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Pulse activity of populations of cortical neurons under microwave exposures of different intensity

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Abstract

In rabbit pulse flows of populations of cortical neurons were investigated prior to, during, and after 1-min microwave irradiation (wavelength 37.5 cm, power density 0.2–40 mW/cm^2). It was found that the microwave irradiation produced shifts in mean values of interspike intervals and in the number of spike bursts. Peculiarities of rearrangements of pulse flows of cortical neurons were conditioned by an intensity of exposures.
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Keywords: Microwave; Cortex; Pulse flow of neuron populations; Interspike intervals; Spike bursts

1. Introduction

The study of brain electrical activity under microwaves is of great practical and theoretical importance. The nervous system plays a key role in the reactions of animals and humans to these penetrating factors. Our investigations are pioneering in the field of brain electrical processes, including events at the neuronal level, under different electromagnetic and magnetic irradiations [5]. Among the physiological mechanisms of the microwave influence on the brain, the direct action on brain structures prevails [1–4]. Unfortunately so far, little attention has been paid to fundamental researches of the functional state of the brain under microwave irradiation. Our review of investigations of biological effects of electromagnetic fields for the last 30 years was conducted on the basis of the database "Medline". Studies carried out in the cortex and neurons are only 3.5% and 0.2%, respectively, of the total number of works on this topic [5].

In our previous investigations it was found for the first time that 1-min microwave irradiation had a little effect on the mean frequency of cortical neuron activity but produced significant shifts in evoked activity [5–8]. Our further works showed that microwaves modified an inner pattern of pulse flows of neurons [5,9–13]. The purpose of the present study was to analyse influence of microwaves of different intensities on the pattern of background activity of populations of cortical neurons, namely: on characteristics of interspike intervals and spike bursts. The consideration of pulse flows of neuronal populations was more desirable than the analysis of the activity of single neurons [14]. The point is that, individual accidental fluctuations are leveled and dominant changes are emphasized in neuronal populations. Preliminary results on this problem partly were presented in our other papers [12,13].

2. Methods

Experiments were carried out in unanesthetized non-immobilized rabbits with electrodes preimplanted under barbitural narcosis into the sensorimotor region of the cortex for recording the neuronal spike activity. The electrodes were glass micropipettes with a tip diameter of 20 μm filled with 1% agar in saline. During experiments, the animal was gently restrained in a wooden frame. The animal's head was exposed to the microwave field (wavelength 37.5 cm, power density 0.2–0.3, 0.4, 0.5, and 40.0 mW/cm^2). The long axis of the animal's head was parallel to the *E* vector. The exposure time, as in our previous studies [1–13], was 1 min.

Three 1-min recordings of the neuronal activity, obtained before, during, and immediately after the microwave expo-

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sure, were subjected to computer analysis. The mean values of interspike intervals and the number of spike bursts were evaluated. To analyse the burst activity, time threshold levels 5, 10, and 20 ms were used. All examined parameters were calculated for 20-s time intervals.

Results obtained during microwave exposure and for first minute after exposure were compared with the initial data using White–Fisher criterion. Distinctions between the numbers of neuronal recordings, containing changes, in different experimental series were estimated by means of *t*-criterion for selective portions of variants. Besides coefficients of correlation between investigated indices before exposures, during them, and after their cessation were calculated.

In the control experiments without microwave exposures, the characteristics of the neuronal activity were considered analogously.

3. Results

The experimental data were obtained from two hundred fifty-six 3-min recordings of neuronal activity. The intensity of 1-min microwave irradiation was 0.2–0.3 mW/cm^2 (52 exposures), 0.4 (50 exposures), 0.5 (40 exposures), and 40.0 mW/cm^2 (64 exposures). In the control experiments, fifty 3-min recordings of neuronal activity were made. The total number of analysed 20-s portions of pulse flows was 2304.

3.1. Interspike intervals

The mean interspike interval in the initial pulse flows before microwave exposure was 17.75 ± 0.60 ms.

Total data on values of mean intespike intervals under irradiation are demonstrated in Tables 1 and 2. Changes of neuronal activity may be conditioned by fluctuations of a functional state and another events. Used methods of analysis were very sensitive and were able to catch slight shifts even in the control experiments (Table 1). Table 2 shows that indeed relationships of neuronal reactions and intensities of irradiation are not rigid, but rather statistical.

Table 1
The effect of microwave irradiation on mean interspike intervals (the comparison of values during and after exposures with the initial data is on the basis of White–Fisher criterion)

Changes as percentages	Control	mW/cm^2					
		0.2–0.3	0.4	0.2–0.4	0.5	40.0	0.5–40.0
During exposure							
%	101.1	88.8	95.4	93.4	105.9	106.7	106.2
p	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
After exposure							
%	99.9	78.3	99.2	93.1	108.8	106.1	107.5
p	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2
The effect of microwave irradiation on the number of recorded pulse flows with changes of mean interspike intervals

Changes of intervals	Control	mW/cm^2					
		0.2–0.3	0.4	0.2–0.4	0.5	40.0	0.5–40.0
During exposure							
Increase	52.0	21.2	48.0	34.3	67.5	93.7	83.6
Decrease	48.0	78.8	52.0	65.7	32.5	6.3	16.4
u	0.56	8.85	0.56	6.45	4.52	17.01	15.03
After exposure							
Increase	48.0	7.7	52.0	29.4	72.5	65.6	68.3
Decrease	52.0	92.3	48.0	70.6	27.5	34.4	31.7
u	0.56	14.54	0.56	8.57	5.90	5.07	7.65

Application: statistically significant distinctions between events with increase and decrease of mean interspike intervals are underlined (u>2.58 corresponds to p<0.01).

Rearrangements took place during exposure and after its cessation. Shifts such as a decrease of the mean value of interspike intervals predominated under irradiation of an intensity below 0.4 mW/cm^2. Shifts of the opposite direction prevailed under irradiation of 0.5 mW/cm^2 intensity and above. Obtained results were combined into two groups in accordance with the predominant direction of neuronal changes: (1) under the microwave irradiation of 0.2–0.3 and 0.4 mW/cm^2 intensities, and (2) under the microwave irradiation of 0.5 and 40.0 mW/cm^2 intensities (Tables 1 and 2). This approach emphasized existence of opposite changes of interspike intervals in these experimental series.

Positive correlation relationships were found between investigated indices before exposure, during it, and after its cessation (coefficients of correlation from 0.7 till 0.9, p<0.01).

3.2. Burst activity

The observed burst number depended on time threshold levels of them revelation. The burst number was reduced as the threshold was raised. In initial pulse flows the number of spike bursts revealed by 5-ms level was 1225.32 ± 32.43 per min. The numbers of spike bursts revealed by 10- and 20-ms lever were 820.02 ± 31.11 and 275.45 ± 13.47 per min, respectively.

The total data on effect of microwave exposure upon the burst activity revealed by the used threshold levels are presented in Fig. 1. The microwave irradiation of 0.4 mW/cm^2 intensity aroused minimum shifts in the burst activity. The microwave irradiation of 0.2–0.3 mW/cm^2 intensity little increased the number of short spike bursts mainly after exposure. Simultaneously a decrease of the number of longer spike bursts occurred. The microwave irradiation of 0.5 mW/cm^2 intensity led to increase in the number of spike bursts of short and medium-size duration (threshold levels 5 and 10 ms) during exposure. After exposure, on the contrary, a decrease of the number of these

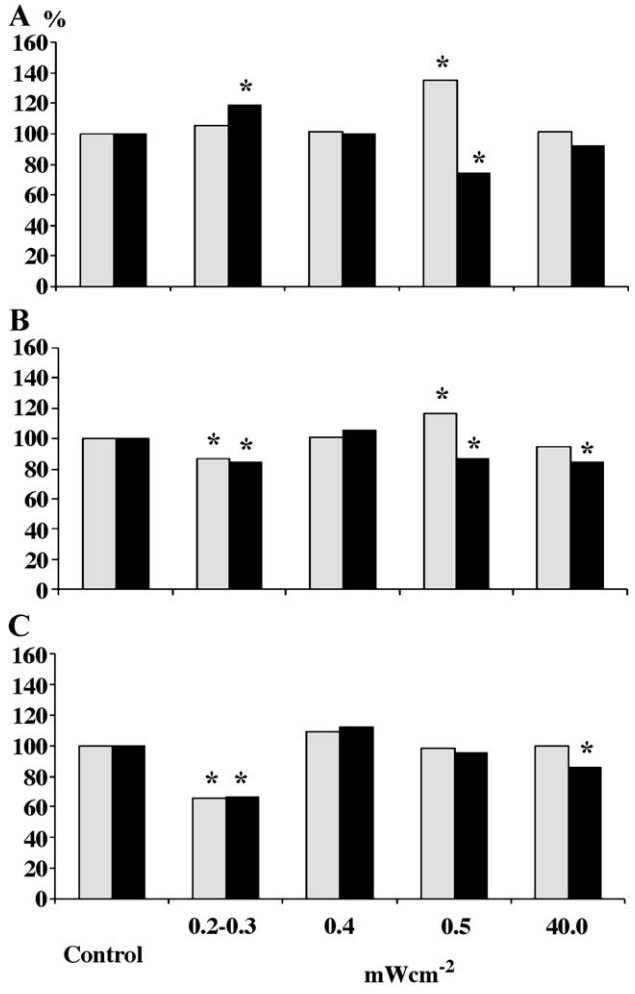

Fig. 1. The effect of microwave irradiation on the number of spike bursts. The time threshold of spike bursts identification is 5 ms (A), 10 ms (B), and 20 ms (C). Light columns are data during exposure; dark columns are data after exposures. Initial data for each experimental part were taken as 100%. Asterisks denote statistically significant changes, $p < 0.01$ (the comparison of values during and after exposures with the initial data is on the basis of White–Fisher criterion).

spike bursts arose. The microwave irradiation of 40.0 mW/cm^2 intensity decreased the number of the spike bursts of different duration after exposure.

Positive relationships existed between the number of the spike bursts of all considered kinds before exposure, during it, and after its cessation (coefficients of correlation from 0.7 till 0.9, $p < 0.01$).

4. Discussion

The modern interpretation of cognitive functions makes us pay close attention to the patterns of spike activity of neurons, in particular of neurons of the cortex. The electrophysiological, morphological, and neurotransmitter aspects of structural and functional organization of the sensorimotor cortex in detail were considered in our monograf [14]. Neurons of this region are known to exhibit altered activity which reflects the integration of information. The organization of the sensorimotor cortex makes it possible to consider it as a self-regulated system with direct and feedback connections creating neuronal circuits [14,15]. There are external and internal modulations of cortical unit circuits [14,16]. Inhibitory interneurons tightly regulate the firing patterns and integrative properties of pyramidal neurons. For the action of cortical circuits, the decisive feature is functioning of cortical GABA-ergic interneurons which possess different characteristics in upper and lower layers of the cortex [17–20].

In our investigations, for the first time, effects of microwaves on neuronal activity were studied. In present work, influence of microwave irradiation of different intensity upon pulse flows of populations of cortical neurons was considered. Objects of analysis were the mean values of interspike intervals and the number of spike bursts revealed by different time threshold levels. Characteristics of interspike intervals are more informative than mean spike frequency [21]. As for burst activity, this kind of neuronal activity is supposed to connect with the encoding of information and to play a special role in detection of signal parameters and moreover in synaptic plasticity [22].

The results of our investigations throw light on some features of the pattern of pulse flows of populations of cortical neurons in intact conditions and under microwave irradiation. It was established that 1-min microwave exposures produced shifts in pulse flows of populations of cortical neurons. Changes of values of interspike intervals and the number of spike bursts occurred through irradiation. Possibility of different effects of microwave exposures took place. Characteristics of rearrangements of pulse flows of neurons were conditioned by intensity of irradiation.

The microwave irradiation of 0.4 mW/cm^2 intensity aroused minimum rearrangement of neuronal pulse flows. The irradiation of 0.2–0.3 mW/cm^2 intensity produced a decrease of mean spike intervals and a decrease of the number of longer spike bursts with a slight increase of the number of short spike bursts. The irradiation of 0.5 and 40 mW/cm^2 intensity mainly led to shifts of opposite direction. An increase of mean spike intervals took place. By the way, rearrangements of burst activity were complex. An increase of the number of spike bursts of short and medium-size duration during the exposure of 0.5 mW/cm^2 intensity was accompanied by a decrease of the number of these bursts after exposure. The irradiation of 40 mW/cm^2 intensity aroused only some decrease of the number of longer spike bursts after exposure.

Correlation analysis of characteristics of neuronal pulse flows showed little significance of initials values of investigated indices in direction and expression of effects of microwaves of used intensities. At sufficient stability of experimental conditions, leading role in appearance of special features of changes of cortex neuronal activity

belongs to parameters of irradiation. Dependence of neuronal rearrangements upon intensity of irradiation was nonlinear.

5. Conclusion

It was first been established that 1-min microwave exposures produced shifts in pulse flows of populations of cortical neurons. Rearrangements of pulse flows included changes of values of interspike intervals and the number of spike bursts.

Characteristics of microwave effects were determined by intensity of irradiation. Nonlinear dependence of neuronal reactions on the parameters of microwave irradiation was observed. Discovered phenomena are real basis of alteration in cognitive functions under electromagnetic irradiation.

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References

[1] R.A. Chizhenkova, The role of different brain formations in EEG-reactions of rabbits to a constant magnetic field and electromagnetic fields of ultra-high and super-high frequencies, Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova 17 (1967) 313–321.

[2] R.A. Chizhenkova, Electrical reactions of the rabbit cerebral cortex to various electromagnetic fields, Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova 17 (1967) 1083–1090.

[3] R.A. Chizhenkova, Potentials of the rabbits brain on exposure to electromagnetic field, Fiziologicheskii Zhurnal SSSR imeni I. M. Sechenova 53 (1967) 514–519.

[4] Yu.A. Kholodov, S.N. Lukyanova, R.A. Chizhenkova, Electrophysiological analysis of the reactions of the central nervous system on electromagnetic fields, in: V.S. Rusinov (Ed.), Electrophysiology of the Central Nervous System, Plenum Press, New York, 1968, pp. 253–260.

[5] R.A. Chizhenkova, Neuronal activity under microwave exposure, in: M.H. Repacholi, N.B. Rubzova, A.M. Muz (Eds.), Electromagnetic Fields: Biological Effects and Hygienic Standardization, World Health Organization, Geneva, 1999, pp. 389–395.

[6] A. Chizhenkova, Background and evoked activity of neurons in the visual cortex of rabbits after action of super-high frequency field, Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova 19 (1969) 495–501.

[7] R.A. Chizhenkova, Slow potentials and spike unit activity of the cerebral cortex of rabbits exposed to microwaves, Bioelectromagnetics 9 (1988) 337–345.

[8] R.A. Chizhenkova, A.A. Safroshkina, T.V. Pylyukh, Low-intensity microwaves affect the patterns of spike flows of cortical neuron populations, In: Microwave in Medicine, 2nd International Scientific Meeting, University of Rome, 11–14 Oct., 1993, Rome, Italy, 1993, pp. 289–292.

[9] R.A. Chizhenkova, A.A. Safroshkina, Effect of low-intensity microwaves on the behavior of cortical neurons, Bioelectrochem. Bioenerg. 30 (1993) 287–391.

[10] R.A. Chizhenkova, A.A. Safroshkina, Electrical reactions of the brain to microwave irradiation, Electro- Magnetobiol. 15 (1996) 253–258.

[11] R.A. Chizhenkova, Burst activity in pulse flows of cortex neuron populations under low-intensity microwaves, in: Millennium Workshop on Biological Effects of Electromagnetic Fields, University of Ioannina, NCSR ''Demokritos'', Heraklion, 17–20 Oct., Crete, Greece, 2000, pp. 104–108.

[12] R.A. Chizhenkova, Pulse flows of populations of cortical neurons under microwave: interspike intervals, Radiatzionnaya biologia, Radioekologiya 41 (2001) 705–711.

[13] R.A. Chizhenkova, Interspike intervals in background activity of populations of cortical neurons under microwave exposures of different intensity, in: Biological Effects of EMFs. 2nd International Workshop. Proceedings, University of Ioannina, NCSR "Demokritos", Rhodes, 7–11 Oct., Rhodes, Greece, 2002, pp. 629–632.

[14] R.A. Chizhenkova, Structural–Functional Organization of the Sensorimotor Cortex, Nauka, Moscow, 1986.

[15] R.A. Chizhenkova, V.Yu. Chernukhin, Nonlinear relations between some parameters of the pattern of pulse flows of cortical neurons, J. Biol. Phys. 26 (2000) 65–73.

[16] P.S. Katz, W.N. Frost, Intrinsic neuromodulation: altering neuronal circuits from within, Trends Neurosci. 19 (1996) 54–61.

[17] R. Metherate, J.H. Ashe, GABA-ergic suppression prevents the appearance and subsequent fatigue of an NMDA receptor mediated potential in neocortex, Brain Res. 699 (1995) 221–230.

[18] J.F.M. Van Brederode, W.J. Spain, Differences in inhibitory synaptic inputs between layer II–III and layer V neurons of the cat neocortex, J. Neurophysiol. 74 (1995) 1149–1166.

[19] M.C. Angulo, J.F. Staiger, J. Rossier, E. Audinat, Distinct local circuits between neocortical pyramidal cells and fast-spiking interneurons in young adult rats, J. Neurophysiol. 89 (2003) 943–953.

[20] J. Trettel, E.S. Levine, Endocannabinoids mediate rapid retrograde → pyramidal neuron synapses of the neocortex, J. Neurophysiol. 89 (2003) 2334–2338.

[21] J.L. Johnson, Discharge frequencies and intervals between pulses: are they always the same? Brain Res. 666 (1994) 125–127.

[22] J.E. Lisman, Bursts as a unit of neural information; making unreliable synapses reliable, TINS 20 (1997) 38–43.