Microwave-Induced Changes in Synaptoarchitectonics at Different Levels of the Visual Analyzer

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It is shown that interneuronal bonds at different levels of the visual analyzer are highly sensitive to microwaves (60 mW/cm^2, 10 min). Evidence of this is seen from the early degeneration and reduced numerical density of synapses and, in the cortical substance, in the reduced total length of active zones of contacts. The rate of recovery of the synaptic apparatus decreases in the following order: retina → external geniculate bodies → visual cortex.

Key Words: *visual analyzer, synapses, microwaves*

Microwaves, acting selectively on the nervous tissue, induce substantial electrophysiological and neuromorphological changes in the peripheral, intermediate, and central parts of the visual analyzer [4,7,9,11]. However, the nature of alterations in the synaptic apparatus at different levels of the visual system and their contribution in microwave-induced visual disorders have been little studied.

The aim of the present study was to investigate the degree of damage and the dynamics of reparir in the synaptoarchitectonics in the retina, external geniculate bodies (EGB), and visual cortex (VC) after a single irradiation with microwaves of thermogenic intensity.

MATERIALS AND METHODS

Experiments were performed on 62 mature random-bred guinea pigs of both sexes. Thirty-six of these animals were exposed to whole-body lateral microwave radiation (60 mW/cm^2, 2375 MHz frequency, 12.6 cm wavelength) during 10 min in an echoproof chamber, the longitudinal axis of the animal being parallel to the magnetic vector of the electromagnetic wave. Measurements on multichamber phantoms showed that the specific absorbed power accounted for about 8 W/kg. Sham-irradiated guinea pigs served as controls and were kept together with the experimental animals under the same vivarium conditions with the usual illumination regime.

The animals were perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) a few minutes, 6 hours, and 1, 5, 10, 25, and 60 days after irradiation. The dorsal ocular wall and dorsal nuclei of the EGB and VC were removed for electron microscopy. Control materials were obtained simultaneously. The materials were postfixed in 1% osmium tetroxide and embedded in araldite. For selective analysis of the paramembrane specialilzation of subsynaptic units the dehydrated objects were contrasted with phosphotungstic acid (PTA) without preliminary treatment with osmium tetroxide [2,5]. Ultrathin slices were prepared with an LKB-3 ultratome, contrasted with uranyl acetate and lead citrate, and examined and photographed with a JEN-100 CX-2 electron microscope.

For quantitative synaptoarchitectonic study 12-15 visual fields of the medial zone of the internal plexiform layer of the central retinal areas, as well as of the dorsal nuclei of the EGB and layer IV of the VC from each irradiated and control animal were photographed. Electron microphotograms with a final magnification of 30,000 and with a neuropil area of 45 μ^2 were obtained. The numerical density of synapses and the percentage of reactively and destructively altered contacts were evaluated by electronograms of the

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osmium-treated preparations. PTA-contrasted preparations were used for estimating the number of PTA-positive synapses and the number of symmetrical, asymmetrical, straight, and curved contacts. The number of synapses of various length in the active contact zone was determined by counting the number of points of intersection with a standard test grid. The total length of active contact zones per 45 μ^2 neuropil was calculated. The quantitative data were processed statistically using the Student t test.

RESULTS

Microwave irradiation of thermogenic intensity induces early (during the first few minutes) reactive changes and substantial damage of synapses, retinal neuron terminals, and visual centers 6 and 24 hours postirradiation, reducing the numerical density of contacts. In the internal pleximorph layer of the retina, the numerical density of the usual distinct synapses 6 hours after microwave irradiation accounts for 79% of the control value ($p<0.05$), 37% of them undergoing degenerative changes, mostly of the light type. In the VC the respective parameters also differ reliably ($p<0.01$) from the control and account for 75 and 44%, respectively. The above decrease in the numerical density of synapses in the retina occurs primarily due to symmetrical straight synapses, while in the visual centers it is mainly on account of asymmetrical curved ones. Unchanged synapses in the retina, EGB, and VC constitute 59, 43, and 49% of the control values. Taking into account the fact that tape contacts more resistant to microwaves account for 5-7% in the internal pleximorph layer and the great majority in the external pleximorph layer of the retina, the total number of preserved synapses in the peripheral part of the analyzer considerably exceeds that in the visual centers. The total length of the active contact zones in the VC, but not in the retina and EGB, is reliably reduced (to 75% of the control, $p<0.05$). Consequently, the degree of microwave-induced damage of interneuronal bonds in the visual centers (especially in the cortex) surpasses that in the retina.

Notable among the early microwave-induced changes are changes in the number, distribution, size, and shape of synaptic vesicles (Fig. 1), swelling and destruction of presynaptic mitochondria, and degeneration primarily of the light (Fig. 2) but also of the dark and focal types. These alterations are unspecific and occur in response to various factors [1,5].

The system of subsynaptic units of the active zone and, especially, the PTA-positive dense projections of asymmetrical contacts, known to play an important role in synaptic transmission, are very sensitive to microwaves. The sharpness of the contours of the dense projections diminishes (Fig. 3), their height

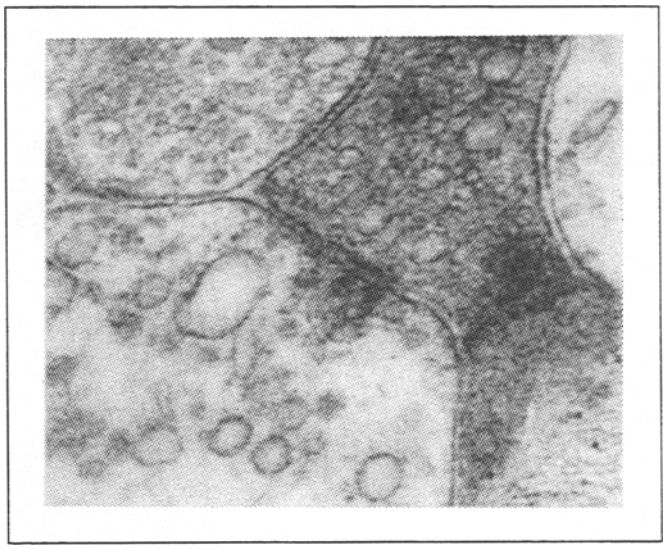

Fig. 1. Enlargement and changes in the form of synaptic vesicles in the internal pleximorph layer of the retina during the first few minutes after microwave irradiation. ×36,000

becomes more variable, and the share of contacts with high dense projections (more than 60 nm) decreases as early as during the first few hours and days after microwave irradiation.

The rate of recovery of the structural and functional state of the synaptic apparatus after irradiation decreases from the peripheral to the central part of the analyzer. The number of synapses with reactive and degenerative alterations and the numerical density of contacts return to the control level by the 5th day in the retina and by the 10th day in the visual centers.

Stereometrical ultrastructural analysis revealed different mechanisms of reorganization of the synaptoarchitectonics at various levels of the visual system. In the retina, the most prompt and effective mechanisms are compensation hypertrophy of preserved contacts,

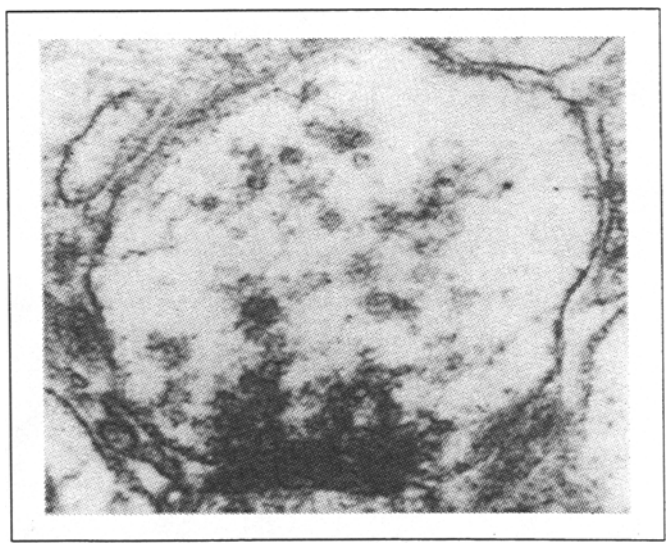

Fig. 2. Light degeneration of a synapse in the visual cortex 6 hours after microwave irradiation. ×48,000

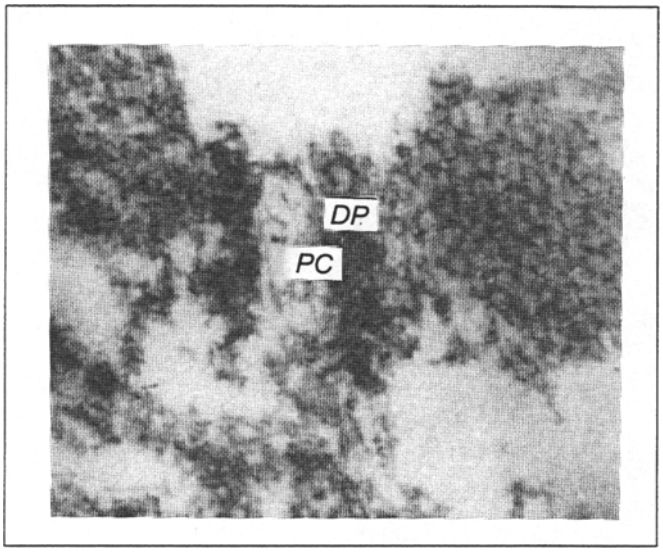

Fig. 3. Indistinct and uneven contours of dense projections of asymmetrical synapses in the visual cortex during the first few minutes after microwave irradiation. *DP* - dense projections; *PC* - postsynaptic consolidation. Phosphotungstic acid contrasting. ×58,00

and an increased share of high-information synapses with an extended active zone (7-8 and 9-16 cross points with the grid, Table 1). A somewhat delayed repair is seen in the VC due to synaptogenesis and increased number of straight contacts with a very short active zone (1-2 cross points).

The observed differences in the mechanisms and efficiency of repair evidently have to do with differences in the initial organization of the synaptoarchitectonics at various levels of the visual analyzer. In the retina, the number of symmetrical synapses constituting the "synaptic reserve" and representing a source of actively functioning asymmetrical contacts is higher than in the visual centers [3,10]. Therefore, the transformation of immature symmetrical synapses into functionally mature synapses followed by their hypertrophy is most characteristic for the retina and evidently is sufficient for compensation of the synaptic damage.

In the visual centers compensatory hypertrophy of synapses surviving after microwave irradiation also occurs, especially early postirradiation. However, at later stages synaptogenesis becomes the predominant phenomenon. It manifests itself in the formation of desmosomelike juvenile contacts with a very short, "point" active zone. It should be emphasized that the above ratio between hypertrophy and synaptogenesis in the VC is characteristic for the recovery period after thermogenic microwave and mean-lethal x-ray exposure [6]. However, hypertrophy may play a key role in the reorganization of the synaptoarchitectonics of the brain cortex caused by other factors, for instance, acute ischemia [8].

Thus, interneuronal bonds at various levels of the visual analyzer are highly sensitive to microwaves of thremogene intensity. This is evidenced from the early degeneration of synapses, the reduction of their numerical density, and, in the cortex, also the reduction

TABLE 1. Number of Synapses with Different Length of Active Contact Zone and Its Total Length per 45 μ^2 Neuropil in the Internal Pleximorph Layer of the Retina and Layer IV of the Visual Cortex of Guinea Pigs after Thermogenic Microwave Irradiation

Time	Number of cross points on test grid					Total length of active contact zone per 45 μ^2 neuropil, $\times 10^2$ nm
	1-2	3-4	5-6	7-8	9-16	
Retina						
Control I	2.8±0.3	2.8±0.3	0.6±0.1	0.04±0.04	-	17.4±1.3
1 min	2.5±0.3	2.6±0.3	0.6±0.2	0.09±0.06	0.01±0.01	16.5±1.4
6 hours	1.6±0.2**	2.5±0.3	0.6±0.2	0.06±0.04	-	14.7±1.4
1 day	1.4±0.3**	1.8±0.3*	1.2±0.3**	0.3±0.1*	0.1±0.07*	17.9±1.7
5 days	1.6±0.2**	2.3±0.4	0.9±0.3	0.04±0.04	-	15.6±1.5
Control II	2.5±0.4	2.4±0.4	0.8±0.2	0.08±0.3	-	16.3±2.1
10 days	1.3±0.4*	2.6±0.3	0.6±0.3	0.08±0.08	-	14.3±1.4
25 days	1.3±0.03**	2.4±0.4	0.7±0.2	0.04±0.04	0.04±0.04	14.1±1.4
60 days	2.6±0.5	3.1±0.4	0.9±0.3	0.3±0.1*	0.07±0.07	21.4±3.4
Visual cortex						
Control I	1.9±0.4	3.5±0.5	1.6±0.5	0.07±0.07	-	25.1±2.7
1 min	2.4±0.4	3.9±0.3	1.0±0.2	0.1±0.06	-	23.5±1.4
6 hours	1.7±0.4	2.1±0.4*	1.4±0.3	0.09±0.09	-	22.7±1.6
1 day	1.6±0.2	2.9±0.3	1.1±0.2	0.1±0.1	-	19.0±1.6*
5 days	2.3±0.3	3.2±0.4	1.4±0.2	0.17±0.09	-	24.3±1.9
Control II	2.0±0.5	0.7±0.3	1.6±0.3	0.06±0.02	-	26.4±2.3
10 days	4.2±0.6*	4.2±0.5	1.1±0.2	0.14±0.09	-	23.7±1.9
25 days	1.7±0.3	2.9±0.3	1.0±0.2	0.12±0.06	-	19.3±1.7*
60 days	2.8±0.3	3.6±0.3	1.2±0.2	0.23±0.3	-	25.6±1.6

Note. Differences from control reliable at *$p<0.05$, **$p<0.01$.

of the total length of active contact zones. The rate of regeneration of the synaptic apparatus diminishes in the following order: retina → EGB → VC. The mechanisms of reorganization of the synaptoarchitectonics are different at different levels of the visual system: in the retina, the most prompt and effective regeneration is achieved through hypertrophy of contacts and an increased number of synapses with an extended active zone, while in the VC the somewhat delayed regeneration is predominantly effected through synaptogenesis.

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