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Millimeter Wave Effects on Electrical Responses of the Sural Nerve In Vivo

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Millimeter wave (MMW, 42.25 GHz)-induced changes in electrical activity of the murine sural nerve were studied in vivo using external electrode recordings. MMW were applied to the receptive field of the sural nerve in the hind paw. We found two types of responses of the sural nerve to MMW exposure. First, MMW exposure at the incident power density $\geq$45 mW/cm^2 inhibited the spontaneous electrical activity. Exposure with lower intensities (10–30 mW/cm^2) produced no detectable changes in the firing rate. Second, the nerve responded to the cessation of MMW exposure with a transient increase in the firing rate. The effect lasted 20–40 s. The threshold intensity for this effect was 160 mW/cm^2. Radiant heat exposure reproduced only the inhibitory effect of MMW but not the transient excitatory response. Depletion of mast cells by compound 48/80 eliminated the transient response of the nerve. It was suggested that the cold sensitive fibers were responsible for the inhibitory effect of MMW and radiant heat exposures. However, the receptors and mechanisms involved in inducing the transient response to MMW exposure are not clear. The hypothesis of mast cell involvement was discussed. Bioelectromagnetics 31:180–190, 2010.

Key words: murine sural nerve; spontaneous electrical activity; mast cells

INTRODUCTION

Millimeter waves (MMW) have been used in Eastern Europe for the therapeutic treatment of over fifty medical conditions including cardiovascular diseases, wound healing, pain relief, etc. [Rojavin and Ziskin, 1998]. High success rates were achieved with application of three ''therapeutic'' frequencies: 42.25, 53.37, and 61.22 GHz at incident power densities of 10–30 mW/cm^2. However, Western medicine is very skeptical toward this treatment. One of the major reasons is the absence of a scientific theory based on a known physiology that would explain how local MMW exposure of the skin produces a systemic effect.

MMW are almost totally absorbed within the superficial layers of the skin, that is, the epidermis and dermis [Alekseev et al., 2008]. Therefore, the initial effect of MMW is limited to structures located in the outer layers of the skin. The epidermis and dermis are highly innervated [Hilliges et al., 1995; Johansson, 1995] and one of the possible mechanisms of MMW action on human and animal organism may be an activation or sensitization of cutaneous nerve terminals and subsequent involvement of the nervous system in producing the systemic effects of MMW [Rojavin and Ziskin, 1998].

In a number of experiments the ability of humans to detect low intensity MMW exposure has been reported [Lebedeva, 1993, 1997]. It was speculated that MMW perception could involve some types of mechanoreceptors and nociceptors. In other articles the ability of MMW exposure to modulate cutaneous nerve activity was studied using the c-Fos protein expression method [Novikova et al., 2008a,b]. It was shown that MMW exposure of the skin activated hypothalamic cells.

MMW-induced hypoalgesia can be considered as indirect evidence of an involvement of the nervous system in mediating these effects [Rojavin et al., 2000; Radzievsky et al., 2008]. The maximal hypoalgesic effect of MMW was achieved by exposing the densely innervated skin areas of animals (nose, paw) [Radzievsky et al., 2000]. Transection of the sciatic nerve in mice eliminated the hypoalgesic effect of

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MMW induced by exposure of the hind paw [Radzievsky et al., 2001]. This indicated that the neural response to the stimulation of cutaneous nerve endings of the paw played a critical role in the MMW-induced hypoalgesic effect.

Another way to study MMW effects on nerves is to use adequate neural models. The effects of 40–52 GHz exposure on the compound action potential (CAP) conduction of an isolated frog sciatic nerve was examined by Pakhomov et al. [1997a,b] and small changes in the CAP conduction were found. However, the time needed for a nerve recovery following electrical stimulation was decreased. Enin et al. [1991] reported that MMW irradiation (55, 61, and 73 GHz) reduced sensitivity of the rat skin to mechanical stimulation. Electroreceptors in skates were also sensitive to low-intensity 37–55 GHz irradiation [Akoev et al., 1995]. MMW exposure caused biphasic changes in the firing rate of mollusc pacemaker neurons [Alekseev et al., 1997]. The transient responses of these neurons to irradiation were due to hyperpolarization of the cell membrane caused by thermal activation of the sodium pump. These results indicate that MMW irradiation, at commonly used intensities, affects the firing rate of pacemaker neurons and seems to be sufficient for activating sensory receptors and other nerve endings located in the upper layers of the skin.

The sensory neurons that innervate the skin are found in the trigeminal and dorsal root ganglia. The epidermis and dermis include specialized and free nerve endings of Aβ, Aδ, and C-fibers. Cutaneous receptors are identified according to their responses to different stimuli. They are divided into three major classes: mechanoreceptors, nociceptors, and thermoreceptors. Within each class, several different subtypes of receptors are distinguished [Kress et al., 1992; Koltzenburg et al., 1997; Cain et al., 2001]. Recently, great progress was achieved in uncovering the transduction mechanisms of sensory receptors. In most cases, stimuli directly activated specific ion channels. For example, six members of transient receptor potential ion channels (TRPV1, TRPV2, TRPV3, TRPV4, TRPM8, and TRPA1) have been found to respond to a wide range of temperatures [Dhaka et al., 2006; Lumpkin and Caterina, 2007; McKemy, 2007]. Some (TRPV1, TRPV2, TRPV4) can be activated by mechanical, chemical, and osmotic stimulations [Lee and Caterina, 2005; Dhaka et al., 2006]. These ion channels were found to be expressed in sensory neurons and could be responsible for thermal, mechanical and noxious sensations.

The available literature data on MMW neural effects and the recent progress in study of transduction mechanisms of sensory receptors provide a rational and solid basis for performing electrophysiological studies of the effects of MMW on cutaneous nerve endings. This knowledge is important for understanding the mechanisms of MMW action on cutaneous nerve endings and for determining the involvement of the peripheral nervous system in systemic MMW effects.

The aim of the present study was to find out whether MMW exposure was able to activate cutaneous sensory receptors in the murine hind paw. It was not clear, a priori, what type of receptors would be responsive to MMW exposure. Therefore, electrophysiological measurements of nerve activity in vivo were performed on the main sciatic nerve branches.

MATERIALS AND METHODS

Animals

We used male Swiss Webster mice, body weight approximately 20–30 g (Taconic Farms, Hudson, NY). The animals were housed in plastic cages in the Central Animal Facility at Temple University. The Institutional Animal Care and Use Committee of Temple University approved the experimental protocols. All experiments were conducted under anesthesia with a mixture of ketamine (95 mg/kg), xylazine (10 mg/kg), and acepromazine (0.7 mg/kg) delivered via intraperitoneal injection. Supplemental doses were given as needed to maintain areflexia.

Nerve Electrophysiology

Anesthetized mouse was placed on a non-metallic heating pad held at 38 °C. The paw temperature was 35.8 ± 0.8 °C ($n = 17$). In some experiments, to reduce the paw temperature the paw was placed next to the heating pad on a platform kept at room temperature or on a pad held at 18 °C and the paw temperatures were 32.6 ± 0.6 °C ($n = 14$) or 27.6 ± 0.7 °C ($n = 4$), respectively. The lowest baseline paw temperature was used for measurements of the temperature dependence of the static response of nerves. For all paw temperatures used, the rectal temperature remained unchanged at 37.0 ± 0.2 °C ($n = 9$).

Hair was removed from the ipsilateral hind limb, and an incision was made on the dorsal aspect of the lower leg in the skin overlying the sciatic nerve. The skin was sutured to a plastic ring to form a basin for mineral oil. Different branches of the sciatic nerve were dissected from connective tissue and placed on a pair of silver-wire electrodes for bipolar recording (Fig. 1a). The electrodes were maneuvered by a micromanipulator. The nerve was cut 3–5 mm from the spinal cord. The basin was filled with mineral oil

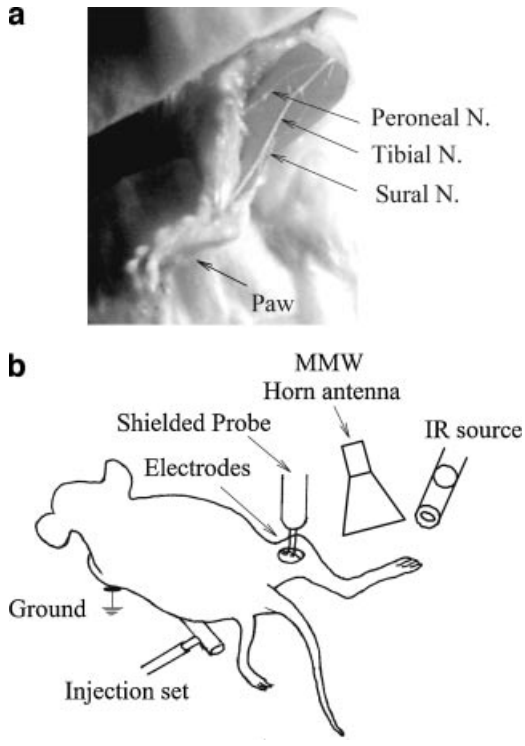

Fig. 1. **a**: The branching pattern of the murine sciatic nerve and (**b**) experimental setup of nerve electrical recording. The electrodes were attached to the sural nerve at the site shown by arrow in (a) pointing to the sural nerve.

warmed to body temperature. The signal from the electrodes was amplified (Iso-DAM8A, WPI, Sarasota, FL) and recorded using a personal computer-based acquisition system, Spike2 (Cambridge Electronics, Cambridge, UK) at a sampling rate of 10 kHz. The experimental setup is shown in Figure 1b.

MMW Exposure

The ipsilateral hind paw was exposed to 42.25 GHz using a rectangular horn antenna or open-ended waveguide placed 5 mm from the skin surface. The incident power density (IPD) distributions on the skin surface obtained from the horn antenna and open-ended waveguide were bell shaped with a peak in the center of the beam. They were well described by a Gaussian type function [Alekseev and Ziskin, 2003]. For characterization of MMW intensity we used the peak values of the IPD. A G4-141 generator (Istok, Fryazino, Russia) was used as the source of MMW energy with an output power of up to 40 mW. We used continuous wave (CW) or pulse wave (PW) exposures. In PW mode the MMW signal was modulated with square wave pulses at 1 kHz using a built-in modulator of the G4-141 generator. In the experiments if not specified otherwise, we used CW exposures.

To obtain higher output power, the signal from the G4-141 generator was amplified using a MMW amplifier AMP-22-01120 (Milllitech, Northampton, MA). The IPD varied from 0 to 260 mW/cm^2. The maximal temperature rise ΔT was dependent on the baseline skin temperature. With the same IPD, when the baseline skin temperature increased, the maximal ΔT decreased. The effect of higher baseline temperatures on ΔT could be due to the cooling effect of increased blood flow [Alekseev et al., 2005]. At an IPD $= 220$ mW/cm^2 and a skin temperature of 35.8 °C, the temperature rise was 6.2 ± 0.6 °C. At a skin temperature of 32.6 °C, $\Delta T = 7.8 \pm 0.7$ °C.

Radiant Heat Exposure

To reproduce the thermal effects of MMW we used a radiant heat source. This source contained a spiral coil made of 0.4 mm diameter Nichrome wire that was located in front of a metal reflector. It was heated with 0–9 V DC. The required beam size was obtained by passing the infrared (IR) beam through two diaphragms. The necessary temperature rise (0–8 °C) in the exposed area of skin was obtained by varying the distance between the IR source and paw, and the voltage of the power supply.

Temperature Measurements

Temperature was measured using an Amber-4256 infrared camera (Amber Engineering, Goleta, CA) or a copper-constantan thermocouple of the IT-23 type (Physitemp, Clifton, NJ). The thermocouple was placed in the paw orthogonal to the E-vector of the MMW field. The IR camera was not used for temperature measurements during radiant heat exposures because the reflected IR signal distorted the measuring IR signal. Temperature measurements in the paw showed good agreement between both heating and cooling kinetics following MMW and radiant heat exposures.

Drugs

Capsaicin, a TRPV1 agonist used for activation of polymodal nociceptor fibers, and compound 48/80, an agent used for depletion of mast cells, were obtained from Sigma–Aldrich (St. Louis, MO). Capsaicin was dissolved in 96% ethanol to yield a concentration of 20 mg/ml. Before application, it was diluted by Dulbecco's Phosphate-Buffered saline (DPBS) (Mediatech, Herndon, VA) to a concentration of 10 mg/ml. A drop of capsaicin solution (5 µl) was applied topically to the skin. Compound 48/80 was dissolved in DPBS at a concentration of 0.5 mg/ml. Ten microliters of this solution was injected into the paw subcutaneously.

Data Analysis

The responses of nerves to MMW and radiant heat exposures were analyzed on the basis of firing rate, that is, the number of spikes or impulses per second. We determined the mean values of firing rate ± SEM for recordings of electrical activity before, during and following exposures. The pre-exposure values of firing rate were used as control. The changes of the firing rate during exposure and following the cessation of exposure were compared with baseline firing rates before exposure. The data were analyzed using the Student's *t*-test or two-way analysis of variance (ANOVA); $P < 0.05$ was considered statistically significant. The results obtained with the nerve of the same animal were well reproducible. For statistical analysis we used the data obtained from different animals; n stands for the number of animals.

RESULTS

Nerve Identification

We tested the responses of three main nerves and several smaller branches of the sciatic nerve to MMW exposure (Fig. 1a). The records of the electrical activity of most nerves were noisy. The spike amplitudes were very small. Only one branch of the sciatic nerve exhibited electrical activity with distinct impulses and responded with changes in the firing rate following MMW and radiant heat exposures. The nerve generated an irregular pattern of spike activity with impulses of different amplitudes at the average firing rate of 1.8 ± 0.7 imp/s ($n = 17$). To identify the receptive fields of this nerve we applied different stimuli (touch, prick, and acetone drop) to the foot that activate mechanical, nociceptive and thermal receptors, respectively. The nerve responded to all stimuli by a notable increase in the firing rate (Fig. 2) only when the stimuli were applied to the lateral side of the foot along the border between the hairy and glabrous skin including the little toe. This allowed us to identify this nerve as the sural nerve [Leem et al., 1993; Koltzenburg et al., 1997]. All results presented below were obtained with sural nerves.

MMW Exposure

We found two types of responses of the sural nerve to MMW exposure. First, MMW exposure of the receptive field of the nerve produced inhibition of the spontaneous electrical activity (Fig. 3). The effect increased with increasing intensity. A statistically significant reduction of the firing rate ($P < 0.05$) was observed at the IPD = 45 mW/cm^2. In this case the temperature rise, ΔT, at the end of 100 s exposure was

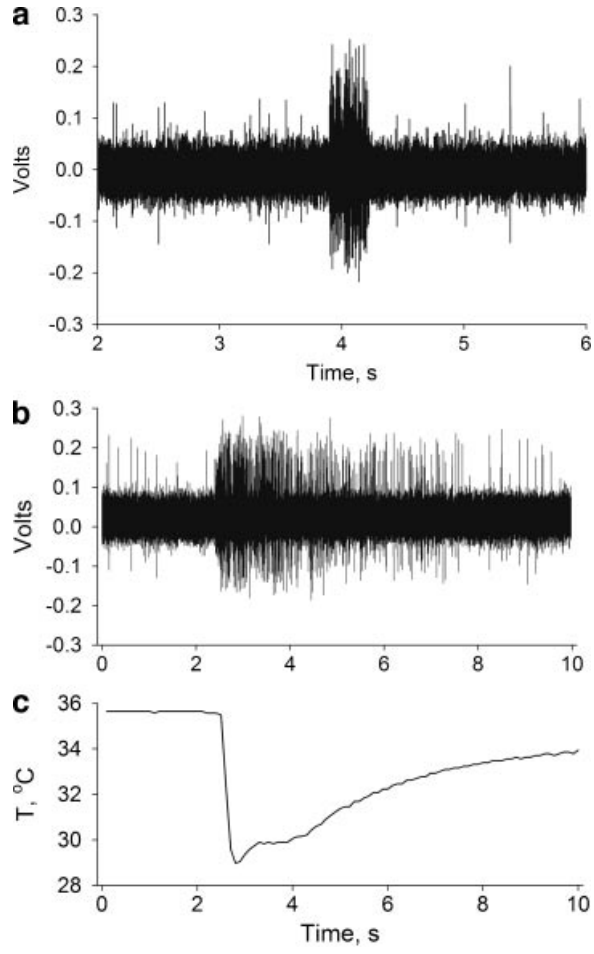

Fig. 2. **a**: Discharge of the sural nerve resulting from light touch and (**b**) fast cooling of the receptive field located on the foot. **c**: Temperature changes during fast cooling produced by an acetone drop (5 μl). Baseline skin temperature was 35.8 °C.

1.5 ± 0.3 °C ($n = 17$). At the greatest exposure intensity tested (IPD = 220 mW/cm^2, $\Delta T = 6.2 \pm 0.6$ °C) the firing rate was reduced to $44 \pm 12\%$ ($n = 17$) relative to control. The inhibition of the firing rate was more pronounced toward the end of exposure. Long lasting exposure (up to 10 min) at the IPD = 10, 20, and 30 mW/cm^2 did not produce any detectable changes in the firing rate of the sural nerve.

Second, we observed a transient increase of the firing rate immediately following the cessation of exposure. This effect was induced at higher intensities of MMW exposure and lasted 20–40 s. At the IPD = 220 mW/cm^2 the maximal value of the transient increase in the firing rate exceeded the control level by $225 \pm 35\%$ ($n = 17$). The threshold intensity for this effect was 160 mW/cm^2. It is well known that cold receptors respond to rapid cooling with a similar transient increase of firing rate [Braun et al., 1980; Schafer et al., 1988; Heinz et al., 1990]. To differentiate

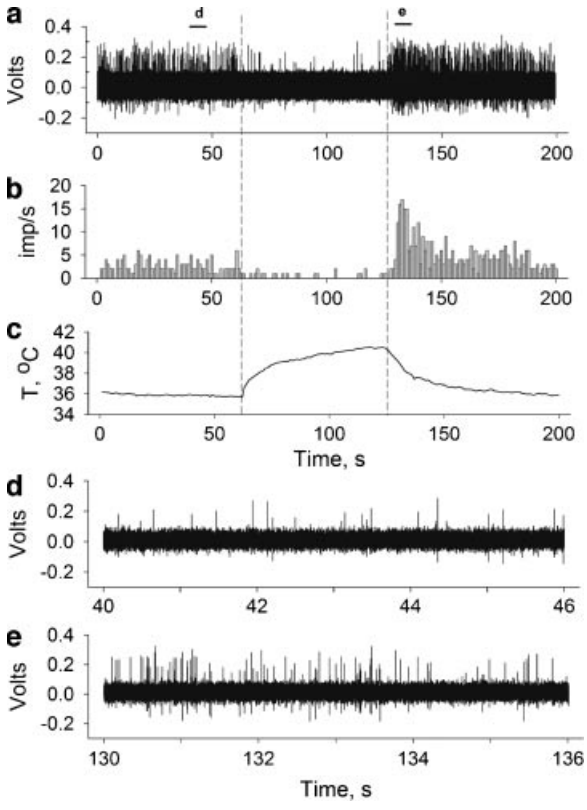

Fig. 3. Effect of MMW exposure on the firing rate of the sural nerve. **a**: Electrical recording. **b**: Firing rate, impulses per second. **c**: Skin surface temperature measured with a thermocouple located in the area of receptive field of the nerve. **d**, **e**: Expansions of the electrical recordings of the nerve discharge before and after exposure, respectively. The areas of expansion d and e are marked by short horizontal lines on the top of the electrical recording in (a). Vertical cursors show the beginning and end of MMW exposure at 220 mW/cm^2.

between the transient increases of the firing rate induced by MMW exposure and by cooling, we designate the terms ''transient response'' for response induced by MMW exposure and ''dynamic response'' for response induced by rapid cooling.

To examine how the baseline skin temperature of the paw influenced the transient response of the nerve to the cessation of MMW exposure we lowered the paw skin temperature from 35.8 to 32.6 °C. This resulted in an insignificant ($P = 0.84$) change of the transient response ($231 \pm 57\%$, $n = 14$). The time intervals to reach the maximal transient response were 14.8 ± 7.8 s ($n = 17$) and 15.5 ± 5.6 s ($n = 14$) at 35.8 and 32.6 °C, respectively. The difference between the time intervals was also statistically insignificant ($P = 0.77$).

Radiant Heat Exposure

MMW exposure at high intensities is accompanied with notable increase in skin temperature. Therefore, the changes in firing rate induced by MMW exposure may be due to the temperature rise in the skin. To evaluate the temperature effect we exposed the receptive field of the sural nerve to a radiant heat source. The intensity of the radiant heat was set to obtain a temperature rise equal to that produced by MMW exposure. Figure 4a shows the temperature rises obtained during MMW and radiant heat exposures of the skin. In both cases the kinetics of the temperature rise and decay of the two sources were very close. Figure 5 shows that radiant heat exposure reduced the firing rate of the nerve, similar to the MMW exposure. However, we did not observe a transient increase of the firing rate after the cessation of radiant heat exposure even at maximal exposure intensities. For comparison between the effects of MMW and radiant heat exposures we used the same mouse. The threshold temperature rise for observing the inhibition of the firing rate was 1.7 ± 0.3 °C ($n = 12$), that is, close to the

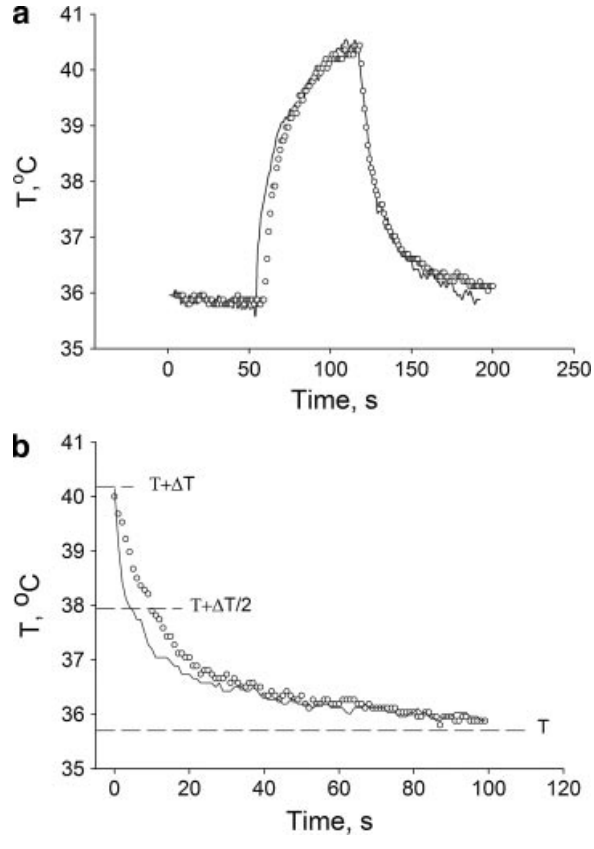

Fig. 4. Heating of the skin in the area of the receptive field of the sural nerve by radiant heat (solid lines) and MMW (open circles) exposures. **a**: Temperature rise. **b**: Kinetics of the temperature decay. The rate of cooling following the peak MMW exposure at the IPD = 200 mW/cm^2 was slowed by gradually reducing the MMW power. T is the baseline skin temperature, ΔT is the maximal temperature rise produced by both exposures.

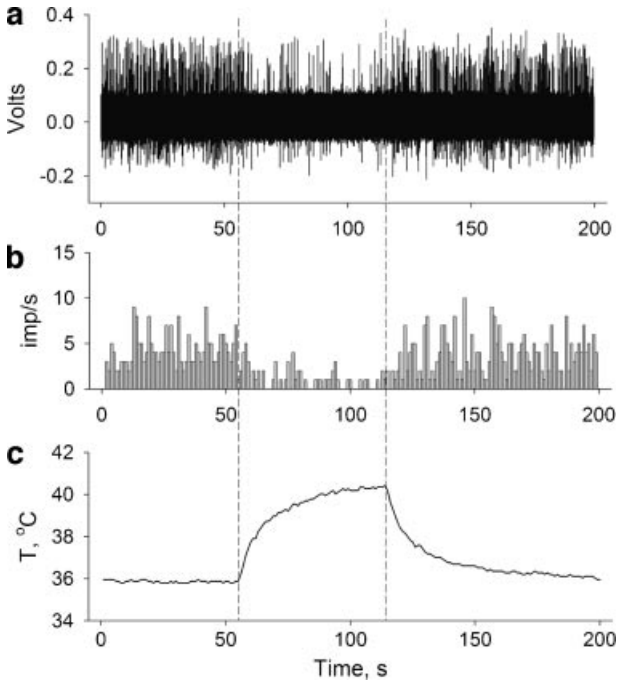

Fig. 5. Effect of radiant heating on the firing rate of the sural nerve. **a**: Spontaneous activity. **b**: Firing rate, impulses per second. **c**: Skin surface temperature measured with a thermocouple located in the area of receptive field of the nerve. Vertical cursors show the beginning and end of radiant heat exposure.

temperature rise which was achieved during MMW exposure at threshold intensities. During radiant heating by $\Delta T = 6.2\,^{\circ}\mathrm{C}$ the firing rate was reduced to $40 \pm 19\%$ ($n = 12$). This value was not statistically different from the similar MMW effect. Thus, the inhibition of the spontaneous electrical activity of the nerve during MMW exposure could be explained purely by the skin temperature rise.

Thermal Sensitivity of Nerve Firing

Most cutaneous receptors do not display ongoing activity in the absence of intentional stimuli except cold receptors which exhibit an ongoing discharge at normal skin temperatures. As shown in Figure 2, rapid cooling of the nerve with an acetone drop induced a strong transient increase in its firing rate, typical of the dynamic response of cold receptors, which is also commonly associated with a transient grouped discharge [Schafer et al., 1988]. Using radiant heat exposure we determined the static response of the nerve to thermal stimulus. The different intensities of radiant heat were applied to the receptive field of the nerve for 3–5 min. The initial skin temperature was 27.6 °C. The mean firing rate was determined for the last minute of exposure when the firing rate reached the new steady-state level and was stable (static response).

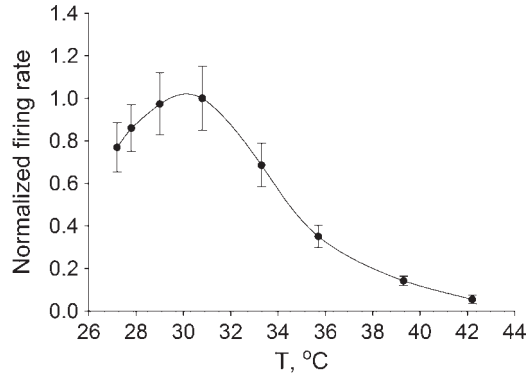

Fig. 6. Static firing rate of the sural nerve at maintained skin temperatures. Baseline firing rate was averaged over a period of 60 s prior to temperature change.

Figure 6 shows the dependence of the static response on foot skin temperature obtained from 4 mice. The maximal firing rate occurred at 30 °C and decreased with increasing the skin temperature. This type of static temperature dependence is typical for cold receptors [Schafer et al., 1986; Schafer et al., 1988; Heinz et al., 1990]. Hence, the inhibitory effects of MMW and radiant heat exposures result from the inhibition of cutaneous cold receptor activity due to the skin heating. The fibers of cold receptors exhibit periodic burst discharges during both the static and dynamic activity at skin temperatures $\leq 25\,^{\circ}\mathrm{C}$ [Braun et al., 1980; Schafer et al., 1988; Heinz et al., 1990]. The discharge patterns of the nerve in the control and following cessation of MMW exposure (Fig. 3d,e) did not show any burst activity at both baseline skin temperatures examined (35.8 and 32.6 °C). It may be that the high baseline skin temperature was not favorable for the burst discharge.

Following the cessation of MMW or radiant heat exposures, skin temperature passively drops at a rate depending on the thermal conductivity of the skin, blood flow, and thermal exchange between the skin and air [Alekseev et al., 2005]. With both types of exposures the temperature decay was the same. Radiant heat cessation did not induce the dynamic response of the nerve typical for cold sensitive fibers. This can be explained by the slower dissipation of heat rather than the cooling rate required for eliciting the dynamic response ($\tau < 5$ s) [Iggo, 1969]. Hence, the transient response of the nerve to the cessation of MMW exposure could not also be attributed to the dynamic response of the cold receptors.

In an attempt to clarify whether the temperature decay rate was critical for inducing the transient response, we varied the cooling rate at the end of MMW exposure (Fig. 4b). The temperature decay rate was nonlinear and could not be described by a single

exponential [Alekseev and Ziskin, 2003]. Therefore, it was characterized by the time to reach 50% of the final temperature, t_{50}. The average value of t_{50} for MMW and radiant heat exposures producing a maximal temperature elevation of 6.2 °C was 6.4 ± 1.3 s ($n = 9$). For this period of time the temperature dropped by 3.1 °C. In one set of experiments we purposely reduced the rate of temperature decay by gradually decreasing the MMW intensity with an attenuator (Fig. 4b). The temperature decay rate reduced from $t_{50} = 3.5$ to 9.5 s. This resulted in a small reduction of the magnitude of the transient response of the nerve (6%). In contrast, the dynamic response of cold receptors to cooling following radiant heating was not induced, even at the most rapid decay rate of $t_{50} = 3.5$ s. This indicated that the temperature decay rate at the range of $t_{50} = 3.5–9.5$ s was not critical for observing the transient response to the cessation of MMW exposure.

Effects of Different Modes of MMW Exposure

To understand the mechanisms underlying the transient response of the nerve it is important to identify nerve fibers involved in this effect. They may be cold or polymodal receptors or various mechanoreceptors. For example, cold receptors could respond to the successive application of thermal stimulus by reduction of their firing rate. To determine the sensitivity of the transient response to MMW we initially induced this response by exposing the receptive field of the nerve to 220 mW/cm^2 and then, 5 s later, we applied the second pulse at 130 mW/cm^2 (Fig. 7). The intensity of the second pulse was lower than the threshold intensity of the transient response. Nevertheless, the second pulse strongly inhibited the transient response within the first 3–5 s exposure. Skin temperature decreased by 2 °C following cessation of the first pulse and remained essentially at the same level during the second pulse. Thus the transient response was inhibited mainly by MMW exposure, not by temperature change. In contrast to the transient response, the dynamic response of cold receptors to fast cooling with acetone remained intact during MMW exposure. This implied that the mechanisms underlying MMW-induced transient response were different from those suggested for dynamic response of cold receptors [Reid and Flonta, 2001; Viana et al., 2002].

In five experiments we used pulse modulated MMW exposure, which allowed us to reduce the temporal-average intensity in half while maintaining the pulse intensity at a level equal to the intensity of CW exposure. These experiments were designed to find out which of the two parameters of PW exposure, pulse intensity or temporal-average intensity, is critical for eliciting the transient response. Figure 8 shows the

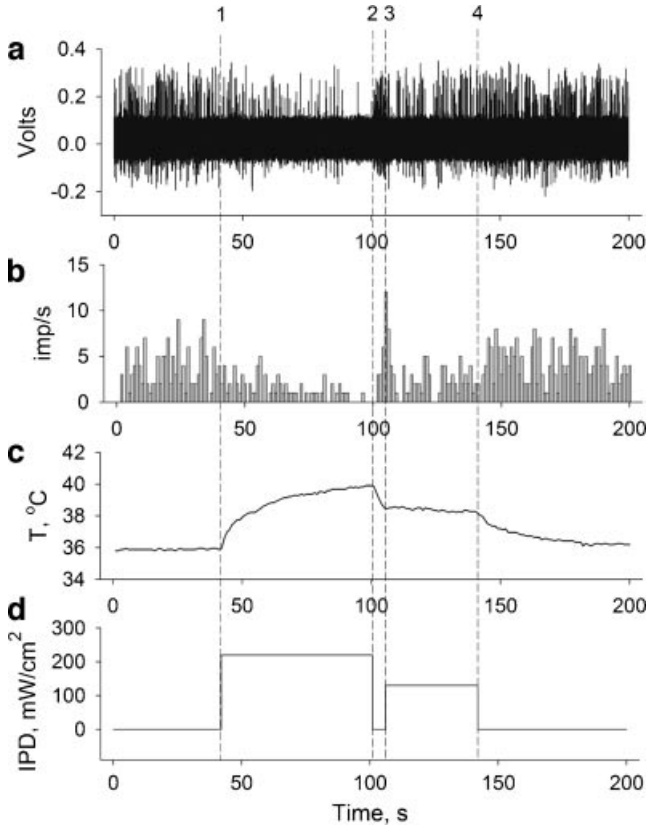

Fig. 7. Effects of successive MMW exposures on the firing rate. **a**: Electrical record. **b**: Firing rate. **c**: Skin temperature changes. **d**: Diagram of MMW exposure. Vertical cursors show the beginning and end of MMW exposures. The interruption time between two exposures (cursors 2 and 3) was 5 s. The IPDs of the first and second pulses were 220 and 130 mW/cm^2.

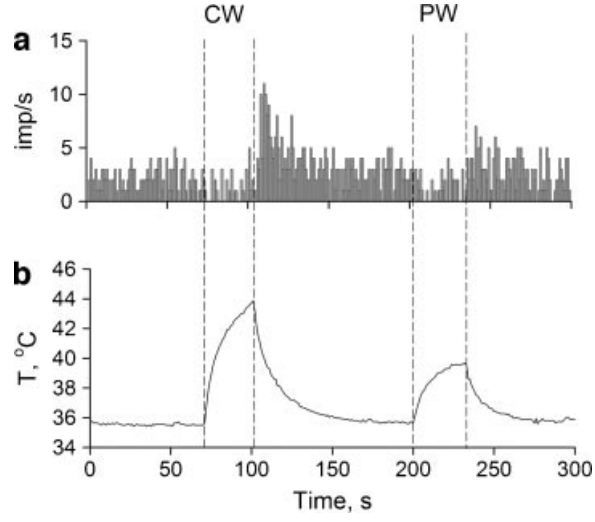

Fig. 8. Effects of CW and PW exposures on the firing rate of the sural nerve. **a**: Firing rate, impulses per second. **b**: Skin temperature changes. The pulsed and CW IPD = 260 mW/cm^2. The temporal-averaged IPD of PW exposure = 130 mW/cm^2.

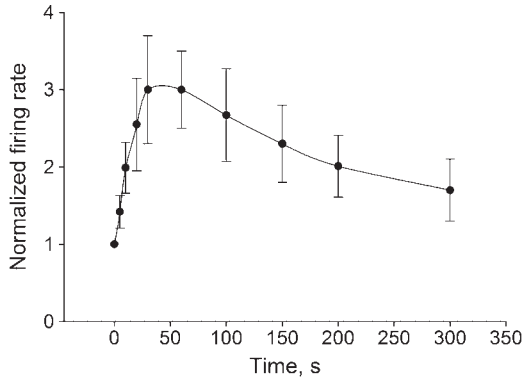

Fig. 9. Dependence of the maximal transient response of the sural nerve on MMW exposure duration. IPD = 220 mW/cm^2.

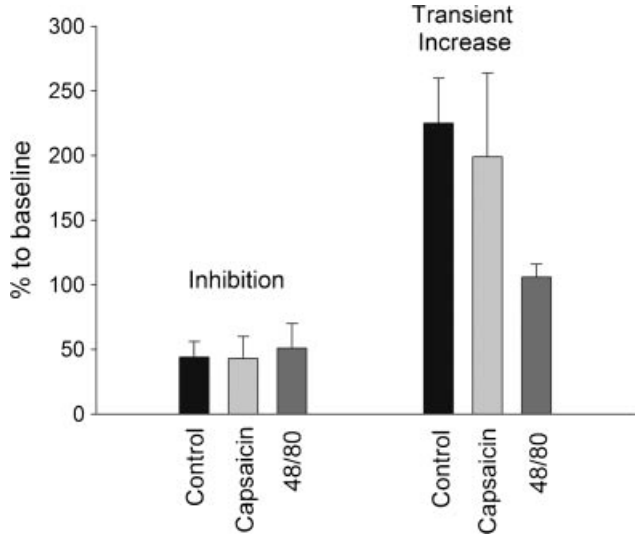

Fig. 10. MMW effect on sural nerve firing modified by drugs. In control (no drugs), in experiments with capsaicin, and compound 48/80, n was 17, 8, and 4, respectively. The difference between the inhibition effects with and without drugs was statistically insignificant ($P > 0.4$). The difference in the transient responses before and following capsaicin treatment was also statistically insignificant ($P > 0.5$). The transient response was eliminated by compound 48/80 ($P < 0.05$ relative to baseline). Baseline level is 100%.

effects of CW and PW exposures at the same CW and pulse IPDs equal to 260 mW/cm^2. PW exposure was not able to induce a statistically significant transient response. These results demonstrated that the temporal-average intensity was the main parameter of exposure for inducing the transient response.

Figure 9 shows the dependence of the transient response on the duration of MMW exposure obtained from five mice. The magnitude of the response reached its maximal value in 30–60 s. By further increasing the exposure duration, the effect exhibited a tendency to decline, which is typical for desensitizing nerves. In the absence of exposure the transient response fully recovered in 7–10 min.

Effects of Drugs

It is well recognized that capsaicin can selectively activate cutaneous nociceptors in the skin by binding to the TRPV1 receptor [Caterina et al., 1997]. At low concentrations capsaicin sensitizes fine afferent nociceptors (C- and Aδ-fibers) [Seno and Dray, 1993]. Repeated application at high concentrations produces an opposite effect, that is, desensitization of nociceptors [Kenins, 1982]. To examine the role of capsaicin-sensitive receptors in inducing the transient response of the nerve we applied 5 µl of capsaicin (10 mg/ml) topically to the receptive field of the nerve. Five to 10 min following capsaicin application, the firing rate of the nerve increased $152 \pm 45\%$ ($n = 8$) relative to the control level. Exposure to MMW inhibited the firing rate relative to the new control level to the same extent as before the treatment ($P = 0.84$). Capsaicin application did not increase the transient response (Fig. 10). Hence, capsaicin-activated fibers were not involved in the transient response.

It is well known that compound 48/80 results in depletion of mast cells [Kowalski and Kaliner, 1988; Drummond, 2003]. Ten microliters of compound 48/80 was injected subcutaneously in the receptive field of the nerve. Initially the injection produced a significant increase in the firing rate. After 10–15 min, when the firing rate was stabilized at $192 \pm 57\%$ ($n = 4$) of the control level, we exposed the paw to MMW at the IPD = 220 mW/cm^2. We observed inhibition of the firing rate typical for the untreated skin but the transient response disappeared entirely (Fig. 10). This indicated that the presence of intact mast cells in the skin was important in inducing the transient response of the nerve to MMW exposure.

DISCUSSION

Both the radiant heat and MMW exposures produced inhibitory effects on the spontaneous electrical activity of the sural nerve. At normal skin temperatures, cold receptors are the main contributors to the spontaneous activity [Schafer et al., 1988; Leem et al., 1993; Carr et al., 2003]. In rodents, cold receptors are common in the receptive field of the sural nerve [Schafer et al., 1988; Leem et al., 1993]. They respond to the temperature rise by reducing their spontaneous activity. Hence, the inhibitory effect of radiant heat and MMW exposures was most likely due to the response of cold receptors to skin heating. Heating of the skin up to 43 °C by either source did not activate discharge of warm receptors or mechano-heat polymodal receptors;

the latter having a thermal threshold $\geq$42 °C [Cain et al., 2001; Zimmermann et al., 2009]. It seems that the exposure intensities used in this study were not high enough to activate nociceptors. It is also possible that the nociceptors generated small amplitude spikes from the whole nerve. However, this was not the case because the brushing and pricking at the receptive field of the nerve induced typical responses to these stimuli. The absence of warm receptor activity in our recordings was in agreement with previous studies that showed the lack of warm receptors in the receptive field of the sural nerve of rodents [Schafer et al., 1988; Leem et al., 1993].

Transient increase in the firing rate was observed only after the cessation of MMW exposure. Radiant heating to the same temperature and with the same temperature decay rate was not able to induce the transient response. This indicated that the mechanism of MMW action was different than radiant heat. The experiments with two successive MMW exposures implied that different receptors might be involved in the transient response. They are most likely cold receptors and low-threshold C-mechanoreceptors which show transient discharge following removal of a maintained stimulus (heat, constant force) [Leem et al., 1993]. High intensity MMW electromagnetic field near nerve terminal membranes may be one of those stimuli capable of activating nerve endings.

We propose three mechanisms that may be responsible for eliciting the transient response of the sural nerve to MMW exposure cessation: (1) thermal, (2) membrane polarization, and (3) mast cell degranulation. In accordance with the thermal mechanism, we suppose that cooling of the skin following cessation of MMW exposure might induce the dynamic response of cold receptors. Though the surface temperature kinetics during MMW and radiant heat exposures showed a good agreement between each other, there might be some temperature difference within the skin at the locations of cold nerve terminals at these two exposures. However, some results provide evidence against this mechanism. First, the transient response was not very sensitive to the changes of the temperature decay rate. Because the cooling of tissue is a passive process, the cooling rate at the same location within the skin would be the same. Second, the transient response lasted 20–40 s which was very long for the dynamic response of cold receptors. Third, the dynamic response of cold receptors is strongly dependent on baseline skin temperature [Braun et al., 1980; Schafer et al., 1988; Heinz et al., 1990]. On the contrary, the transient response to MMW cessation remained essentially unchanged when the baseline skin temperature was lowered.

As recently shown, the cold sensation by specific thermoreceptor nerve endings can result from the closing background K^+ channels, causing depolarization of the nerve terminal membrane [Viana et al., 2002]. In this case, the temperature elevation produces an opposite effect, that is, hyperpolarization of the membrane and inhibition of nerve firing. Within the membrane, the hypothesis we propose is that MMW exposure might also hyperpolarize the nerve terminal membrane of cold or other receptors by a non-thermal mechanism. In cold receptors it would be hyperpolarization in addition to thermal. The cessation of MMW exposure would produce rapid depolarization of the membrane and transient increase of firing rate. At present, our results do not allow us to suggest specific mechanisms of direct action of MMW on nerve endings.

The third possible pathway of activation of cutaneous nerve terminals by MMW may involve degranulation of mast cells. We propose that MMW initially degranulate mast cells which release neuromediators. Recently, a similar mechanism was proposed for explanation of electric and magnetic field effects in humans [Gangi and Johansson, 2000]. It is known that mast cells are located very close to nerve terminals [Skofitsch et al., 1985; Botchkarev et al., 1997]. The released neuromediators (histamine, substance P, serotonin, etc.) can easily reach nerve terminals and excite them. However, during MMW exposure we did not find any signs of activation of the nerve. This might be due to the thermal or non-thermal hyperpolarization of the membrane which significantly reduced the activation of nerve terminals by neuromediators. However, following the cessation of exposure, neuromediators could effectively interact with nerve terminals and activate them. It seems that the higher intensity was required for degranulation of mast cells while the activation of nerve terminals by neuromediators might be inhibited at lower intensities. Some results indirectly support this hypothesis. For example, depletion of mast cells by compound 48/80 eliminated the transient response of the nerve. The second application of MMW produced very fast inhibition of the transient response induced by the first MMW application. Long exposure reduced the transient response indicating that the receptors involved in this effect underwent desensitization.

To suggest a reasonable mechanism of MMW action on nerve terminals, it is necessary to identify the individual nerve fibers responsible for MMW effect and to determine receptors and mediators that play a major role. The transient response appears to be specific to MMW because the radiant heating did not reproduce this effect. The transient response was induced at exposure intensities much higher than those used in

therapy. Therefore, the involvement of nerve fibers generating the transient response in therapeutic effects is not obvious, and further experimental study of the biological effects of high intensity MMW is needed.

We showed that MMW exposure at therapeutic intensities of 10–30 mW/cm^2 did not alter the firing rate of the sural nerve innervating the murine hind paw. This may indicate that the low-intensity MMW exposures used in therapy do not produce direct stimulation of cutaneous afferents. However, we cannot rule out other possible effects of MMW on nerve endings such as sensitization or desensitization which change the sensitivity of nerve endings to external stimuli. We found several articles describing neural effects of MMW on rat in vivo [Enin et al., 1991; Novikova et al., 2008a,b] that can be compared with our results. Enin et al. recorded electrical activity of mechanoreceptor fibers of the sciatic nerve and did not find that MMW induced activity of the fibers. The exposure intensities were $\leq$8 mW/cm^2. In two other articles, rats were exposed to MMW at knee areas [Novikova et al., 2008a,b]. Using the c-Fos protein expression method the authors showed activation of hypothalamic cells. However, it is difficult to evaluate the actual threshold intensity for this effect because the IPD values were not provided, only the output power of the generator. These effects might occur at exposure intensities comparable with those used in our experiments.

We found that inhibition of the firing rate occurred at intensities of MMW exposure $\geq$45 mW/cm^2 which produced a temperature rise of the skin of $\geq$1.5 °C. We characterized MMW intensity by the peak IPD. However, only the average IPD is provided in most MMW therapy reports. During therapeutic treatment, MMW exposures at the average IPD of 10–30 mW/cm^2 could increase skin temperature by 1.5–2.0 °C. The distribution of the IPD radiated by a horn antenna is not uniform. The peak intensity at an average IPD of 10 mW/cm^2 may exceed 23 mW/cm^2 [Alekseev and Ziskin, 2003]. Therefore, at higher average exposure intensities, that is, at 20–30 mW/cm^2, the peak IPD can exceed the threshold intensity (45 mW/cm^2) for this effect. Thus, the inhibition of the firing rate of the nerve may play some physiological role in MMW effects. However, such an effect would be purely thermal and similar to the changes in the firing rate of the nerve induced by conventional heating.

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