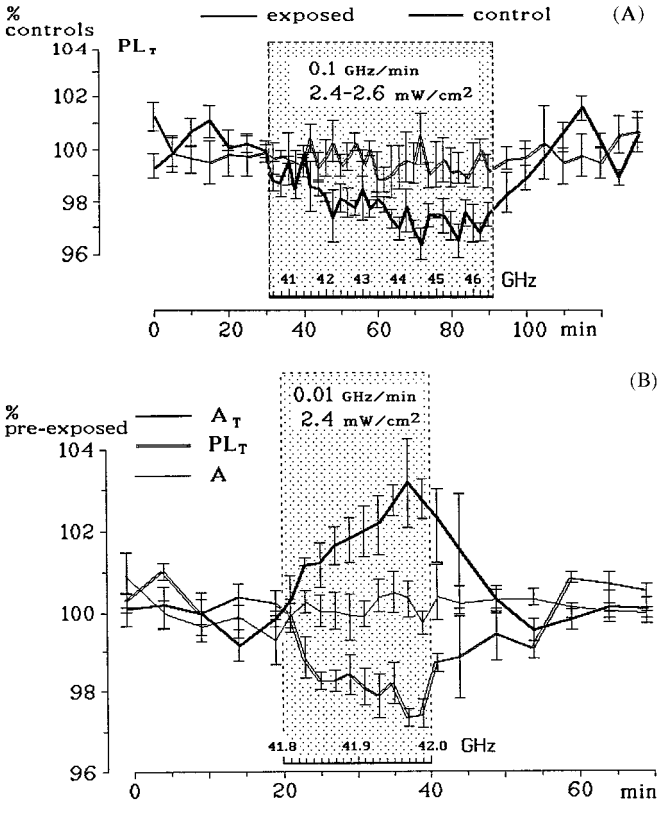

Fig. 2. Millimeter wave-induced alterations of compound action potential (CAP) conduction compared with (**A**) control experiments or with (**B**) the pre-exposure values. In both parts, CAP parameters are shown as percentage of the average preexposure value. Each datapoint is the mean ± SE from four to six independent experiments. Other designations are the same as in Fig. 1.

merely by general heating of the preparation by microwaves. To verify it, we studied the effects of conventional heating on nerve performance (Fig. 3). As can be seen from this graph, the pattern of the conventional heating-induced CAP changes (concurrent decrease of *L*, *PL*, Pl_t, and increase of A_t, while *A* stays almost stable) is the same as observed with the MMW exposures (Fig. 2).

Alterations of CAP indices due to small temperature changes (1–2 °C) could be approximated well by linear functions (Figure 4). This chart makes it possible to establish quantitatively whether the MMW-induced CAP changes could be regarded as produced by MMW

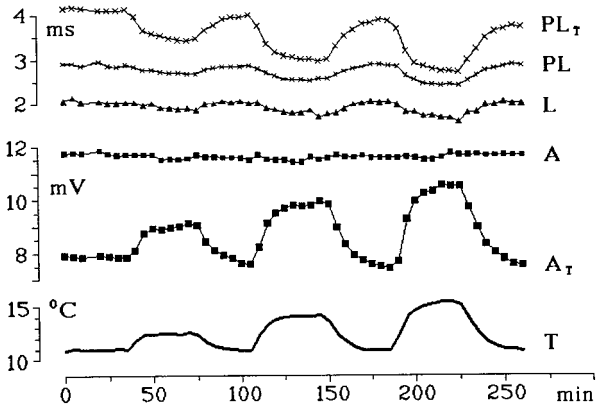

Fig. 3. Alterations of an isolated nerve performance by conventional heating. The lower trace, T, is the preparation temperature; other designations are the same as in Figure 1.

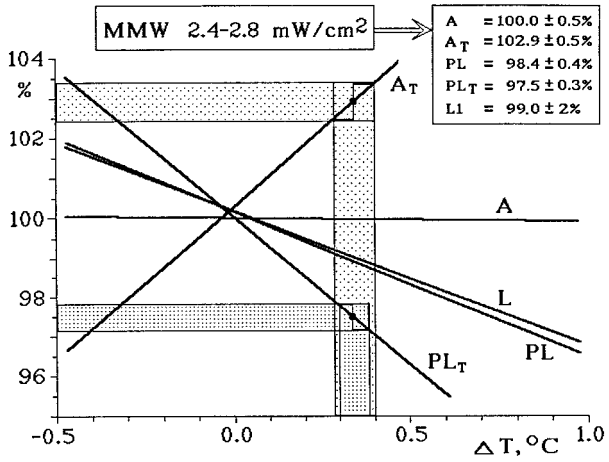

Fig. 4. Comparison of nerve performance alterations caused by millimeter-wave irradiation and conventional heating. The abscissa is the temperature change (°C) and the ordinate is the CAP parameters, in percent, to their values at the initial temperature. The lines A_t, A, L, etc., are obtained by differentiation and linear approximation of the data shown in Figure 3. A window at the top summarizes CAP changes observed under irradiations at 2.4–2.8 mW/cm^2. Dotted horizontal sectors on the chart show the range of values for A_t and PL_t observed under MMW irradiation; vertical dotted columns show the range of the corresponding values of temperature elevation. Other designations are the same as in Figure 1. See text for more explanation.

heating. At the initial temperature (zero temperature change), all CAP parameters were taken as 100% (they are not exactly 100% on the graph, because approximations were based on real experimental data and this introduced a small error). Experiments with MMW established that exposure at 2.4–2.8 mW/cm^2 increases A_t to 102.9 ± 0.5% and decreases PL_t to 97.5 ± 0.3% (these values are marked by two horizontal dotted bars on the graph). These changes can be explained by heating only if their magnitudes correspond to one and the same temperature rise. One can see from the graph that the corresponding temperature rise is indeed the same and equals 0.3–0.4 °C (it is marked by vertical dotted bars).

The same analysis was performed for other CAP parameters, and the correspondence of changes observed under MMW exposure and under conventional heating was essentially perfect. Thus, all MMW effects found in the LRS series could be attributed to microwave heating of the preparation. In turn, the level of MMW heating of the nerve could be measured indirectly from the MMW-induced alterations of CAP. We infer heating of the nerve preparation by 0.3–0.4 °C at an IPD of 2.4–2.8 mW/cm^2.

It is important to note that this use of the nerve itself as a thermometer did not reveal any substantial variations of temperature rise at different frequencies of irradiation. Within the accuracy of such measurements, heating depended on the IPD only. This fact not only confirms the correctness of our IPD measurements, but also shows the absence of any ''geometrical resonances'' which, hypothetically, could lead to a markedly increased MMW absorption and heating at certain ''resonance'' frequencies.

Our failure to find any frequency-specific effects in the LRS series did not necessarily mean the absence of such effects. Possibly, the LRS procedures used were not sensitive enough to reveal the effects. In earlier studies with 915 MHz microwaves [Pakhomov et al., 1992], use of a functional test with a high-rate stimulation detected nerve state changes that were otherwise undetectable. Therefore, this sensitive test was used next to study the effects of several selected MMW frequencies.

High-Rate Stimulation (HRS) Experiments

The principal difference between LRS and HRS is that LRS by itself does not alter the nerve functional state, while HRS does. Under HRS, the interval between pairs of stimuli is not long enough for complete recovery after conduction of the preceding CAPs, thus leading to nerve fatigue with gradual decrease of CAPs' amplitude and conduction velocity (Fig. 5).

After positioning the exposed and control nerves in the chambers, they were continually stimulated at 4 pairs/s until a reasonable stabilization of CAPs was

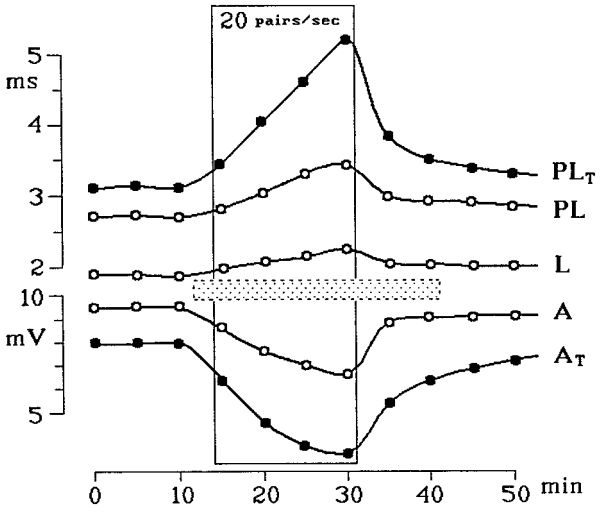

Fig. 5. Pattern of changes in compound action potential parameters and recovery in experiments with high-rate stimulation (20 paired pulses/s from the 14th until the 31st min of experiment; otherwise the rate is 4 paired pulses/s). Exposure or sham exposure (dotted bar) starts at 12 min and continues for 30 min. See Figure 1 for other designations.

obtained (60 min at least). Nerves with unstable or multi-peak potentials were usually discarded. Successful nerve preparations underwent from three to seven experiments lasting for 50 min each, with intervals sufficient for nerve recovery. MMW or sham exposure began on the 12th min of an experiment and continued for 30 min; HRS was turned on after 2 min of exposure and continued for 17 min. CAPs were recorded every 5 min, that is 3 datapoints before, 6 datapoints during, and 2 datapoints after exposure (Fig. 5). LRS was applied throughout a series of experiments with no interruptions other than HRS; LRS also continued between the experiments, when no data were collected.

MMW exposures were performed at a power density of either 2.0–2.7 mW/cm^2 (41.42, 42.04, 45.90 GHz, and 51.41 GHz), or 0.24–0.28 mW/cm^2 (41.22 and 50.91 GHz), as summarized in Table 2. The acceptable inaccuracy of adjustment of the radiation frequency was within 20 MHz from the indicated nominal values. As demonstrated in the LRS series, and confirmed by the Luxtron thermometer measurements, nerve heating at 2.0–2.7 mW/cm^2 was about 0.3–0.4 °C, and at 0.2–0.3 mW/cm^2 it was negligible. Different exposure regimens and sham exposures were randomized, except for the first experiment in a series, which was always performed without irradiation. The data from this first experiment were used only for evaluation of the individual nerve's ability to tolerate HRS and were not included in the statistical analysis. The same procedures, excluding irradiation, were simultaneously applied to the parallel control preparations.

Data processing was organized so as to maximally deemphasize individual differences between the nerve preparations and unforced functional changes during a series of experiments. This was accomplished by introduction of relative indices (*RI*) of the HRS tolerance, which were calculated as following:

$$RI = ({}^{e}V_n/{}^{e}V_i)/({}^{c}V_n/{}^{c}V_i) \cdot 100\%,$$

where ${}^{c}V_i$ is the value of a CAP parameter (amplitude, latency, etc.) before the HRS start in the first experiment in a series; ${}^{c}V_n$ is the value on the *n* min of the first experiment (during or after the HRS); ${}^{e}V_i$ and ${}^{e}V_n$, are the respective values in a succeeding experiment when HRS was accompanied by either MMW or sham irradiation. Calculation of relative indices from original data is also illustrated in Figure 6. If the nerve performance during an experiment with MMW irradiation is the same as in the first experiment in a series (which was always performed without irradiation), the relative indices will stay at 100%. If a relative index differs from 100%, this difference may indicate an aftereffect

TABLE 2. Millimeter-Wave Irradiation Experiments with the High-Rate Stimulation Protocol

			Relative index values in exposed nerves (mean ± SE, %, at 30 min)[a]				
			Conditioning action potential			Test action potential	
Intensity (mW/cm^2)	Frequencies tested (GHz)	No. of experiments (exposed + parallel control)	Amplitude	Peak latency	Latency	Amplitude	Peak latency
Sham	—	6 + 6	102.1 ± 2.9	90.4 ± 2.9	95.5 ± 1.2	92.9 ± 4.1	81.2 ± 4.5
0.24–0.28	41.22, 50.91	11 + 11	105.8 ± 1.2	90.3 ± 1.6	95.8 ± 1.0	109.3 ± 4.4*	92.1 ± 2.9
2.0–2.7	41.42, 42.04, 45.90, 51.41	22 + 22	107.5 ± 1.7	93.0 ± 2.7	94.4 ± 0.8	109.8 ± 3.8*	92.0 ± 4.6

[a]The 30-min time point corresponds to 16 min of the high-rate stimulation and 18 min of irradiation. The data within the intensity ranges were pooled together irrespective of the frequency of the radiation.
*$P < 0.02$ compared with the sham exposure. Differences in data values between the respective parallel control groups (not included in the table) were not statistically significant.

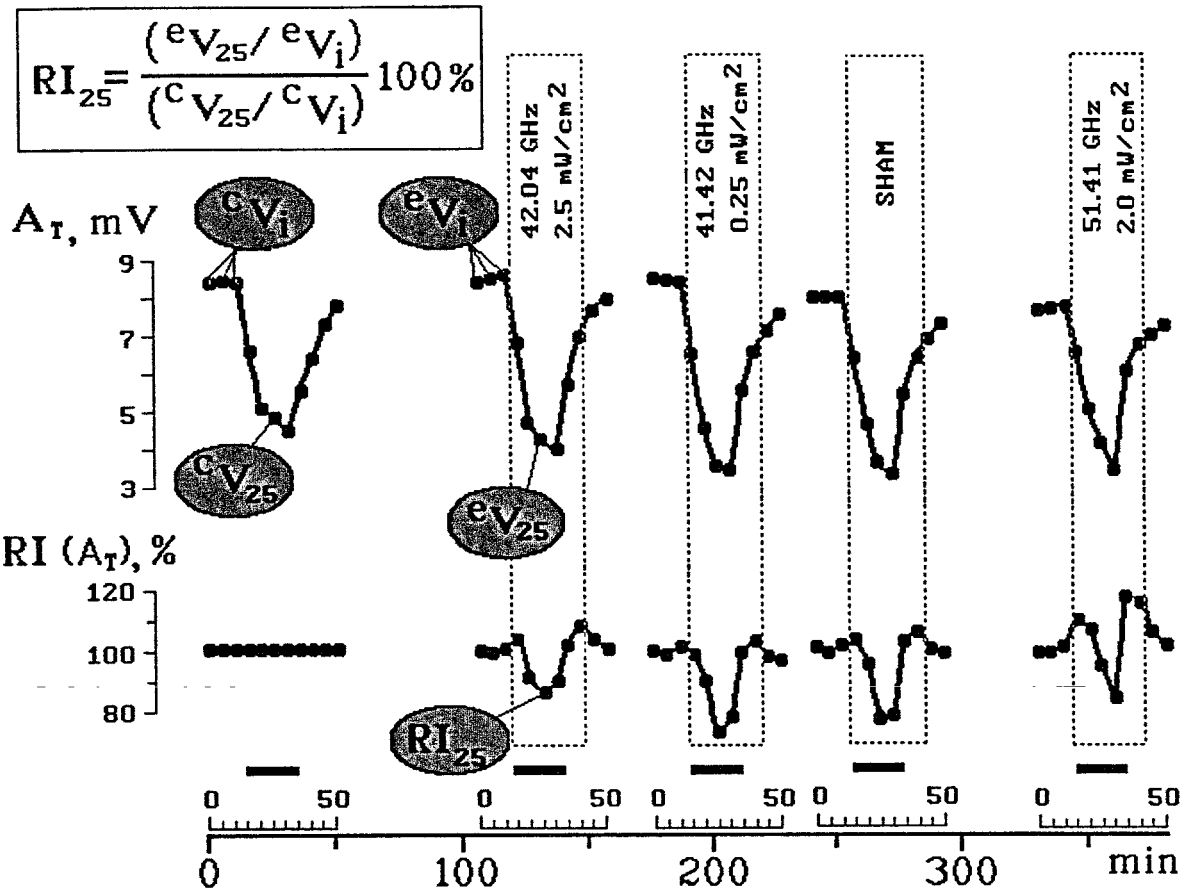

Fig. 6. Isolated nerve performance in a series of high-rate stimulation (HRS) experiments with one nerve preparation and the analysis of data. Horizontal axes: elapsed time (below) and time scales for each HRS experiment. Periods of the HRS (20 paired pulses/s) are shown by horizontal bars; otherwise, the rate was 4 paired pulses/s. Dotted line boxes indicate periods of microwave and sham exposure. Vertical axes: amplitude of the test compound action potential (A_t, mV), and its relative index $RI(A_t)$, in percent. The *RI* is used to assess quantitatively the individual nerve's HRS tolerance in each of the HRS experiments. For example, for a 25-min datapoint of the second experiment in the series, the relative index RI_{25} is calculated by the equation at the top of the graph. ^cV_i is the initial A_t value in the first experiment in a series (average of 3 datapoints before the HRS); $^cV_{25}$ is the A_t value on the 25th min of the experiment. ^eV_i and $^eV_{25}$ are the same values in the next experiment with exposure. Similarly, *RI* is calculated for every datapoint in all subsequent experiments with this nerve preparation. Note that *RI* deviation from 100% in these experiments does not necessarily indicate an effect of irradiation, it could come up as an aftereffect of the previous HRS trains, spontaneous changes in the nerve state, etc.

of the previous HRS, an effect of MMW, chance variations, or an impact of other uncontrolled factors. To identify the effect of MMW, if any, we randomized the sequence of exposures and sham exposures and averaged the data from the experiments with different nerve preparations. The statistical significance of the MMW-induced changes was estimated in comparison with the sham-treatment data by a two-tailed Student's *t* test. All the same data processing applied to the parallel control experiments.

A total of 78 experiments on 26 nerve preparations were carried out. For the initial analysis, the data for experiments with the high (2–2.7 mW/cm^2) and low (0.24–0.28 mW/cm^2) incident power densities were pooled together, regardless of the frequency of the radiation. This analysis established that the changes in CAP conduction reached maximum at 30 min into the experiment; that was the last datapoint during the HRS train. The average relative index values for this datapoint are provided in the Table 2. Irradiation at either field intensity induced no statistically significant changes in CAP parameters ($P > .05$), except for the

increase in the relative index of the test CAP amplitude (A_t, $P < .02$). The difference in this parameter between the sham-exposed group and both the exposed groups was the same (about 17%), despite the 10-fold difference in the field intensity. It is important to note that no statistically significant differences were found between the respective parallel control groups.

On the whole, the results indicated that MMW irradiation could attenuate the A_t decrease during the HRS train. The equivalence of magnitudes of this effect in the two MMW-exposed groups inferred that an irradiation parameter other than intensity could be essential for producing this effect. Hence, we analyzed the data separately for each frequency of the radiation.

The mean increase of the A_t relative index by the end of the HRS train ranged from about 6 to 22% for different radiation frequencies (as compared with the sham exposure data). Only minimal changes of 6 to 9% (nonsignificant) were observed under exposure with 42.04 and 41.42 GHz MMW at 2.4–2.7 mW/cm^2, even though the microwave heating was 0.3–0.4 °C. Irradiation at the same incident power, but at a different frequency of 45.9 GHz, caused a 21% increase of this index ($P < .05$). The other tested frequencies produced a statistically significant effect of the similar magnitude (13–22%, $P < .05$), despite reduction of the incident power density to 2 mW/cm^2 (51.41 GHz) and even to 0.24–0.28 mW/cm^2 (41.22 and 50.91 GHz, see Fig. 7).

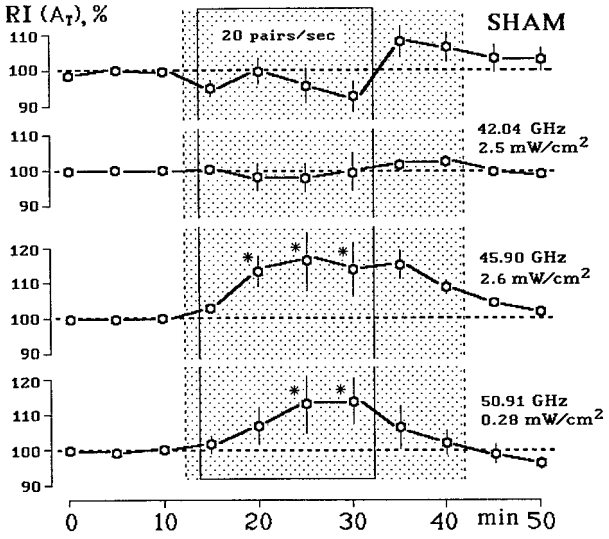

Fig. 7. Effect of millimeter waves on the nerve tolerance to high-rate stimulation. The vertical axes show the relative index of the test CAP amplitude (mean ± SE, five to seven independent experiments in each group). See text and Fig. 6 for the method of the relative index calculation. Periods of high-rate stimulation (20 paired pulses/s) and irradiation are shown by a box and dotted areas, respectively. Radiation frequency and incident power density are given to the right of the graphs. Asterisks indicate significant difference from the sham exposure data ($P < .05$).

DISCUSSION

Our experiments failed to confirm observations of severe MMW-induced alterations of CAP conduction, such as reported by other authors [Burachas and Mascoliunas, 1989; Chernyakov et al., 1989]. For the low-rate electrical stimulation of the nerve, MMW irradiation either did not cause any effect or, at high enough power levels, produced effects that were exactly the same as those produced by the equivalent conventional heating. One should note, however, that irradiation and other experimental conditions used in these two studies were substantially different from those in our work.

Another observed MMW effect, namely the increase of the A_t relative index by the end of the HRS train, deserves a more detailed analysis. This effect testifies to an attenuation of the HRS-induced A_t suppression or, in other words, to an ability of MMW to facilitate nerve recovery after conduction of a spike. This result fits well with the recent findings of Sazonov and Rizshkova [1995] that MMW exposure of the isolated frog sciatic nerve significantly decreases the time needed for CAP restoration after a high-rate (1 kHz) electrical stimulation train. According to our present data, this effect depends on the frequency of the radiation, but has almost the same magnitude at 10-fold different field intensities. These results suggest that the mechanism of the MMW action was different from general heating of the preparation, unless one assumes that at the same IPD level MMW absorption and heating at some frequencies could be more than tenfold greater than at others. This assumption does not seem feasible and also contradicts the results of the LRS experiments: Reverse calculation of the preparation heating from the nerve function changes (''use of the nerve as a thermometer'') revealed no indications of extremely increased or decreased MMW absorption at any particular frequencies.

Instead of general MMW heating, the formation of so-called ''hot spots'' could be a more reasonable explanation for the effect. The spatial distribution and intensity of the local field maxima are very much dependent on the frequency of the radiation [Khizhnyak and Ziskin, 1994], so frequency-specific bioeffects could be produced by different patterns of ''hot spots.'' One should also take into account the possibility of frequency-specific nonthermal interactions, which are often claimed to be characteristic for the MMW band [Chernyakov et al., 1989; Kataev et al., 1993; Rebrova, 1992; and others].

At this point, we consider it more important to

make sure that the observed frequency-specific effect of MMW on nerve function is real, rather than to speculate about underlying physical or physiological mechanisms. Our experimental procedures were organized so as to collect more physiological data and test more exposure regimens while using fewer animals and trying to rule out the possible impact of any factors other than microwaves. However, this made the statistical interpretation of the data very complicated. The experimental procedures had several features that influence the statistical confidence that can be placed in the findings: (1) Several treatment groups were compared with one sham-exposed group. (2) Both MMW and sham exposure experiments were supplemented with respective parallel controls, and there were no considerable differences between the parallel control groups. (3) A_t was not the only parameter measured, and we did not know *a priori* which parameter will reveal an effect. (4) The same effect occurred not just in an isolated timepoint, but in several adjacent and functionally related timepoints of the experiment. Conditions 1 and 3 decrease the statistical confidence that the effect was real, whereas conditions 2 and 4 increase it.

There are established techniques that can address some of these conditions separately (such as the Dunnet's test [1955] to compare several treatments with one control; see Winer [1971] for review), but no known method can quantitatively assess the cooperative impact of all these factors together. At the present time, the reality of this MMW effect cannot be addressed by statistical analysis, no matter whether we use the *t* test or more sophisticated procedures. The confidence levels reported in this paper should be regarded as an estimation only and are not necessarily correct. Instead of statistical manipulations, a solid proof of the effect can only be made by its reproduction in independent experiments.

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Appendix: Calculation of On–Axis Power Density for Horn Antennas

Methods for calibration of field strength-measuring probes/devices include free–space standard field methods, guided wave methods, and transfer probe methods. For calibrating field strength measuring probes/devices in the millimeter wave range, guided wave and transfer probe methods are inappropriate. The dimensions of transmission lines become very small, and dipoles in the transfer probe method become increasingly difficult to fabricate due to the small wavelengths involved. In free–space standard field methods, standard gain horns are commonly used to establish highly accurate electromagnetic field intensities in the frequency range above 1 GHz. We have used horn antennas in the frequency range 37 to 53 GHz and 53 to 78 GHz to generate standard field intensity at various distances from the horn antenna.

The on–axis incident power density (IPD) at an on–axis field point in free–space is computed using the equation

$$S = P_T G/4\pi r^2 \quad (1)$$

where S is the power density (W/m^2), P_T is the net input power into the horn (W), G is the absolute numerical power gain of the horn antenna, and r is the distance from the horn antenna aperture to the on–axis field point (m).

On–axis gain of the horn antenna is calculated from equations given by Larsen [1978] as

$$G(dB) = 10 \log(AB) + 10.08 - R_H(dB) - R_E(dB) \quad (2)$$

where A is the wavelength normalized width of the horn aperture, B is the wavelength normalized height of the horn aperture, R_H is the gain reduction factor due to the H–plane flare of the horn, and R_E is the gain reduction factor due to the E–plane flare of the horn. The gain reduction factors are given by

$$R_H(dB) = (0.01\alpha)(1 + 10.19\alpha + 0.51\alpha^2 - 0.097\alpha^3) \quad (3)$$

$$R_E(dB) = (0.1\beta^2)(2.31 + 0.053\beta) \quad (4)$$

$$\alpha = A^2(1/L_H + 1/r) \quad (5)$$

$$\beta = B^2(1/L_E + 1/r) \quad (6)$$

where L_E and L_H are wavelength normalized values of the slant lengths and r is the wavelength normalized distance from the aperture of the horn to the on–axis field point.

For given horn dimensions, on–axis gain was calculated at each frequency of interest at distances from 40 to 500 mm. The net input power to the horn is determined from forward and reflected power measurements at the feed to the horn using a bidirectional coupler, and the incident power density S is then calculated using the gain computed from equations (2)–(6). All calculations for S are expressed in mW/cm^2 per mW of net input power to the horn.