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DESTRUCTIVE AND REPAIR PROCESSES IN THE HIPPOCAMPUS AFTER PROLONGED EXPOSURE TO NONIONIZING MICROWAVE RADIATION

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Data in the literature on disturbance of CNS functions in subjects exposed to nonionizing radiation [4-7] and the results of the writer's previous investigations [1-3] have necessitated special studies of the pathogenesis of these disturbances and their clinical manifestation.

This paper describes a study of the ultrastructural changes in the brain following prolonged exposure to nonionizing microwave radiation (NIMR) of low intensity. The hippocampus, one of the principal formations of the limbic system which is concerned in many functions of the body including memory and behavior, was chosen as the test object.

EXPERIMENTAL METHOD

Male rats were exposed to the action of NIMR (wavelength 12.6 cm) for 40 min, 3 times a day, with intervals of 3-4 h, and always at the same times of day, for 2 months. The animals of different groups were exposed to NIMR of the following intensity: group 1) 1000 $\mu W/cm^2$, 2) 50 $\mu W/cm^2$, 3) 25 $\mu W/cm^2$, 4) 10 $\mu W/cm^2$. The animals were irradiated on the Luch-58 apparatus with dosimetric control in an anechoic chamber, in which the rats were kept in special cages. A group of intact rats served as the control. Each group consisted of 100 animals, in which various physiological, biochemical, and histochemical parameters were determined at different times.

For the electron-microscopic investigations the brain was perfused, and thereafter subjected to the normal procedures for investigation of the ultrastructure of nerve tissue. Three layers of the hippocampus (Ammon's horn) were subjected to electron microscopic analysis: the alveus layer, the layer of polymorphic cells, and the layer of pyramidal cells.

EXPERIMENTAL RESULTS

In animals exposed to NIMR with an intensity of 1000 $\mu W/cm^2$ the state of the structural elements of most fibers differed from the control in the alveus layer, which consists mainly of myelinated and unmyelinated nerve fibers conducting impulses to and from the hippocampus.

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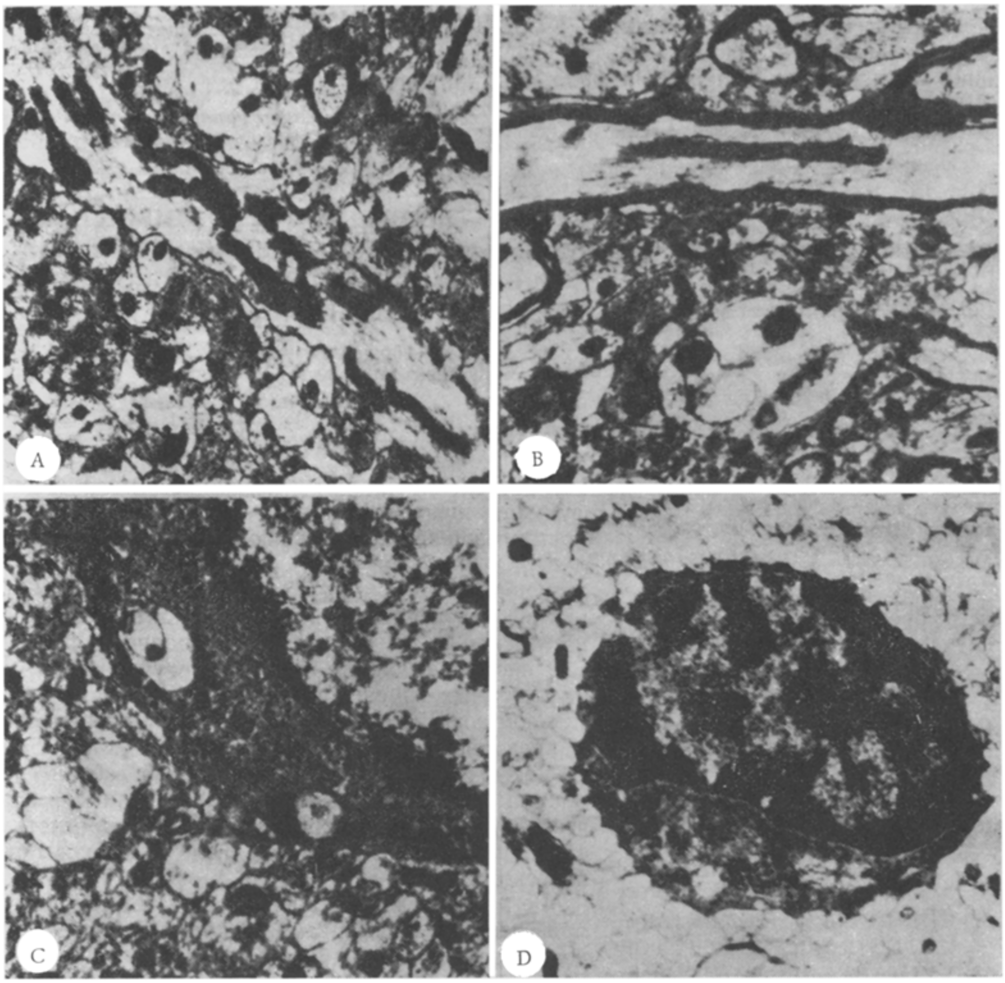

Fig. 1. Ultrastructural changes in rat hippocampus following exposure to NIMR with an intensity of 1000 $\mu W/cm^2$ for 2 months. A) Alveus layer: swelling of mitochondria in longitudinal section through unmyelinated nerve fiber, increase in their electron density, 10,800 ×; B) layer of polymorphic cells: considerable swelling and lengthening of mitochondrion in longitudinal section through myelinated nerve fiber, local demyelinization and thinning of neurofibrils, 16,000 ×; C) vacuolation of cytoplasm and change in structure of chromatin in cell of polymorphic layer, 15,800 ×; D) state of microglial cell of polymorphic layer, 14,600 ×.

Both the membranes of the nerve fibers and the neurofibrils of the axons, including their organelles, showed changes. The clearest changes were found in the mitochondria (Fig. 1A). The mitochondria were considerably enlarged and showed changes in their shape (twisted and very long) and electron density: The mitchondria were dark, and the structure of their membranes, the cristae, and septa could not be identified. The number of mitochondria was increased. Local demyelinization was observed in the form of translucency of the myelin layer. The Ranvier nodes and Schmidt—Lantermann incisures could not be identified. Uneven thinning of the neurofibrils and disturbance of their integrity were observed. Nearly all the nerve fibers were vacuolated. The vacuoles differed in shape and size. They were numerous. Sometimes drops similar to lipofuscin or modified lysosomes were found in the substance of the fibers.

Far fewer nerve fibers could be seen in the layer of polymorphic cells than in the alveus layer. Here also, however, fibers with modified structural elements could be distinctly identified. The character of the changes was similar: vacuolation, regressive transformation of myelin, uneven thinning and fragmentation of the neurofibrils, swelling of the mitochondria, an increase in their electron density, etc. (Fig. 1B). Nerve and glial cells whose structural elements also were considerably altered, were observed in this same layer. More marked changes were observed in the nerve cells (Fig. 1C). They contained vacuoles of vari-

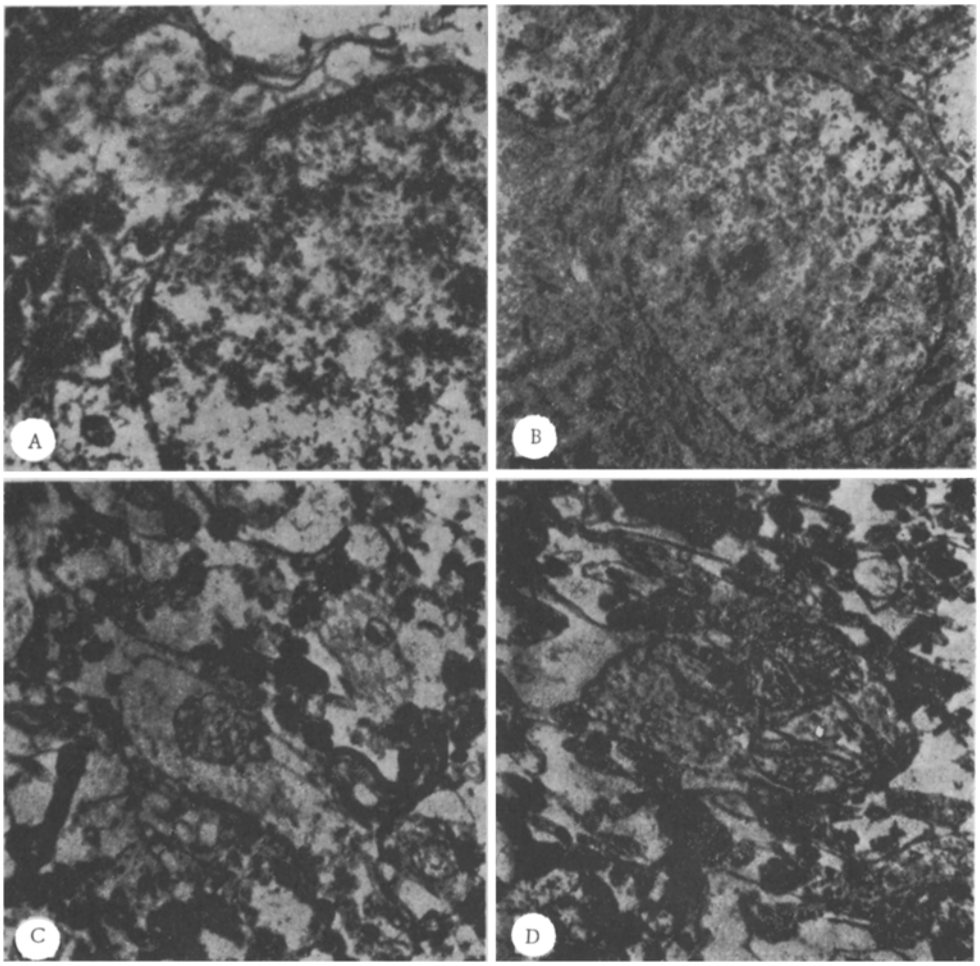

Fig. 2. Ultrastructural changes in hippocampus of rat following exposure to NIMR with intensities of 25 μW/cm² (A, B) and 10 μW/cm² (C, D) for 2 months. A) Part of cell body in pyramidal layer: accumulation of changed mitochondria in one part of cytoplasm and their absence in another part, 10,000 ×; B) near-normal state of cell ultrastructure in polymorphic layer, 4600 ×; C) transverse section through nerve fiber (alveus layer) with slight changes in mitochondrion, 9500 ×; D) unmyelinated nerve fiber of polymorphic layer, in which two mitochondria with different degrees of destruction of cristae and septa can be clearly distinguished, 16,000 ×.

ous shapes and sizes, in some parts of the cytoplasm the organelles had almost completely disappeared, pathological mitochondria were present in other parts of the cytoplasm, chromatin granules in the center of the nucleus showed considerable dispersion and disappearance and were concentrated near the membrane (translucency of the nucleus); ectopia of the nucleoli was observed. The cytoplasmic and nuclear membranes were ill-defined. Structural elements of the glial cells were changed by a lesser degree than those of the nerve cells. They had a more compact nucleus and somewhat translucent cytoplasm (Fig. 1D).

In the pyramidal cell layer the ultrastructure of the nerve cells also was altered. The character of the changes was similar to that discovered in the previous layers of this formation. However, their intensity and the frequency of their distribution were less than in the alveus layer and in the layer of polymorphic cells. Neurons with a nearly normal state of the nucleus were more often found here, although the cytoplasm of these neurons was vacuolated and contained pathologically changed mitochondria, but no other organelles, as shown by translucency of part of the cytoplasm.

In animals exposed to NIMR with an intensity of 50 μW/cm², vacuolation, local translucency, and disappearance of the myelin sheath, with uneven thinning and fragmentation or almost complete liquefaction of the neurofibrils were observed in the alveus layer in both myelinated and unmyelinated nerve fibers. Against this background pathologically changed mitochondria were more clearly defined. They were enlarged and had increased electron density. Their cristae, septa, and membranes could not be distinguished. No other organelles could be seen.

Changes in the ultrastructure of the cytoplasm and nucleus found in the layer of polymorphic cells were similar to those observed in the polymorphic layer of the hippocampus in animals in the previous series of experiments. However, the ultrastructure of the neuroglial cells was less severely disturbed.

Ultrastructural changes in the layer of pyramidal cells were similar in character to those in the corresponding layer of the hippocampus in animals in the previous series of experiments. However, the karyoplasm in most neurons was almost normal in appearance, with a normal state of the nucleoli. Such nuclei were observed even in neurons whose cytoplasm was modified.

In animals exposed to NIMR with an intensity of 25 and 10 $\mu W/cm^2$ less marked ultrastructural changes were found than in animals exposed to NIMR of higher intensity, and some almost normal cells could be seen in them (Fig. 2). Integrity of the neurofibrils was preserved more often in animals exposed to NIMR with an intensity of 25 $\mu V/cm^2$ than in animals of the other groups. After exposure to radiation with an intensity of 10 $\mu W/cm^2$, besides dark (with increased electron density) mitochondria others of a paler appearance were observed, whose structure could be partially made out, and they seemed to be almost normal. The endoplasmic reticulum was more clearly defined in this case (although it was modified) than in animals in the previous series of experiments, and neuroglial cells — macro- and microphages — also were visible. Their cytoplasm contained many inclusions of different types, and this was responsible for their electron density.

Sometimes structures resembling synapses were found in the animals of these groups. However, in view of the absence of many structures characteristic of synapses, these formations were not taken to be definitely synapses.

Prolonged exposure to NIMR with intensities of between 1000 and 10 $\mu W/cm^2$ (40 min each time, 3 times a day for 2 months) causes changes in hippocampal ultrastructure. The character of the changes discovered indicates processes in two opposite directions, namely destruction and repair, taking place at times in the same cell. The intensity of these processes depends on that of NIMR. The changes discovered in hippocampal ultrastructure may in all probability be reflected in its function and they may constitute one component in the pathogenesis of the early disturbances in persons exposed to this environmental factor.

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