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Effects of the Action of Microwave-Frequency Electromagnetic Radiation on the Spike Activity of Neurons in the Supraoptic Nucleus of the Hypothalamus in Rats

S. M. Minasyan, G. Yu. Grigoryan, S. G. Saakyan, A. A. Akhumyan, and V. P. Kalantaryan

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Acute experiments on white rats anesthetized with Nembutal (40 mg/kg, i.p.) were performed with extracellular recording and analysis of background spike activity from neurons in the supraoptic nucleus of the hypothalamus after exposure to electromagnetic radiation in the millimeter range. The distribution of neurons was determined in terms of the degree of regularity, the nature of the dynamics of neural streams, and the modalities of histograms of interspike intervals; the mean neuron spike frequency was calculated, along with the coefficient of variation of interspike intervals. These studies demonstrated changes in the background spike activity, predominantly affecting the internal structure of the spike streams recorded. The major changes were in the duration of interspike intervals and the degree of regularity of spike activity. Statistically significant changes in the mean spike frequencies of neuron populations in individual frequency ranges were also seen.

KEY WORDS: hypothalamus, supraoptic nucleus, electromagnetic radiation, spike activity.

Along with the traditional areas in which electromagnetic energy has been used (television, radio communications, etc.), increasing attention is being paid to the possibility of using this radiation in biology and medicine. Studies of the biological actions of low-intensity electromagnetic radiation at microwave frequencies (millimeter range) are of particular interest; these have a variety of effects on the body affecting systems processes such as adaptation and sensitization [6, 20].

The central nervous system has been shown to have the greatest reactivity to the actions of electromagnetic radiation [6, 13, 15, 23]. However, few studies have addressed the effects of millimeter waves on the nervous system – the main regulatory system with significant influences on all processes occurring in the body.

The functional state of the central nervous system is often assessed by using the background spike activity of neurons. Measurements of spike frequency provide only a partial reflection of the nature of the spike stream, while identification of parameters based on analysis of interspike intervals provides a more informative assessment of spontaneous activity in different physiological processes [18].

Studies of changes in the background rhythms of hypothalamic neurons are of clear interest, considering the great importance of the hypothalamus in the mechanisms of adaptation and maintenance of homeostasis in the body [1], and also considering the fact that the extralemniscal somatosensory system, which is responsible for the conduction of non-specific and weak stimuli such as electromagnetic radiation has extensive links with the limbic system, hypothalamus, and other parts of the brain [6, 7].

The present study describes a comparative analysis of the background spike activity of neurons in the supraoptic nucleus of the hypothalamus after single exposures to low-intensity electromagnetic radiation at different frequencies, with the aim of assessing the functional state of the central

Faculty of Human and Animal Physiology and Faculty of Microwave Radiophysics and Telecommunications, Erevan State University, 1 Alek Manukyan Street, 375025 Erevan, Republic of Armenia.

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nervous system and identifying a number of features in the action of millimeter-range radiation on the brain.

METHODS

Experiments were performed in acute experimental conditions on white rats anesthetized with Nembutal (40 mg/kg, i.p.). Spike activity was recorded using glass microelectrodes (tip diameter 1 μm, resistance 3–5 MΩ) filled with 2 M NaCl. The stereotaxic orientation of the electrodes was in accord with coordinates from an atlas [21]. Animals were divided into two groups: a control group (six rats) and an experimental group (12 rats). Rats of the experimental group were subjected to single exposures to electromagnetic radiation with millimeter waves in a continuous generation regime. The source of monochromatic millimeter radiation was a G4-141 microwave generator emitting radiation in the frequency range 37.50–53.57 GHz. The stability of the generated frequency was ±0.05%. The frequency of the output signal was controlled with a model Ch2-25 frequency meter. The maximum output power measurable with the M5-49 thermistor head was 53 mV.

Considering that the biological effect appears some period of time after the start of irradiation and that the optimum duration of exposure is quite long (from 20 min to 1 h) [5], we selected an intermediate exposure period of 40 min. Irradiation was performed at room temperature (19–22°C). Two different radiation frequencies were used – 42.2 GHz (at a power density of 0.19 mW/cm^2) and 50.3 GHz (at a power density of 0.48 mW/cm^2). These frequencies were selected on the basis of the hypothesis regarding the biological relevance of the natural electromagnetic background, which in the millimeter range has a marked banded structure due to the presence of molecular absorption by atmospheric oxygen and water vapor [3]. The frequency of 50.3 GHz was also selected because of the resonance properties of the water component of the biological environment typical of all tissues in normal conditions [10]. The animal's head was irradiated in the vertical direction at a distance of 40 cm from the generator horn, i.e., the animal was placed in the far zone of the irradiator to exclude spatial heterogeneity of the field arising from interference between incoming waves and waves reflected from the target [4].

After experiments, electrode locations were verified histologically.

Recording and analysis of spike activity from neurons in the supraoptic nucleus of the hypothalamus were performed using a computer program specifically written for biological signals. Sequential regions of the interspike interval trace were analyzed, each including 1200 action potentials. The stationary spike activity of the neurons recorded was initially determined from the shapes of sliding frequency plots; the stationary nature of spike activity was also assessed using the non-parametric Kolmogorov–Smirnov test. The structure of the stationary spike stream was assessed in terms of a set of interspike intervals and their distribution. When sets were stationary, normalized first-order histograms of interspike intervals were plotted along with autocorrelograms to the eighth order, reflecting the probability that a spike would appear at different time points. The shapes of these plots identified three groups of neurons with different degrees of regularity in their spike streams: group 1 neurons were characterized by eight well marked peaks, which is regarded as indicating a predominance of a regular component in the neuron spike activity (Fig. 1, *A*, *I*); group 2 had autocorrelograms in which only 2–3 peaks were marked, after which there was a plateau (Fig. 1, *A*, *II*). These neurons were assigned to a group with intermediate-regularity activity; group 3 neurons had autocorrelograms characterized by the absence of marked peaks, reflecting a relatively uniform level of the probability at which a spike would appear at different time points (i.e., a plateau) (Fig. 1, *A*, *III*). Neurons with this type of activity were regarded as irregular. Neurons with non-stationary activity were placed in a separate group.

The dynamic structure of spike streams was identified by calculating serial correlation coefficients, where differences from zero were regarded as significant at $p < 0.05$. The correlation coefficients of sequential interspike intervals were calculated for the overall set of adjacent intervals, for pairs of interspike intervals with one, two, and more intervals (i.e., correlation coefficients to the 50th order).

Analysis of serial correlograms of stationary and non-stationary spike streams from neurons in the supraoptic nucleus of the hypothalamus yielded four major types of dynamics for interspike intervals: 1) random sequencing of interspike intervals (all serial correlation coefficients were equal to zero); 2) local changes in discharge frequency (serial correlation coefficients had only positive and zero values); 3) train-group activity (serial correlation coefficients of both signs were seen, along with zero values); and 4) monotonic changes in discharge frequencies (serial correlation coefficients had only positive values).

For stationary neurons, histograms of interspike intervals were constructed and their shapes were used to define mono-, bi-, and polymodal neurons (Fig. 1, *B*, *I*, *II*, *III*).

Values were calculated for the major statistical parameters of the background spike activity: the mean spike frequency and the coefficient of variation of interspike intervals. Discharge frequencies were used to divide the spike activity of neurons into three groups: 1) neurons with low-frequency spike activity (<10 spikes/sec); 2) intermediate-frequency neurons (10–30 spikes/sec); and 3) high-frequency neurons (>30 spikes/sec).

The significance of changes in the distributions of interspike intervals of neurons in the supraoptic nucleus of the hypothalamus in terms of the degree of regularity, dynamic types of spike activity, the modality of histograms of interspike intervals, and the distribution of neurons

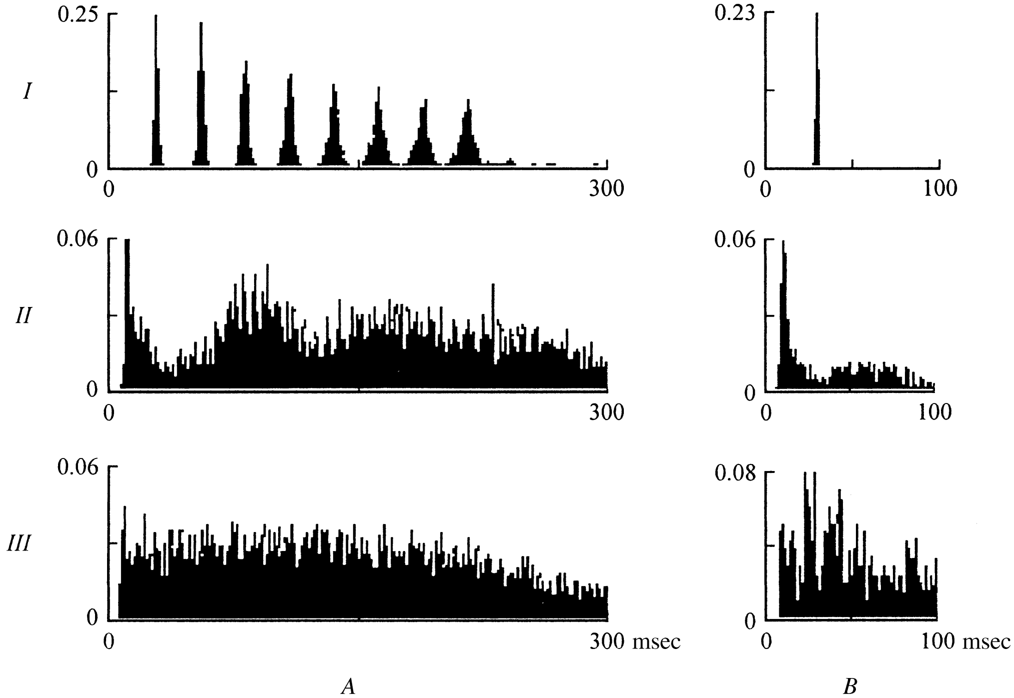

Fig. 1. Types of autocorrelograms and histograms of interspike intervals in the spike streams recorded here. *A*) Autocorrelograms of neurons with different levels of regularity: *I*) with a regular component in spike activity; *II*) with intermediate-regularity activity; *III*) with irregular activity; *B*) types of histograms of interspike intervals with different modalities: *I*) monomodal histograms; *II*) bimodal histograms; *III*) polymodal histograms. The abscissa shows the time of spike formation; the ordinate shows the probability of spike formation.

among the different frequency ranges after exposure to microwave radiation as compared with controls was assessed using the χ^2 test. The significance of changes in the major statistical parameters of background spike activity was assessed using Student's test. The significance of changes in mean spike frequencies in different frequency ranges after exposure to electromagnetic radiation was assessed using White's test [9].

RESULTS AND DISCUSSION

The background activity of 228 rat hypothalamus supraoptic nucleus neurons was studied – 78 in normal conditions, 76 after irradiation at 50.3 GHz, and 73 after exposure to irradiation at 42.2 GHz.

Autocorrelation analysis identified types of spike activity with different degrees of regularity (Fig. 2, *A*). Analysis of autocorrelograms of rat hypothalamus supraoptic nucleus neurons recorded in normal conditions demonstrated a predominance of cells with irregular activity (79.5%); the proportion of neurons with intermediate-regularity spike activity was 11.5%.

Exposure to low-intensity electromagnetic radiation was followed by significant ($p < 0.01$) shifts in the distributions of neurons in terms of the degree of regularity of spike activity. Thus, irradiation at 42.2 GHz decreased the proportion of cells with intermediate-regularity activity by a factor of 3.1, while the proportion of irregular neurons decreased by a factor of 1.9. The proportion of non-stationary neurons (9.6%) showed no significant changes as compared with the proportion in normal conditions (9%). Radiation at 50.3 GHz led to increases in the proportion of neurons with intermediate-regularity activity by a factor of 2.8 and a 1.7-fold decrease in the proportion of irregular units. The proportion of non-stationary neurons increased 1.8-fold, reaching 15.8%. As regards regular neurons, the proportion of these was 13.7% after irradiation at 42.2 GHz and 5.3% after irradiation at 50.3 GHz; none were seen in normal conditions.

Calculation of serial correlation coefficients and construction of serial correlograms allowed four major dynamic patterns of interspike intervals to be identified (Fig. 2, *B*). Thus, more than half of spike streams recorded in normal conditions were characterized by local changes in discharge frequency (53.6%); 33.3% of cells showed periodic changes in discharge frequencies in the form of train-group activity. Neurons with randomly following interspike intervals and monotonic changes in discharge frequency accounted for 2.6% and 7.7% respectively. Exposure to low-intensity

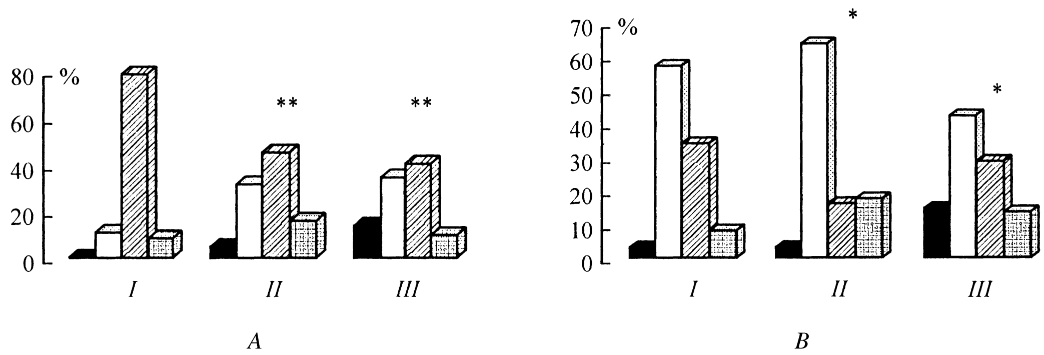

Fig. 2. Plots of the distributions of neurons in the supraoptic nucleus of the hypothalamus in normal conditions and after exposure to microwave irradiation. *A*) Plots of the distribution of neurons in the supraoptic nucleus of the hypothalamus in terms of the degree of regularity of their spike activity. Black columns show regularly discharging neurons; white columns show intermediate-regularity neurons; cross-hatched columns show irregular neurons; dotted columns show non-stationary neurons. **Significant differences in distributions (χ^2, $p = 0.01$). *B*) Plots of the distributions of neurons in the supraoptic nucleus of the hypothalamus in terms of the type of dynamics of interspike interval sequencing. Black columns show neurons with random sequencing of interspike intervals; white columns show neurons with local changes in discharge frequencies; cross-hatched columns show neurons with train-group activity; dotted columns show neurons with monotonic changes in discharge frequency. *Significant differences in distributions, (χ^2, $p < 0.05$). The abscissa shows measurements in normal conditions (*I*), irradiation at a frequency of 50.3 GHz (*II*), and irradiation at a frequency of 42.2 GHz (*III*); the ordinate shows the proportion of neurons, %.

waves led to significant ($p < 0.05$) changes in the dynamics of interspike interval sequencing. Irradiation at 42.2 GHz showed, as compared with normal conditions, a 1.3-fold decrease in the proportion of neurons with local changes in activity and a 1.2-fold decrease in the proportion with train-group activity, along with a 5.8-fold increase in the proportion of background-active units with random sequencing of interspike intervals and a 1.8-fold increase in those with monotonic changes in discharge frequency.

Exposure to radiation at 50.3 GHz decreased the proportion of neurons with train-group activity by a factor of 2.1, also producing 2.2-, 1.5-, and 1.1-fold increases in the proportions of units with monotonic, random, and local changes in discharge frequency, respectively.

Analysis of histograms of the interspike intervals of neurons in the supraoptic nucleus of the hypothalamus yielded the following distribution of recorded spike streams in terms of their modality. Thus, 78.8% of neurons were polymodal in normal conditions, with significantly fewer mono- and bimodal neurons (4.2% and 17% respectively). Exposure to electromagnetic radiation at a frequency of 42.2 GHz increased the proportion of monomodal neurons to 10.6% (2.5-fold) and produced some decrease in the proportion of polymodal neurons, to 72.7%. The proportion of bimodal neurons showed virtually no change from that in normal conditions. Radiation at 50.3 GHz decreased the proportion of polymodal cells to 68.8%; the proportions of mono- and bimodal units increased 1.5-fold (to 6.2% and 25%, respectively).

The mean frequency of background discharges of neurons in the supraoptic nucleus of the hypothalamus was 22.7 ± 1.9 spikes/sec. Exposure to radiation at a frequency of 42.2 GHz increased the mean frequency to 25.8 ± 2.5 spikes/sec, an increase to 23.3 ± 2.1 spikes/sec being seen after exposure to radiation at a frequency of 50.3 GHz.

Analysis of the distributions of neurons in the supraoptic nucleus of the hypothalamus in different frequency ranges showed a predominance of intermediate-frequency (10–30 spikes/sec) cells recorded in normal conditions (50.7%), and the mean discharge frequency was 18.6 ± 1.0 spikes/sec. The proportions of low-frequency (<10 spikes/sec) and high-frequency (>30 spikes/sec) units were 22.5% and 26.8%, respectively. The mean frequency of low-frequency neurons was 6.5 ± 0.4 spikes/sec and the mean frequency of high-frequency neurons was 44.8 ± 2.8 spikes/sec.

Exposure to radiation was followed by statistically significant (White's test) differences in the mean spike frequencies in individual frequency populations of neurons. The proportion of low-frequency neurons after irradiation at 42.2 GHz was 25.8%, with a mean frequency of 6.6 ± 0.5 spikes/sec. The proportion of cells with intermediate spike frequencies decreased to 39.4%, with a significant decrease in the mean spike frequency to 17.7 ± 1.1 spikes/sec ($p < 0.05$). There was some increase in the proportion of high-frequency units to 34.8%, with a significant increase in the mean spike frequency to 49.1 ± 3.2 spikes/sec ($p < 0.05$).

Exposure to electromagnetic radiation at 50.3 GHz produced the following changes in the distributions by frequency range. There was a decrease in the proportion of cells discharging at low frequency to 20.3%, with a significant decrease in the mean spike frequency to 5.4 ± 0.7 spikes/sec ($p < 0.05$) in this range; there was a decrease in the proportion of high-frequency cells, to 23.4%, with a significant increase in the mean spike frequency to 47.4 ± 4.2

spikes/sec ($p < 0.05$). The proportion of neurons with intermediate spike frequencies increased to 56.3%, with a significant increase in the mean frequency to 19.7 ± 0.9 spikes/sec ($p < 0.05$).

Irradiation at millimeter wavelengths at a frequency of 42.2 GHz was followed by a significant decrease in the coefficient of variation of interspike intervals to 85.6 ± 3.3% ($p < 0.05$). Exposure to electromagnetic radiation at a frequency of 50.3 GHz was followed by an insignificant decrease in the coefficient of variation to 91.2 ± 4.5%, from 94.6 ± 3.3% in background conditions.

Our studies of the background spike activity of neurons in the supraoptic nucleus of the hypothalamus using the analytical methods described above allowed us to define the dynamic structure, regularity, and modality of the spike streams recorded, along with their statistical characteristics, in normal conditions and after exposure to low-intensity electromagnetic radiation. These studies identified changes in the background spike activity, predominantly affecting the internal structure of the spike streams recorded, particularly the degree of regularity and the nature of the dynamics of interimpulse interval sequencing. There were no significant changes in the mean frequency of neuron spike activity. Similar effects were reported from Chizhenkova's studies [16, 19]. Nonetheless, the changes in the mean neuron spike frequency in the individual frequency ranges were statistically significant in terms of White's test.

Microwave radiation is almost completely absorbed in the epidermis and upper layers of the skin, acting on superficially located receptors and free nerve endings [5, 7]. In this light, it can be suggested that changes in nerve cell activity appear to be induced by increases in afferent spike activity from areas with elevated sensitivity to electromagnetic radiation [23]. As increases in spontaneous activity are regarded as the result of the effects of transmitters on extrasynaptic receptors [12], the effects of microwave radiation may be due to modification of the states of various of the brain's transmitter systems at the level of the individual neuron.

Autocorrelation analysis of the background spike activity, which allows assessment of the possible periodicity in changes in excitability, showed that both the control and experimental groups were dominated by neurons with irregular spike activity. This appeared to be the result of the interaction of neurons in networks, i.e., it is a systems, "network," effect [11], reflecting the arrival of streams of different types of information in the supraoptic nucleus [2]. Decreases in the proportions of irregular neurons after exposure to microwave radiation provide evidence of a significant weakening of the "network" effect.

According to published data [5], the nature of the actions of millimeter waves on the body depends on biotropic measures of the radiation, such as frequency. This is why the course of the biological reaction can be different at different irradiation frequencies. Thus, the results obtained here showed that irradiation at 42.2 GHz has a more marked effect on the regularity of neuron spike activity as compared with irradiation at 50.3 GHz, as evidenced by the significant decrease in the proportion of irregular neurons and the increase in the proportion of regular and intermediate-regularity neurons.

Radiation increased the proportion of neurons with non-stationary spike activity, an observation which is in accord with published data [16].

The dynamic structure of spike streams recorded in both series of experiments was dominated by neurons with local changes in discharge frequency. This activity, which showed irregular sequencing of interspike intervals, may be determined by pre- and postsynaptic modulation of inhibitory (GABA) and excitatory (glutamate) inputs of neuronal signals in the supraoptic nucleus of the hypothalamus [22, 24]. Single exposures to low-intensity electromagnetic radiation in the millimeter range resulted in increases in the proportion of background-active cells with local and monotonic changes in discharge frequency, which may be induced by increases in the frequencies of spontaneous EPSP and IPSP, associated with activation of metabotropic glutamate receptors on the presynaptic cell bodies and with dendritic glutamatergic and GABAergic neurons in the supraoptic nucleus [22, 24]. Decreases in the proportion of neurons with train-group activity may be evidence related to the sedative effects of millimeter waves, also noted by other authors [7]. This effect was most marked after irradiation at 50.3 GHz.

The results obtained here show that single sessions of irradiation of animals' heads with low-intensity electromagnetic radiation in the millimeter range are accompanied by insignificant changes in mean spike frequency. Modification of the spike frequency within the normal range and the absence of significant changes provide evidence of the subthreshold (in terms of its biological significance) nature of the actions of low-intensity electromagnetic radiation on the functioning of the central nervous system, whose responses depend on the initial functional state [8]. It remains possible that the external absence of changes in neuron activity in a number of cases is merely the consequence of mutual canceling of changes in the opposite directions.

The increase in the degree of regularity in the background activity of neurons in the supraoptic nucleus of the hypothalamus was accompanied by a parallel decrease in the mean coefficient of variation of interspike intervals. The significant reduction in the coefficient of variation of interspike intervals after irradiation at 42.2 GHz is evidence that the spike stream acquires a high degree of structuring [17], which is supported by the increase in the proportion of regular neurons. It is possible that modulation of the background activity of brain neurons by millimeter waves produces some degree of change in the functional status of the central nervous system and the whole body, affecting fundamental aspects of viability.

Thus, our studies demonstrated shifts in the initial parameters of the background spike activity of neurons in the supraoptic nucleus of the hypothalamus after exposure to low-intensity electromagnetic radiation in the millimeter range. Nonetheless, comparison of the extents of these effects in relation to the radiation frequency indicates that there were no significant changes of the "resonance" type. The rearrangements in the background rhythms seen after single exposures to electromagnetic radiation are evidence of non-specific brain responses [14].

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