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HYPOALGESIC EFFECT OF MILLIMETER WAVES IN MICE: DEPENDENCE ON THE SITE OF EXPOSURE

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Summary

Based on a hypothesis of neural system involvement in the initial absorption and further processing of the millimeter electromagnetic waves (MW) signal, we reproduced, quantitatively assessed and compared the analgesic effect of a single MW treatment, exposing areas of skin possessing different innervation densities. The cold water tail flick test (cTFT) was used to assess experimental pain in mice. Three areas of exposure were used: the nose, the glabrous skin of the right footpad, and the hairy skin of the mid back at the level of T5-T10. The MW exposure characteristics were: frequency = 61.22 GHz; incident power density = 15mW/cm^2; and duration = 15 min. The maximum hypoalgesic effect was achieved by exposing to MW the more densely innervated skin areas – the nose and the footpad. The hypoalgesic effect in the cTFT after MW exposure to the murine back, which is less densely innervated, was not statistically significant. These results support the hypothesis of neural system involvement in the systemic response to MW.

Key Words: millimeter waves, pain, mice, innervation density

Despite the availability of analgesics in medical practice, pain management, especially management of chronic pain syndromes, continues to be a controversial problem. It is estimated that 35-40 million Americans suffer from intractable pain, and annual expenditures for the management of chronic pain are over 50 billion dollars (1).

Sedation and pain relief are the most common effects of millimeter wave (MW) therapy, a procedure that has been used in several Eastern European countries in thousands of patients (2). This method is based on the biological effects resulting from the exposure of a patient's skin to electromagnetic millimeter waves (range of frequencies – 37-78 GHz; incident power density levels - <25 mW/cm^2). Successful use of MW for the treatment of diseases, ranging from gastrointestinal and cardiovascular pathology to local dermatitis and wound healing, has been

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reported in numerous articles and reviews (2-6). However, the physiological mechanisms involved in these treatments are still unclear, and the clinical use of MW therapy far exceeds experimental studies in this field. Most MW treatment modes suffer from the fact that they were developed empirically, the reported results, although very impressive, were not obtained in a double blind manner, and were analyzed qualitatively rather than quantitatively. Nevertheless, pain relief is indeed a reproducible effect of MW treatment, and after appropriate double-blind experimental studies, MW treatment could become a valuable noninvasive alternative or a supplementary method of treatment for various pathologic conditions characterized by pain.

The hypoalgesic effect of MW treatment, besides its clinical value, could also be used for experimental studies in animals, where the results could be evaluated quantitatively. This could lead to a better understanding of the mechanisms involved and ultimately to the development of an optimal treatment strategy and an analysis of the mechanisms involved. However, to date, there have been no reported attempts to reproduce and quantitatively assess the influence of MW radiation on nociception in laboratory animals.

There are three main approaches in the selection of areas to be exposed during MW treatment in patients. Most authors have reported exposure of the skin over the sternum, large joints, occipital area, and forehead (7-13). Another approach is the local exposure of skin directly over the disease foci: postoperative wounds (14), over joints affected by rheumatoid arthritis (15), and over various breast areas in patients with breast tumors (16). The third approach makes use of acupuncture points for the sites of exposure (17-20). However, to the best of our knowledge, all of the above-mentioned treatment modes were developed empirically, and appropriate comparative studies were never performed.

Several MW exposure sites were used in non-pain related animal experiments: hip (21), back (22;23), "medulla oblongata projection" area (24), and areas over pathologic foci (25;26). We were unable to identify references to any original studies showing why these particular areas had been chosen. Only in one of the studies the "anti-stress effect" of MWs had been compared after exposure of occipital, left hip, and right hip areas in rats (27). Overall, the occipital area was claimed to be the most effective site.

Based on a hypothesis of neural system involvement in the initial recognition and further processing of MWs signal (2), the main objective of the present study has been to reproduce, quantitatively assess and compare the hypoalgesic effect of MW treatment, exposing areas of murine skin possessing different innervation densities.

Methods

Animals. Male Swiss albino mice (20-25 g) were obtained from Taconic Co. (Germantown, NY). Animals were housed in the Central Animal Facility at Temple University School of Medicine, under controlled temperature and light/dark cycle conditions, with food and water freely available. The University IACUC approved the protocol.

Exposure procedure. To prevent the influence of electromagnetic "noise", exposure of mice to MW radiation was conducted in a shielded chamber made of 0.5-inch thick low-carbon steel sheets. The generator of the MW (Model G4-142; made in the former USSR), the power meter (ML 4803A; Anritsu, Japan), and the spectrum analyzer (Hewlett Packard 8565B, USA) were located outside the shielded area. Electromagnetic MW exposure characteristics were: frequency – 61.22 GHz; incident power density – 15 mW/cm^2; duration – 15 min.

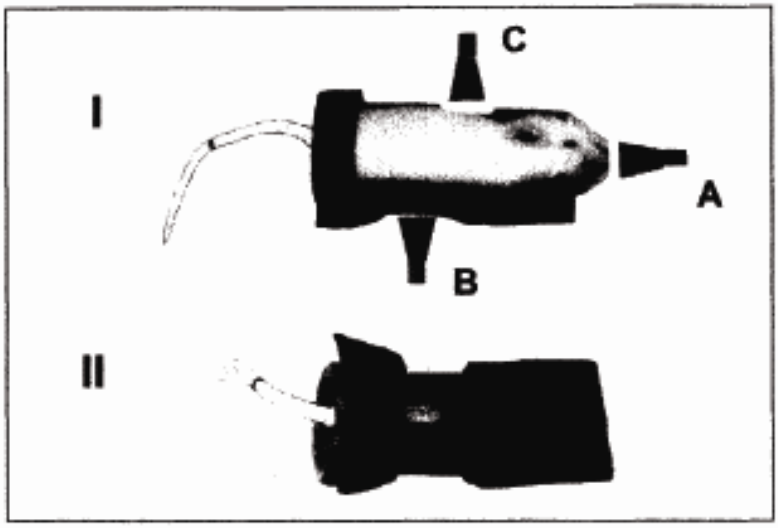


Fig.1.

I. Mouse positioned in a specially designed plastic restrainer prior to the experiment. The murine nose and mid back are uncovered for exposure purposes. The mouse tail and right paw are placed outside the restrainer. A, B and C – indicate the areas used for MW exposures. **II.** The plastic tube in **I** is rotated to reveal exteriorization of right paw. The paw was fixed outside the restrainer with thin Scotch tape that absorbs less than 3% of MW energy at the given frequency. To ensure that only the paw received MW energy, a black protective flexible microwave-absorbing shield was placed between the paw and the restrainer.

Animals were assigned to groups in a random order. Before exposure, each mouse was restrained in a plastic tube (mouse nose and right paw uncovered for exposure purposes; mouse tail placed outside the restrainer) (Fig.1). Because of possible stress induced by restraint, which can affect tail-flick data (28), the mice were exposed in pairs. One mouse was placed under the horn antenna for exposure to MW (experimental groups), while the second one did not receive MW treatment, but was also restrained (sham groups). Taking into consideration the innervation density differences of various parts of a murine body (29), three areas of exposure were used in our experiments: the nose, the glabrous skin of the right footpad, and the hairy skin of the mid back at the level T5 – T10.

Cold water tail flick test. Since our previous findings demonstrated possible involvement of opioids in the systemic response to MW exposure (30,31), we employed the cold water tail flick test (cTFT) in mice as the antinociceptive assay. This test has been designed to assess quantitatively the antinociceptive actions of various drugs and experimental conditions and is appropriate for investigating opioid-mediated effects. (32). Furthermore, it has been shown that cold-induced pain mimics chronic types of pain better than other experimental methods (33). This could be of an additional value, taking in consideration resistance of chronic types of pain to the traditional methods of treatment.

The test is performed as follows: **a)** Two individuals are involved. One exposes the mice, and the other conducts the cTFT test, unaware of which mouse was exposed to MW. **b)** A cold water bath containing melting ice (1.0±0.5°C) is used. **c)** To prevent possible damage to the mouse tail, a 60 s cut-off time is utilized. **d)** Mice undergo the cTFT on two consecutive days. On the first day the mice are placed in restrainers and the test is conducted two times with a 15-min break. The nociceptive threshold was the time taken for the mouse to remove or flick its tail. Results from these tests were discarded ("training"). On the second day, the first test was recorded as a baseline. Following 15 min of MW or sham exposure, each mouse was tested four more times: immediately after the exposure, and 15, 30 and 45 min after the exposure. In this way, every animal served as its own control. This timetable was developed from our preliminary experiments (data not shown), and had provided results with minimal variability. **e)** Each mouse was used only once.

Data analysis. Raw data were converted to percent of each mouse's baseline response, and the average percent change was calculated for each mouse. Post-treatment group means and means for each time point were compared to those of the sham group means using the Wilcoxon rank-sum test. A level of $p < 0.05$ was taken to indicate statistical significance.

Results

Using the cTFT, the antinociceptive effect of MW treatment was monitored after a single exposure of each selected murine body area: nose, paw and back (Fig. 2). Single MWs treatment resulted in more than a two-fold increase in latency in response to the cold pain following the nose and paw exposures, and more than a 50% increase of this index after back exposure. However, while the differences in cTFT responses to MWs after the nose and paw exposures were both statistically significant ($p<0.01$ and $p<0.05$, respectively), the antinociceptive effect following back exposure to MW was statistically insignificant as compared with the results of cTFT in sham-exposed mice.

Despite the significant increase in group average latencies to cold pain after the nose and paw were exposed to MW, individual responses varied in these groups. After a single exposure to MW, according to the results of the cTFT, mice may be subdivided into four categories:

a. "Supersensitive" – mice, whose average time in the cTFT after treatment increased more than twice.
b. "Sensitive" - mice, whose resistance to cold pain increased from 50 to 200% as compared with baseline.
c. "Insensitive" – animals, whose time in the cTFT changed within ± 50% of baseline.
d. "Inverse-sensitive" – mice that had a decrease in resistance to cold pain by more than 50% from their original response.

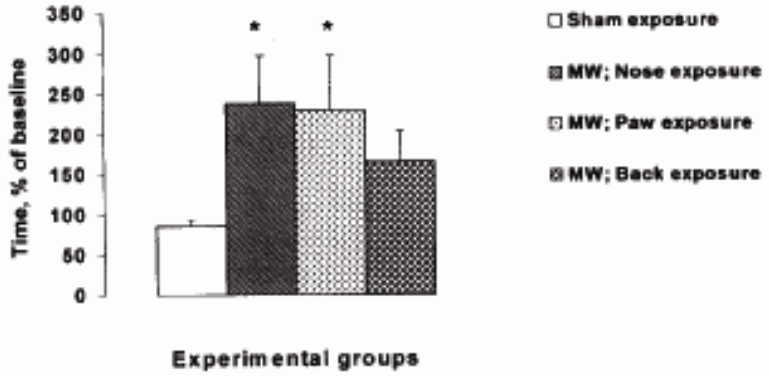


Fig. 2.

Analgesic effects of a single exposure to MW. Cold-water tail flick test. Areas of exposure: nose (n=28), paw (n=14), and back (n=14). Sham exposure, n=54. Electromagnetic exposure parameters: frequency, 61.22 GHz; incident power density, 15 mW/cm^2; duration of exposure, 15 min. Each column represents the group mean + SE. Values with a statistically significant difference ($p<0.05$) from the sham control data are marked with asterisks.

Under the conditions of our experiment, after nose and paw exposures, about 40% of the mice were classified as "supersensitive" to MW treatment, almost 5 % were classified as "sensitive" to the treatment, about 10% were "inverse-sensitive", and the rest did not demonstrate any significant changes in the cTFT after exposure to the active MW generator (Fig 3).

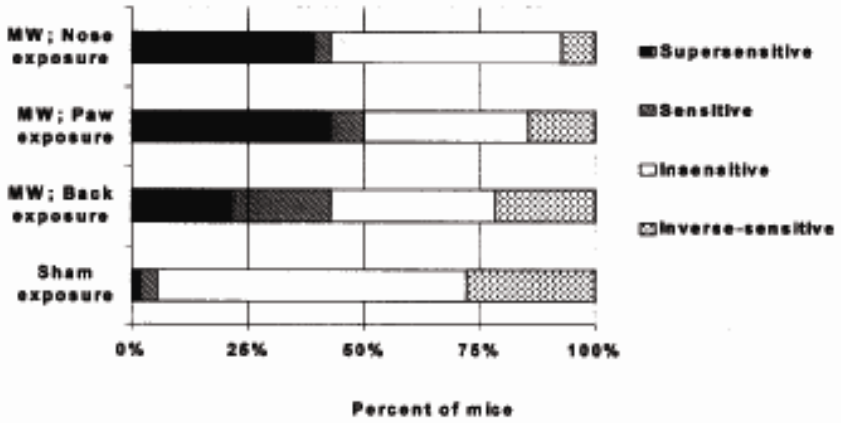


Fig. 3.

Individual variability in analgesic effects of a single exposure to MW (see explanations in the text). Areas of exposure: nose (n=28), paw (n=14), and back (n=14). Electromagnetic exposure parameters: frequency, 61.22 GHz; incident power density, 15 mW/cm^2; duration of exposure, 15 min. Data are shown as % of the total number of animals in each group.

The effect of MW treatment in the cTFT was detected immediately after the 15-min murine nose exposure and was statistically significant during the entire observation period (Fig 4a). The same tendency can be observed after the paw exposure (Fig 4b). However, half the number of animals in this group (fourteen), and approximately the same level of individual variability resulted in a lack of statistically significant differences at the 15 and 30-min time points. Exposure of murine backs to MW did not produce statistically significant changes in the cTFT at any of the time points (Fig 4c).

Further analysis of the data showed that the maximum antinociceptive effect in mice developed in the first half-hour following MW exposures of the nose and paw (Fig.5). In 65% of the mice, the maximum antinociceptive effect occurred at the 15 or 30 min time points. Similar dynamics were found for the group of mice whose paws had been exposed to MW.

Discussion

Numerous authors have noted the sedative and hypoalgesic effects of MW therapy. In gastrointestinal diseases (18;34;35), cardiovascular diseases (36), respiratory system diseases (37;38), and phantom pain (39), two-three sessions of MW therapy usually resulted in alleviation, or in total pain relief in 73-100% of patients. However, it is still unclear which parameters of MW treatment (frequency and power of MWs; duration of the exposure; number of exposures; sites of exposure) are "optimal" for achieving maximum effects. The present study is an attempt to reproduce, quantitatively assess, and compare the hypoalgesia associated with a single MW treatment on the site exposed.

MW are absorbed in water and water-containing media (including biological structures) within the first 0.3 – 0.5 mm from the surface (40;41). This characteristic limits the number of possible cellular targets for MW therapy mainly to structures within the epidermis. Because local exposure to MWs is usually followed by a wide range of biological and therapeutic effects, it has been hypothesized that MW radiation acts through free nerve endings of the epidermis with further involvement of the peripheral and central nervous system (42;43). Results from several clinical and animal studies provide indirect evidence in support of this hypothesis. The ability of neural cells and isolated nerves to respond to MW exposure was shown in several independent in vitro studies (44;45). Kolosova et al. showed that, following sciatic nerve transection, MW treatment accelerates anatomical and functional regeneration (25). Electroencephalographic changes were noted in healthy human volunteers (43) and children with cerebral palsy (46) as a result of exposure to MWs. Positive results of MW treatment have been reported for neurotic depression (47), and psychogenic forms of sexual dysfunction (48).

Results from the present study provide additional support for the hypothesis of neural system involvement in the systemic response to MW exposure. The hypoalgesic effect of MW treatment was greatest following exposure of the nose area, which possesses a very dense sensory innervation from the infraorbital branch of the maxillary division of the trigeminal nerve (49). A somewhat smaller effect was obtained after exposure of the glabrous skin of the mouse footpad, where innervation density is also rather high (50). Exposure of the hairy skin of the back, which is less densely innervated (29), resulted in the lowest analgesic effect. Thus our hypothesis is that if all other conditions are equal, the maximum hypoalgesic effect could be achieved by exposing the most densely innervated skin areas to MW.

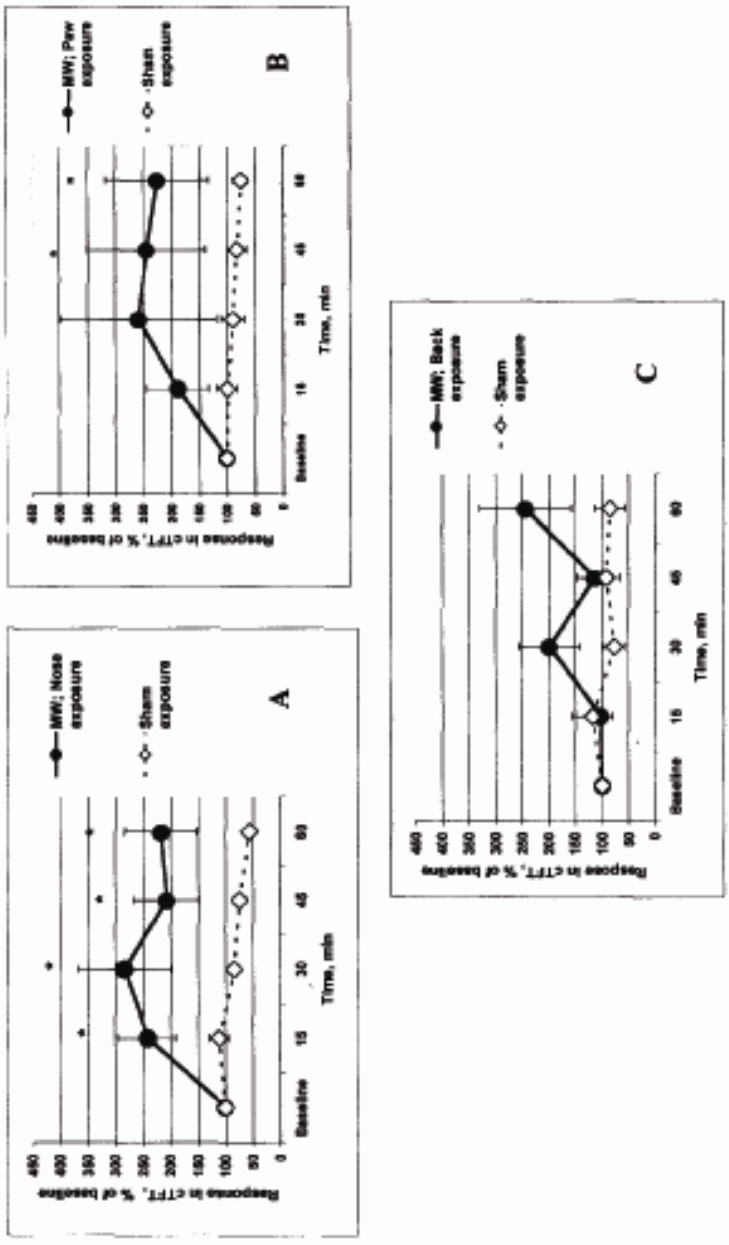
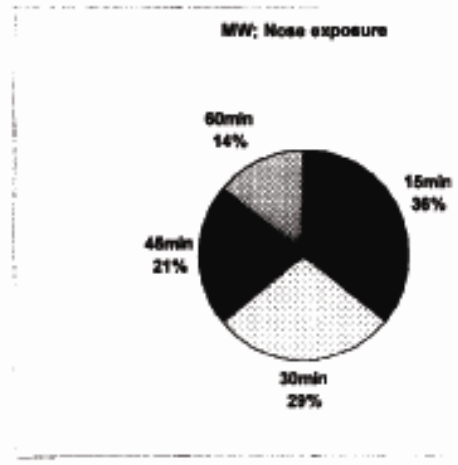


Fig. 4. Analgesic effects of a single exposure to MW. Cold-water tail flick test. Areas of exposure: A. Nose (n=28 in each group), B. Paw (n=14 in each group), and C. Back (n=14 in each group). Electromagnetic exposure parameters: frequency, 61.22 GHz; incident power density, 15 mW/cm^2; duration of exposure, 15 min. Each point represents the mean ± SE of all mice of the group. Values with a statistically significant difference ($p<0.05$) from the sham control data are marked with asterisks.



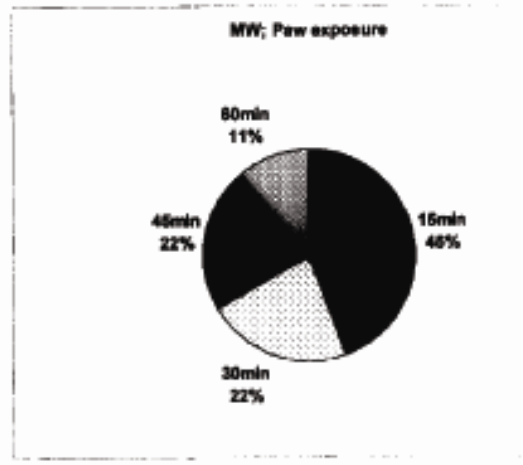


Fig. 5.

Percentage of mice achieving maximal effect in the cTFT at different time points following MW exposures. Sites of exposure: nose (n=28), paw (n=14). Electromagnetic exposure parameters: frequency, 61.22 GHz; incident power density, 15 mW/cm^2; duration of exposure, 15 min.

A rather high rate of individual variability in the reaction to a single MW exposure was an attribute of our experiments. While over 40% of the mice reacted to exposure of their nose and paw to MW by more than doubling their resistance to cold pain, over a third showed no response to treatment in the cTFT. Using a cold pressor test, we previously found a similar distribution in human volunteers after a single exposure to MWs (although a different frequency and power were used) (51). At first glance, these data contradict the reported 70-100% success rate of MW treatment in patients. However, in clinical practice the number of MW therapy sessions varies from 5 up to 30 (11), (52), while in our present study there was a single MW exposure. Importance of the number of MW exposures to experimental outcomes had been reported previously (53). Additional experiments are needed to determine whether multiple exposures could result in a potentiation of the effect in "sensitive" animals, or whether several exposures could increase the number of individuals obtaining a beneficial effect.

The mechanism, or rather, mechanisms involved in the development of biological reactions to MW are still unclear. Most probably, there are multiple substances, structures, and systems that take part in the initial absorption of MWs, in the processing of this signal, and in further formation of various biological effects following MW treatment. In our previous experiments we demonstrated possible involvement of opioids in the antipruritic effect of MW (54), and in the potentiation of ketamine and chloral hydrate anaesthesia by MW (30). It is reasonable to hypothesize that the hypoalgesic effect of MW described in the present study also involves opioids. However, this hypothesis needs to be tested, and will be the subject of a separate study.

Acknowledgment

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