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Millimeter Waves Thermally Alter the Firing Rate of the *Lymnaea* Pacemaker Neuron

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The effects of millimeter waves (mm-waves, 75 GHz) and temperature elevation on the firing rate of the BP-4 pacemaker neuron of the pond snail *Lymnaea stagnalis* were studied by using microelectrode techniques. The open end of a rectangular waveguide covered with a thin Teflon film served as a radiator. Specific absorption rates (SARs), measured in physiological solution at the radiator outlet, ranged from 600 to 4200 W/kg, causing temperature rises from 0.3 to 2.2 °C, respectively. Irradiation at an SAR of 4200 W/kg caused a biphasic change in the firing rate, i.e., a transient decrease in the firing rate (69 ± 22% below control) followed by a gradual increase to a new level that was 68 ± 21% above control. The biphasic changes in the firing rate were reproduced by heating under the condition that the magnitude (2 °C) and the rate of temperature rise (0.96 °C/s) were equal to those produced by the irradiation (for an SAR of 4030 W/kg). The addition of 0.05 mM of ouabain caused the disappearance of transient responses of the neuron to the irradiation. It was shown that the rate of temperature rise played an important role in the development of a transient neuronal response. The threshold stimulus for a transient response of the BP-4 neuron found in warming experiments was a temperature rise of 0.0025 °C/s. *Bioelectromagnetics 18:89–98, 1997.*

Key words: millimeter waves; mollusc neuron; pacemaker activity

INTRODUCTION

It is well known that millimeter waves (mm-waves) cannot penetrate deeply into biological objects because they are almost totally absorbed within 1 mm in the superficial layers of the skin (Gandhi, 1983; Furia et al., 1986). Therefore, the primary effect of mm-waves is limited to structures located within the skin, such as sensory receptors, nerve endings, and immunocompetent cells.

Some neurons and specialized nerve endings, including thermoreceptors, show spontaneous activity [Iggo, 1962; Pierau et al., 1975]. To study the mechanisms of the effects of mm-waves on receptors and nerve endings, we selected pacemaker neurons of the mollusc *Lymnaea stagnalis*, generating a steady rate of pacemaker activity under normal physiological conditions [Jerelova, 1971; Jerelova et al., 1971; Bolshakov and Alekseev, 1992]. The effect of microwaves on molluscan neurons has been reported in a number of papers [Wachtel et al., 1975; Seaman and Wachtel, 1978; Arber and Lin, 1985a,b; Arber et al., 1985; Bolshakov and Alekseev, 1987, 1992].

Temperature responses of some pacemaker neurons of molluscs are similar to the responses of human thermoreceptors [Carpenter, 1981; Carpenter and Alving, 1968]. Based on theory [Barnes, 1984] and on experimental studies [Bolshakov and Alekseev, 1986; Wachtel et al., 1982], the rate of temperature rise can play an important role in the response of excitable cells to heating. Therefore, when evaluating the thermal effect of mm-waves, it is important to take into account not only the increment but also the rate of temperature rise.

The aim of the present investigation was to examine the effect of mm-waves on the firing rate of a pacemaker neuron. It was found that heating produced by mm-wave irradiation strongly affects the firing rate.

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MATERIALS AND METHODS

Preparation of Neurons

Experiments were conducted by using the BP-4 pacemaker neuron of the large parietal ganglion of the mollusc *Lymnaea stagnalis* [Jerelova, 1971; Jerelova et al., 1971]. The large parietal ganglion contains only one BP-4 neuron. This neuron is recognizable by its size (about 200 μm in diameter) and shape (surrounded by small cells). After the surrounding connective tissue was removed, the isolated nerve ring of the mollusc was attached to the wax base of a chamber with cactus needles. The chamber, which was made of Plexiglass, had an effective volume of about 2 ml and was constantly perfused with physiological solution (Fig. 1). The physiological solution contained 80 mM NaCl, 1.6 mM KCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$, and 2 mM Tris, pH 7.5. In some studies, ouabain (Sigma Chemical Co., St. Louis, MO) was used for inhibition of the electrogenic Na-K exchange pump (sodium pump) of the neuron; it was dissolved in the physiological solution at a concentration of 0.05 mM.

Firing Rate Measurements

The membrane potential of the neuron was recorded by using a conventional microelectrode technique [Purves, 1981]. The intracellular glass microelectrode was inserted into the cell body of the BP-4 neuron through the intact ganglion sheath. All electrodes were filled with 2 M KCl and had resistances of 5–10 MΩ. The glass microelectrode was connected electrically by an Ag-AgCl electrode to the input of a TEV-200 preamplifier (Dagan Corp.). The output signal was displayed on an oscilloscope and a chart recorder and was simultaneously recorded by a tape recorder for further off-line computer analysis.

The firing rate was determined by counting the number of spikes within 1 min time intervals. The initial firing rates of the neurons varied between 15 and 50 spikes per min from cell to cell. In each individual experiment, the effect of mm-waves was determined as the change in the firing rate relative to control level in percent. The control firing rate was taken to be the average value during the 10 min interval immediately prior to the irradiation. In the sham experiments, we checked the changes in the firing rate for periods of 1.0–1.5 h, under the same conditions as for the exposure experiments, except that the output power was turned off. In these experiments, the firing rate was stable for 1 h and then slowly decreased at the rate of 3–4% every 10 min. Results are presented as mean ± standard error of the mean (S.E.M.); n indicates the number of neurons.

In total, 39 BP-4 neurons were included in our study of mm-wave effects (Table 1). We used 6 neurons in the sham experiments, 14 neurons in short-term (about 2 min) exposure experiments, 14 neurons in long-term (12, 14, and 22 min) exposure experiments, and 5 neurons in experiments with ouabain. Short-term exposures were used for two types of studies: 1) study of the dependence of a transient response of the neuron on specific absorption rate (SAR; n = 9) and 2) study of the dependence on the distance from the radiator (n = 5). In the first type of experiment, the neuron was irradiated once at each of three different SARs, beginning with the lowest and finishing with the highest level (Table 1). In the second type of experiment, each

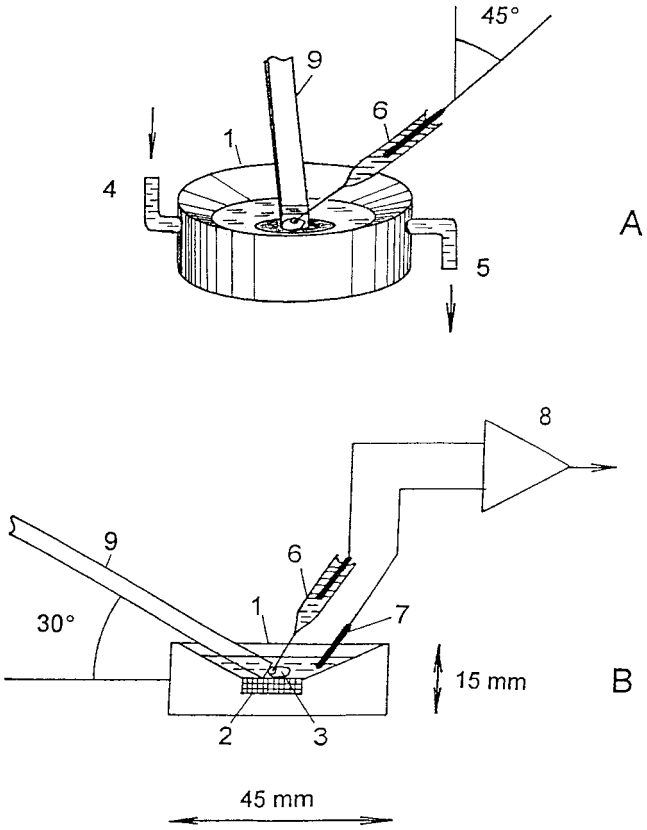

Fig. 1. Experimental arrangement for exposure of a neuronal preparation to millimeter waves (mm-waves) while recording its spontaneous activity by using a glass microelectrode. **A:** Frontal view. **B:** Cross section at right angle to the plane of the page. The bottom of a chamber (1) was covered with wax (2) to attach a neuronal preparation (3). Inlet (4) and outlet (5) are for the physiological bathing solution. Membrane potential was recorded between a glass microelectrode (6) inserted into the neuron body and an Ag-AgCl reference electrode (7) outside the ganglion by using an amplifier (8). The opening of a waveguide (9) covered by a thin Teflon film was used as a radiator. Following insertion of the microelectrode into the BP-4 cell, the radiator was placed into the solution at fixed distances from the cell by using a micromanipulator.

neuron was irradiated once at each of three distances from the radiator (see below). In both cases, the periods between exposures were 10 min. Each neuron was used only once in an experiment with long-term exposure (n = 14). In these experiments, neurons were irradiated at an SAR of 3,150 W/kg (12 min, n = 3; 22 min, n = 2) or at 4200 W/kg (12 min, n = 2; 14 min, n = 7). For statistical analysis of the transient response, we used the data from both short- and long-term exposures at the same SAR (Table 1, Σn).

An additional 29 neurons were used in experiments with warming. Eighteen of these were used to determine the threshold sensitivities of the neurons, three were used to study the steady-state firing rate, and eight were used to study transient responses of the neurons to warming.

mm-Wave Exposure

The cells were irradiated with continuous mm-waves at a frequency of 75 GHz. The Russian-made mm-wave generator, G4-142 (53–78 GHz), was the irradiating source. The open end of a rectangular waveguide, which was covered with a thin waterproof Teflon film, served as a radiator. This waveguide was coupled to the generator output through a flexible waveguide section, allowing for changes in the position of the open end. Using a micromanipulator, the rectangular waveguide was positioned so that the neuron studied was located at the center of the waveguide opening in contact with the Teflon film (Fig. 1). For studying the dependence of the firing rate on the distance of the neuron from the radiator, the neuron was placed along the beam axis at 0.0, 0.5, and 1.0 mm from the waveguide opening. The waveguide opening had a 3.6 × 1.8 mm internal cross section.

The output power was measured with the Russian-made power meter, M3-75. The power was monitored by using a directional coupler connected to the generator output. The maximal power of 12.6 mW could be reduced (by about 40 dB) by using the attenuator built into the generator. The angle between the waveguide and the chamber bottom was 30°. The tip of the electrode was positioned at a 45° angle to the E-vector of the mm-wave field (Fig. 1A). The radiator was placed into the position described above after the electrode was inserted into the cell.

Determination of the SAR

The amount of mm-wave energy absorbed was evaluated by measuring SAR in the solution closest to the waveguide outlet [Alekseev and Ziskin, 1995]. The temperature was measured by using a copper-constantan thermocouple of the IT-23 type (Sensortek Inc.) with an accuracy of ±0.1 °C. The diameter of the sens-

TABLE 1. The Numbers of Neurons Used in Sham and mm-Wave Exposure Experiments

SAR, W/kg	Sham (n)	Experiments With Distance				Long-term Exposure		Σn_i	Ouabain+ Exposure (n)
		Short-term Exposure							
		N	n	N	n	N	n		
0	6	—	—	—	—	—	—	—	—
600	—	n_1, n_2, n_3, n_4, n_5, n_7	6	—	—	—	—	6	—
1550	—	n_1, n_2, n_4, n_6, n_7	5	—	—	—	—	5	—
2800	—	n_1, n_2, n_3, n_6, n_8, n_9	6	—	—	—	—	6	—
3150	—	n_5, n_8, n_9	3	—	—	n_{15}-n_{19}	5	8	—
4200	—	n_3-n_9	7	n_{10}-n_{14}	5	n_{20}-n_{28}	9	21	5

n is the total number of neurons used; N indicated the specific neurons n_i. Data from Σn_i neurons was used for statistical analysis of the transient response.

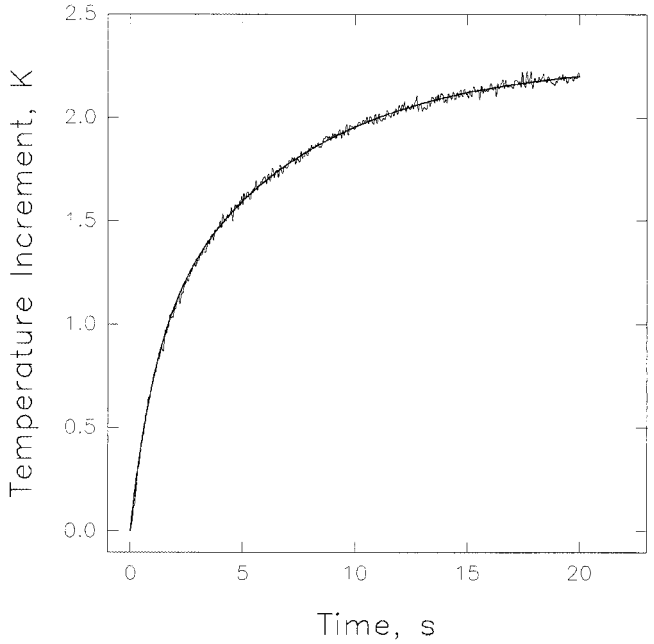

Fig. 2. Temperature elevation kinetic produced by mm-wave exposure at incident power of 12.6 mW in the physiological solution. The superimposed trace is a fit using Equation 1 with $C_1 = 0.38$, $C_2 = 0.62$, $\tau_1 = 1.05\ s^{-1}$, $\tau_2 = 6.67\ s^{-1}$, and $\Delta T°(\infty) = 2.3$ °C. [$\Delta T°(20) = 2.2$ °C].

ing element of the thermocouple was 0.1 mm, and the time constant was 0.005 s. The thermocouple was placed orthogonal to the E-vector in the center of and immediately next to the film covering the waveguide opening in the absence of the neuron.

The influence of the thermocouple on the temperature measurements in the liquid sample exposed to 75 GHz was studied earlier by using an infrared technique [Alekseev et al., 1991]. It was shown that, at a similar location of the thermocouple in the waveguide opening, SARs determined by the thermocouple and by infrared camera were within ±10%. At thermocouple distances from the waveguide of 0.5 and 1.0 mm, there were delays in the temperature rise rate (about 1 s at 1 mm) that prevented accurate determination of the initial temperature rise rate. Therefore, SAR determinations were performed only when the thermocouple was located at the surface of the film covering the waveguide opening.

Temperature changes were recorded by using a 12-bit analog/digital converter (CIO-AD16; Computer Boards, Inc.) at a sampling interval of 50 ms. To avoid the subjectivity of a visual determination of the initial temperature rise rate from the heating kinetics, we developed a different approach. First, we found a function closely fitting the heating kinetics. Then, the initial temperature rise rate was calculated by using the derivative of this function.

TABLE 2. Temperature Increments at the End of 20 s Exposures ($\Delta T°$ (20)), Initial Rates of Temperature Rise ($dT°/dt$) and SARs in the Physiological Solution Corresponding to Different Values of Output Powers (P) of the Generator at 75 GHz. The Thermocouple was Placed in Contact to the Film Covering the Waveguide Opening (for Details see Method). *m* is a Number of Experiments. Results are mean ± SEM

P, mW	$\Delta T°$ (20), K	dT°/dt, K/s	SAR, W/kg	*m*
1.8	0.32 ± 0.04	0.14 ± 0.03	600 ± 114	4
4.6	0.82 ± 0.04	0.37 ± 0.05	1550 ± 232	4
9.0	1.46 ± 0.05	0.67 ± 0.09	2800 ± 392	5
9.7	1.68 ± 0.07	0.75 ± 0.11	3150 ± 472	5
12.6	2.22 ± 0.08	1.00 ± 0.14	4200 ± 588	5

The heating kinetics were described as a function of two exponentials:

$$\Delta T°(t) = \Delta T°(\infty)[1 - C_1\exp(-t/\tau_1) - C_2\exp(-t/\tau_2)], \quad (1)$$

where $\Delta T°(\infty)$ is the steady-state temperature rise, τ_1 and τ_2 are time constants, and C_1 and C_2 are preexponential coefficients. Fitting was done by using the least-squares technique [Guest, 1961]. An example of the fit is shown in Figure 2. The heating kinetics were analyzed for a 20 s time span. The value of $\Delta T°(\infty)$ was determined by setting it equal to k $\Delta T°(20)$, where $\Delta T°(20)$ is the measured temperature elevation at 20 s, and k is a constant given by $1/[1 - C_1 \exp(-20/\tau_1) - C_2 \exp(-20/\tau_2)]$. In total, temperature rises were recorded in 23 trials using five different output power levels (Table 2). Standard error of the estimates for the fits between calculated values and observed temperatures (400 samples for 20 s) ranged from 0.015 to 0.065 °C.

We found that C_1, C_2, τ_1, and τ_2 were independent of the incident power. The values of these parameters (mean ± S.E.M. from 23 experiments) were $C_1 = 0.36 \pm 0.05$, $C_2 = 0.64 \pm 0.04$, $\tau_1 = 1.06 \pm 0.12$ s, and $\tau_2 = 6.73 \pm 0.64$ s.

$$SAR = C\ dT°(t)/dt|_{t=0}, \quad (2)$$

where C is the heat capacity equal to 4200 J/(kg·K) for the physiological solution, and $dT°(t)/dt|_{t=0}$ is the initial rate of the temperature rise [IEEE, 1992]. The initial rate of the temperature rise was determined by differentiating Equation 1 and evaluating the derivative at t = 0:

$$dT°(t)/dt|_{t=0} = k\ \Delta T°(20)(C_1/\tau_1 + C_2/\tau_2). \quad (3)$$

Thus, under these conditions, SAR is proportional to $\Delta T°(20)$, which depends on the power and frequency of the irradiating field. The temperature rise rates and the SARs calculated from Equations 2 and 3 are summarized in Table 2. The different SARs used in our experiments (see Tables 1 and 2) were obtained by changing the source power.

In theoretical studies, Foster et al. [1978] developed the equations determining the temperature elevation resulting when microwaves impinge on layers of tissue. Interestingly, they showed that the time constant for the temperature elevation does not depend on the incident power density but is proportional to the square of the penetration depth. Furia et al. [1986] estimated a skin depth of 0.231 mm at 300 GHz, rising up to 0.782 mm at 30 GHz. The mean value of the shorter time constant in our experiments using 75 GHz irradiation was 1.06 s. According to the theory developed by Foster et al. [1978], this value of the time constant would be the result if the penetration depth was equal to 0.39 mm, a value that is within the range reported by Furia et al. [1986].

Temperature Regulation

The measurements of the firing rate during irradiation were conducted at room temperature (18–20 °C) without temperature control of the experimental chamber. For studying the thermal reactions of the neuron, the temperature was increased by letting the physiological solution flow through a glass heat exchanger, with circulating water heated to a specific temperature. The thermocouple in these experiments was placed within 1 mm of the neuron studied. The necessary temperature rise rate was achieved by carefully controlling the temperature and flow rate of the heated solution through the chamber. The flow rates were varied in the range of 0.5–2 ml/min. In a study of the threshold sensitivities of the neuron to heating, the temperature was increased by 0.3 °C at the rates of 0.0015 (n = 4), 0.0025 (n = 6), 0.04 (n = 5), and 0.07 (n = 3) °C/s. In the experiments modeling thermal effects of mm-waves, the temperature increased by 2 °C, with a temperature rise rate of 0.96 °C/s (n = 8). At a constant temperature, the flow rates used did not produce noticeable changes in the firing rates of neurons.

RESULTS

Effects of mm-Waves

Millimiter-wave exposure caused biphasic changes in the firing rate of the BP-4 neuron. After irradiation was turned on, the firing rate first decreased and then increased to a new steady-state level.

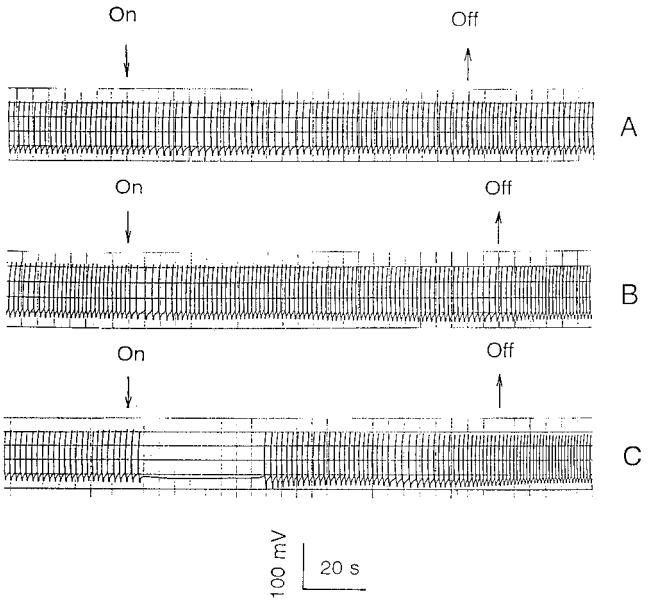

Fig. 3. Effect of mm-wave exposure on the firing rate in a BP-4 neuron at three different specific absorption rate (SAR) values: 600, (B) 1550, and (C) 2800 W/kg. The interval between the (A) arrows denotes the period of exposure (approximately 2 min).

Figure 3 illustrates the appearance of a transient decrease in the firing rate during exposure to mm-waves for short periods (about 2 min) at different output powers. Similar results were obtained with other neurons (n = 9). The decrease in the firing rate developed within the first 10 s of irradiation. With increasing SAR from 600 to 2,800 W/kg, the transient decrease in the firing rate became more pronounced.

The dependence of the transient decrease of the firing rate on SAR is shown in Figure 4. Exposures with SAR values of 4200 W/kg, on average, caused a transient inhibition of the firing rate of BP-4 neurons during the second minute by 69 ± 22% (n = 23). In two neurons, short-term exposures at high levels of SAR (4200 W/kg) stopped pacemaker spike generation completely, and it did not recover. The results from these neurons were also included in the analysis of the statistics. They produced an increase in S.E.M. from 20 to 22%. For calculation of the average, we used the results of the short-term exposures for 14 neurons and the results of the long-term exposures for 9 neurons.

During long-term exposures (12–22 min), a marked increase in firing rate, which is the second phase of the neural response, can be observed (Fig. 5). This phase is slower than the transient decrease. After an initial decrease, the firing rate at an SAR of 4200 W/kg regained its control rate after 3–5 min of irradia-tion and, 6–8 min later, became stable at a level exceeding the control rate by 68 ± 21% (n = 9). The rising phase developed

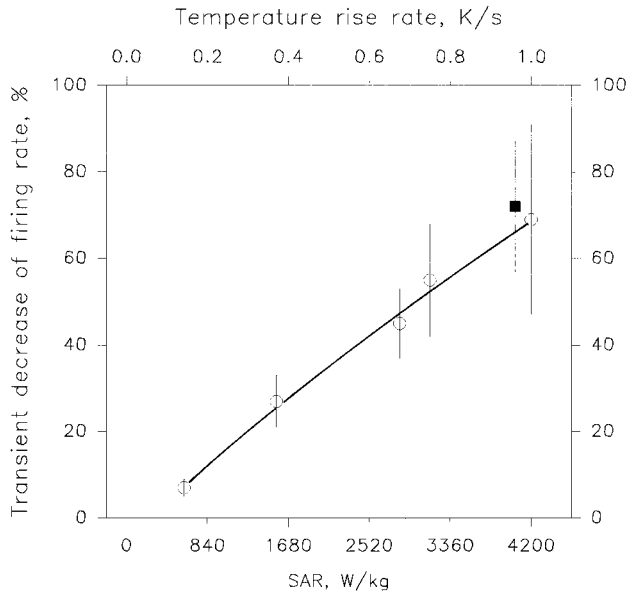

Fig. 4. Dependence of the transient decrease of the firing rate on SAR and initial temperature rise rate resulting from mm-wave irradiation (circles) and warming (square). The changes in transient response are given as percent of the control values. Data points are means, and error bars are standard deviations. The line was drawn by hand.

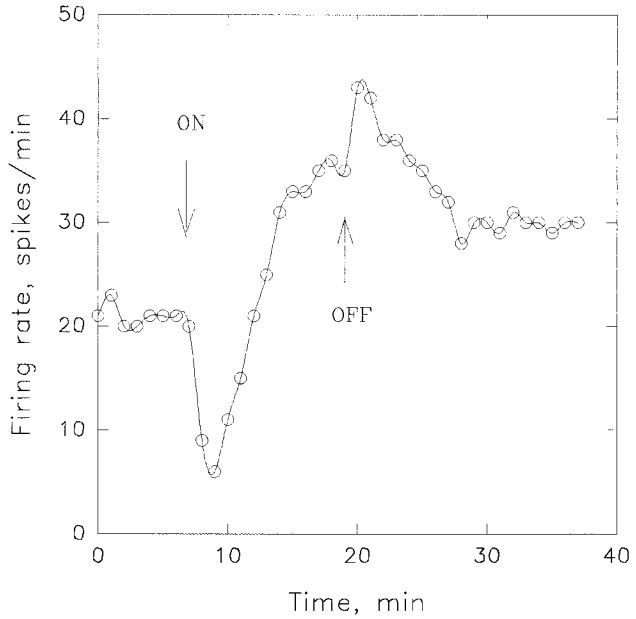

Fig. 5. An example of changes of the firing rate in a BP-4 neuron during and after the irradiation with mm-waves at an SAR of 3150 W/kg. The arrows indicate the beginning and cessation of exposure. The data points are single values from one neuron.

during long-term irradiation (14 min) was well fitted by an exponential with a time constant $\tau = 3.7 \pm 1.9$ min ($n = 7$). The results from the first 2 min of the long-term exposures were the same as the results of the short-term exposures.

When the long-term irradiation was turned off, biphasic changes in the firing rate were observed. Immediately after the irradiation was turned off, a transient increase of the firing rate was recorded. This was followed by a slow decrease of the firing rate to its control level or to a level higher than the control. The magnitude of the postexposure transient increase, compared with the rate just before the cessation of 14 min irradiation at an SAR of 4200 W/kg, was $39 \pm 16\%$ ($n = 7$), which was less than that of the transient decrease after switching on the irradiation. Nevertheless, the more the firing rate decreased at the beginning of the irradiation, the more it increased at the end of irradiation. The correlation coefficient between onset decrease and offset increase (at 14 min) in the firing rate was 0.92 ($n = 7$). The postexposure transient increase was seen in both short- and long-term exposures.

The transient decrease in the firing rate was always accompanied by a hyperpolarization of the neuronal membrane by several millivolts. A possible explanation for the hyperpolarization of the membrane and subsequent decrease in firing rate is an increase in the activity of the sodium pump. We attempted to evaluate this possibility by applying ouabain to the ganglia. Ouabain is a relatively specific inhibitor for sodium pumps in mollusc neurons and in other preparations [Carpenter, 1981; Carpenter and Alving, 1968; Pierau et al., 1975].

Ten minutes after the addition of 0.05 mM ouabain to the external solution, the spontaneous firing rate almost doubled. Following treatment with ouabain, the response to mm-wave irradiation also changed significantly (Fig. 6). At an SAR of 4200 W/kg, the magnitude of the transient response during the second minute of irradiation decreased from $69 \pm 22\%$ ($n = 23$) in the absence of ouabain to $9 \pm 4\%$ ($n = 5$) in the presence of ouabain. This result supports the hypothesis that the sodium pump is responsible for the transient decrease of the firing rate during mm-wave irradiation.

Changes in the Firing Rate on Warming

To evaluate the contribution of the temperature rise during irradiation in producing the effect, we compared the changes in the firing rate on warming produced by mm-wave irradiation and simple heating. For experiments in which the steady-state firing rate was measured as a function of temperature, we used the method developed by Willis et al. [1974]. The temperature of the external solution was increased from 18 to 28 °C in 1 or 2° increments. At every new temperature step, the cell adapted itself to the new environment

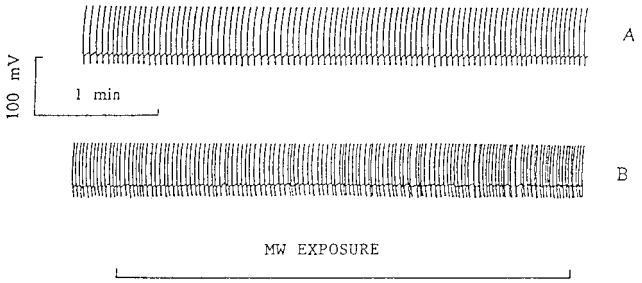

Fig. 6. Effect of mm-waves (SAR = 4200 W/kg) on the firing rate in the presence of ouabain. **A:** The firing rate pattern of the BP-4 neuron without ouabain before irradiation. **B:** The firing rate pattern of the same neuron in the presence of 0.05 mM ouabain and mm-wave influence. The horizontal bar indicates the duration of mm-wave irradiation for B.

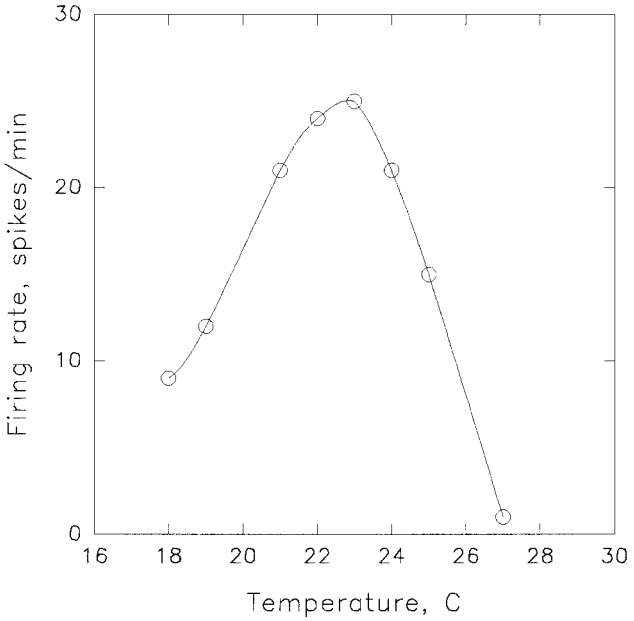

Fig. 7. Temperature dependence of the steady-state firing rate in the BP-4 neuron. All data points are single values from a single neuron.

during 10 min, after which the firing rate was recorded. Figure 7 shows the typical firing rate in one of the three neurons studied under these conditions. While the temperature increased from 18 to 23 °C, the number of spikes increased by about 40%/K until the firing rate reached a maximum level. At higher temperatures, the firing rate dropped off, and the pacemaker activity stopped.

On heating, the firing rate showed transient inhibition that was largely dependant on the rate of temperature rise. We modelled the mm-wave effect on the firing rate simply by heating the external solution by 2.0 °C at an initial rate of 0.96°/s, which corresponds to an SAR of 4030 W/kg. The transient decrease in the firing rate of neurons in these experiments was 72 ± 15% (n = 8), which is within the range of changes in the firing rate during irradiation with a similar SAR (Fig. 4). The steady-state level of increase in the firing rate was achieved in 10 min and was equal to 60 ± 20% (n = 8). Thus, the effect of mm-waves is qualitatively and quantitatively equivalent to simple warming of the neuron. Therefore, the thermal mechanism seems to be the main cause of the effects of mm-waves on the firing rate of the BP-4 neuron.

In a study of the threshold sensitivities of the neuron to temperature changes, the minimal temperature rise rate at which a decrease in the firing rate by about 3% could be recorded was 0.0025 °C/s (n = 6). The difference between control and experimental data was statistically significant at $P < 0.05$.

Dependence of the Effect on Distance From the Waveguide Opening

Alekseev and Ziskin [1995] found that, in mm-wave irradiation of physiological solution, the temperature distribution profile along the beam axis did not match that of power density or SAR. With distance, power density dropped much faster than temperature. This result could be explained as follows: The initial heating at different depths in the solution by mm-waves is proportional to the local power density at these depths. But, due to the high thermal conductivity of the solution, heat is transferred from the superficial layer (0.2–0.4 mm) to the interior, producing a temperature rise in the deeper regions.

Next, we wished to ascertain the relative contributions of the thermal and nonthermal components responsible for the changes in the neuronal firing rate. This was accomplished by noting the firing rate of a neuron at different distances from the waveguide opening. Because the temperature and power density distributions are different, an effect based on a thermal mechanism should show a diminishing response consistent with the temperature drop-off, whereas a nonthermal effect should show a diminishing pattern consistent with power density drop-off.

In this experiment, we measured the changes in the firing rate and temperature after 1 min irradiation at an incident power of 12.6 mW. This exposure produced an SAR of 4200 W/kg in the physiological solution at 0 mm from the waveguide outlet. The dependence of the power density (P) on distance (x) was calculated by using the Lambert-Beer equation [Furia et al., 1986]:

$$P = P_0 \exp(-2x/\Delta), \quad (4)$$

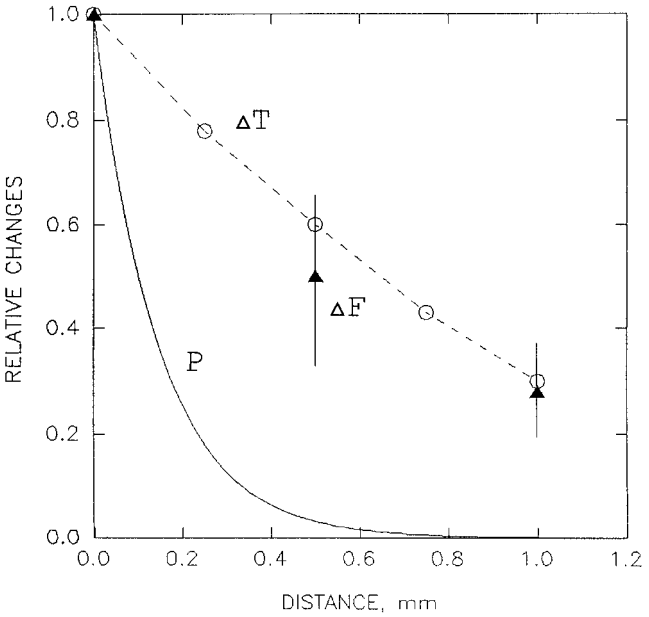

Fig. 8. Power density (P) and changes in the firing rate (ΔF) and in the temperature (ΔT) after a 1 min exposure at 75 GHz and an SAR of 4200 W/kg as a function of distance from the waveguide outlet. The three data points for ΔF (triangles) fall near means of the data points for ΔT (circles). The power density (solid line) was calculated by using Equation 4 with a skin depth of 0.29 mm, which corresponds to an attenuation of 30 dB/mm. ΔF, ΔT, and P are normalized to their maximum values. The error bars are standard deviations of ΔF. Standard deviations of ΔT did not exceed ±10% (not shown).

where P_0 is the power density at x = 0, and Δ is the skin depth. Figure 8 shows the dependence of the temperature rise, change in firing rate, and power density on the distance from the waveguide outlet. The firing rate changes match the temperature profile more closely than the profile of the power density distribution. This result indicates that the response of the neuron was due to temperature elevation and not to a nonthermal response to mm-wave irradiation.

DISCUSSION

We found that the minimum temperature rise rate necessary for recording a transient inhibition of the firing rate of the snail BP-4 neuron is about 0.0025 °C/s. In humans, the threshold stimulus is 0.001 °C/s for warmth receptors and 0.004 °C/s for cold receptors [Iggo, 1962]. It is obvious that the thermal sensitivity of the BP-4 neuron is within the range of the thermal sensitivity of human thermal receptors. For this reason, coupled with the low cost and considerable convenience of using these invertebrates, snail neurons are a good model for studying the thermal effects of mm-waves on thermal receptors.

The *Lymnaea* neuron is not unique in its response to temperature changes. Other molluscs have been reported to have similar thermosensitive neurons [Carpenter, 1973a, 1981; Carpenter and Alving, 1968; Gorman and Marmor, 1970a; Marmor, 1971a; Willis et al., 1974]. It has been shown that the spontaneous firing rate of molluscan pacemaker neurons is a direct function of membrane potential [Carpenter, 1973b]. The membrane potential of excitable cells, including molluscan neurons, can be separated into two components: one that depends on electrogenic activity of the sodium pump and a second that depends on the Na-K permeability ratio (P_{Na}/P_K) [Carpenter, 1981; Gorman and Marmor, 1970a,b, 1974a,b; Marmor, 1971b; Marmor and Gorman, 1970].

The sodium pump produces a hyperpolarizing current across the membrane with increasing temperature. The component of the membrane potential that is dependent on ionic gradients and permeabilities is determined primarily by the ratio P_{Na}/P_K, according to the Goldman equation [Gorman and Marmor, 1970a; Marmor and Gorman, 1970; Moreton, 1968; Mullins and Noda, 1963]. On warming, this component produces depolarization of the membrane because of an increase of the permeability ratio P_{Na}/P_K [Gorman and Marmor, 1970a].

To explain the mechanisms underlying the changes in the firing rate of the *Lymnaea* neuron, we are developing the following model: The transient inhibition of the firing rate is most likely due to the hyperpolarization of the membrane caused by an increased activity of the sodium pump, because its inhibition by ouabain eliminates the transient response of the neuron to irradiation. Figure 3 shows that the transient response develops within the first 10 s of irradiation. It seems that the rate of hyperpolarization closely follows the temperature rise rate (Fig. 2). An increase of the permeability ratio P_{Na}/P_K due to the temperature increase during irradiation leads to the opposite effect—depolarization of the membrane.

We assume that the time course of the increase of the firing rate (τ = 3.7 min) follows the time course of depolarization. Differences in the time courses of the development of hyperpolarization and depolarization produce biphasic changes in the membrane potential and firing rate. At the exposures used (Table 2), when the temperature rise rates are much more than 0.0025 °C/s, hyperpolarization dominates over slow depolarization, giving rise to the maximal magnitudes of the transient response. At temperature rise rates close to

0.0025 °C/s, depolarization of the membrane dominates over hyperpolarization, reducing the transient response.

The dependence of the steady-state firing rate on temperature in the snail neuron (Fig. 7) is qualitatively similar to that of human thermoreceptors except for differences in the temperatures of maximum sensitivity [Ruch, 1979]. The pattern of the firing rate changes in the snail neuron may be dependent on the experimental conditions, including the starting temperature before irradiation. For example, the rate of increase in magnitude of pump current in *Aplysia* neurons is greatest at 12–16 °C [Willis et al., 1974]. Figure 7 shows that the rate of increase in the steady-state firing rate is also dependent on the temperature. Small temperature increments (1-2 °C) at 18–20 °C produce an increase in the steady-state firing rate, whereas, at temperatures higher than 23 °C, we would expect a decrease in the steady-state firing rate at the same temperature increments.

Experiments show that a temperature increase as small as several tenths of a degree is capable of causing recordable changes in the firing rate of the pacemaker neuron, provided that the temperature rise rate is equal to or greater than 0.0025 °C/s. High thermosensitivity of neurons and specialized nerve endings is related to the high cell membrane resistance and electrogenic activity of the sodium pump [Carpenter, 1973a, 1981; Carpenter and Alving, 1968; Pierau et al., 1974]. For example, mechanoreceptors also demonstrate a high thermal sensitivity due to the presence of the sodium pump [Pierau et al., 1974]. Therefore, even small temperature elevations of a degree or two could produce a notable effect on human thermoreceptors and other thermosensitive nerve endings in the skin, provided that the rate of temperature rise is sufficiently great. Such effects could occur as a result of irradiating the skin with the mm-wave therapeutic devices used in the former Soviet Union [Betsky et al., 1989].

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