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Kindling-Induced Hippocampal Cell Death in Rats

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Quantitative analysis of the main cells and interneurons in different layers and fields of Ammon's horn, the hilus, and dentate gyrus was performed two weeks and one month after kindling, i.e., specific electrical stimulation of the ventral hippocampus. At both experimental time points, cells numbers were significantly decreased at all levels of the areas studied except the marginal layer of field CA4. Thus, in this model of experimental epilepsy, the process of epileptogenesis involved both interneurons and main cells in the hippocampus. The possible reorganization of intrahippocampal circuits and their involvement in the increased excitability characteristic of epilepsy are discussed.

KEY WORDS: hippocampus, neurons, electrical stimulation, epileptogenesis.

Epileptic convulsions have significant influences on brain structure. The earliest morphological changes associated with prolonged convulsive activity consist of selective cell death in epileptogenic structures, primarily the hippocampus. The extent of these changes in different clinical and experimental forms of epilepsy depends on the nature and duration of the inducing factors, the time since the most recent convulsion, the age of the individual, phylogenetic characteristics, and many other factors; the temporal and spatial characteristics of hippocampal sclerosis differ in each concrete case [3, 7, 11]. Despite the fact that the development of hippocampal sclerosis in epilepsy has been addressed in many investigations, this process has only been partially studied; results obtained using the same model are sometimes contradictory. Thus, identification of the mechanisms of epileptogenesis requires further detailed investigation of cell death in different parts of the hippocampus. The most widespread clinical type of epilepsy is temporal epilepsy, which is characterized by the development of convulsive activity in structures of the temporal (auditory) lobe. Kindling – specific electrical stimulation of "epileptogenic" brain structures – provides an experimental model of this clinical type. The aim of the present work was to perform quantitative studies of different cell types in the individual layers and fields of the hippocampus itself (Ammon's horn), the hilus, and the dentate gyrus after kindling of the central hippocampus.

MATERIALS AND METHODS

Studies were performed using white mongrel male rats, which were divided into three groups each of five individuals. Group 1 consisted of animals kept in normal animal-house conditions (intact control animals). Rats of group 2 underwent implantation of electrodes into the ventral hippocampus under i.p. sodium ethaminal (40 mg/kg) anesthesia, though no stimulation was applied (sham stimulation controls). Similarly, electrodes were implanted into the ventral hippocampus of animals of group 3 (experimental group); the hippocampus was stimulated seven days after implantation using a fast kindling protocol [9]. At 24 h after the end of stimulation, five test stimuli were applied with 5-min intervals. Animals showing five sequential convulsions were selected for further investigation. Animals were perfused two weeks and one month after completion of test stimulation (group 3) and two weeks and one month

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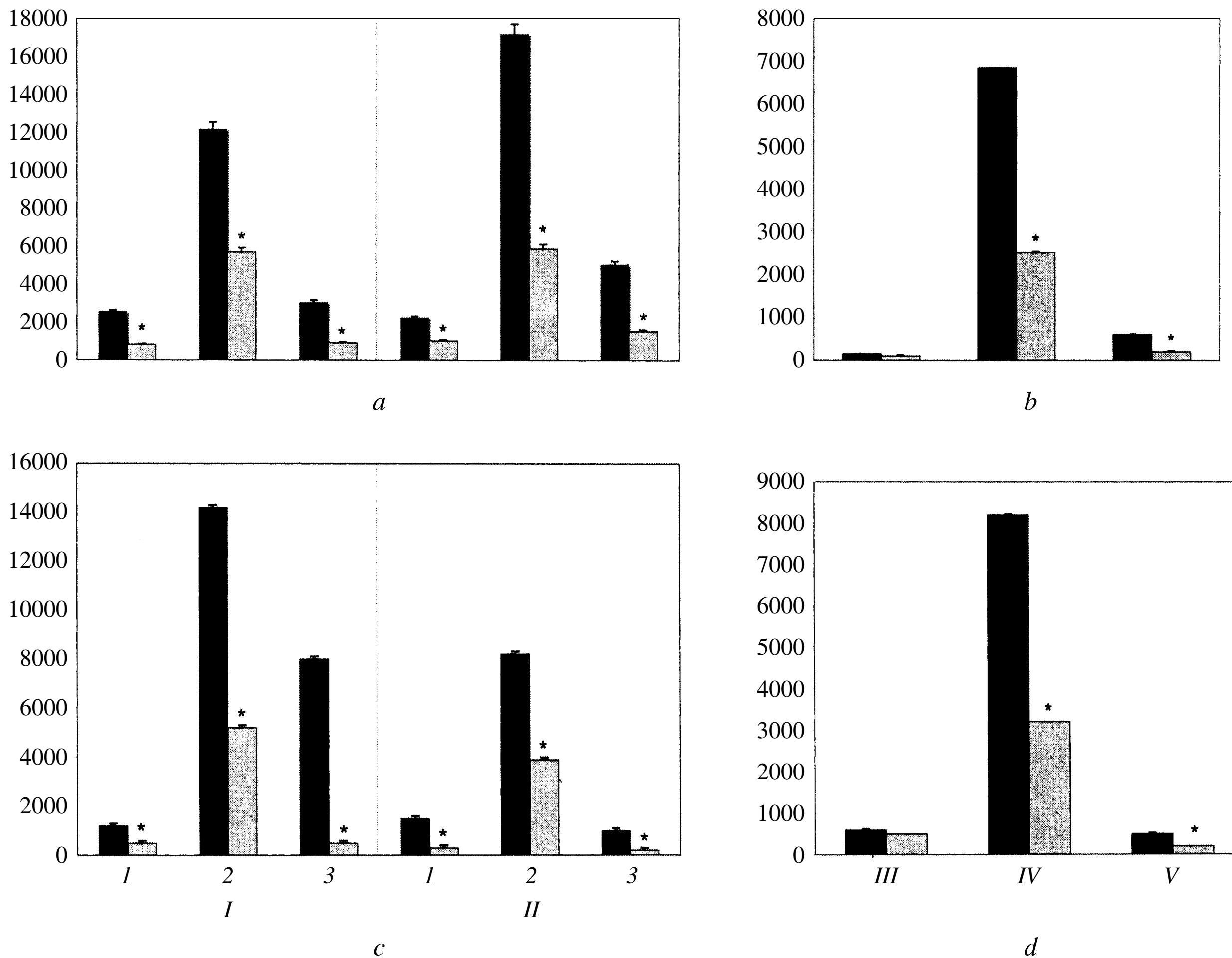

Fig. 1. Numbers of neurons in different areas of the hippocampus two weeks (*a*, *b*) and one month (*c*, *d*) after kindling of the ventral hippocampus of rats. Dark columns show control animals; light columns show experimental animals. On the abscissa: *I*) field CA1; *II*) field CA3; *III*) field CA4; *IV*) dentate gyrus; *V*) hilus; *1*) marginal layer; *2*) pyramidal layer; *3*) radial layer; the ordinate shows the numbers of neurons per unit volume tissue [12]; *significant differences compared with controls; vertical bars show standard errors.

after electrode implantation (group 2). Perfusion was performed under i.p. anesthesia with sodium ethaminal (40 mg/kg) by intra-aortic administration of 4% paraformaldehyde in phosphate buffer pH 7.2–7.4. Brains were then fixed in the same solution and cryoprotected in the appropriate fluids; a cryomicrotome was then used to cut serial sections of thickness 15 μm, which were stained with cresyl violet by the Nissl method. Stereological analysis of neuron counts was performed in the marginal radial layers and the pyramidal cell layer of fields CA1, CA3, and CA4 (the parts of Ammon's horn directly adjacent to the hilus), the hilus, and the dentate gyrus. Quantitative analysis of nerve cells was performed using every fifth section (10 sections from each animal) using an ocular morphometric grid with a ×40 objective and a ×10 ocular. Numbers of cells in hippocampal fields and the hilus were determined using the formula $N = Q^- \times 1/t$, where N is the total number of cells in the relevant volume of brain tissue from which the section was obtained; Q^- is the number of cells in the series of sections under investigation; t is 1/5 [12]. In the dentate gyrus, the number of cells in each section was determined using the formula $N = n \times S$ (n is the number of cells per unit area of the ocular grid, i.e., 0.000625 mm^2, and S is the area of the dentate gyrus calculated using the ocular grid). The statistical significance of results was assessed using the Minitab (Basic Study) program.

RESULTS

Two weeks after electrical stimulation of the ventral hippocampus, the number of neurons in each hippocampal field decreased as compared with the number in intact control animals. In particular: in field CA1, the number of cells in the

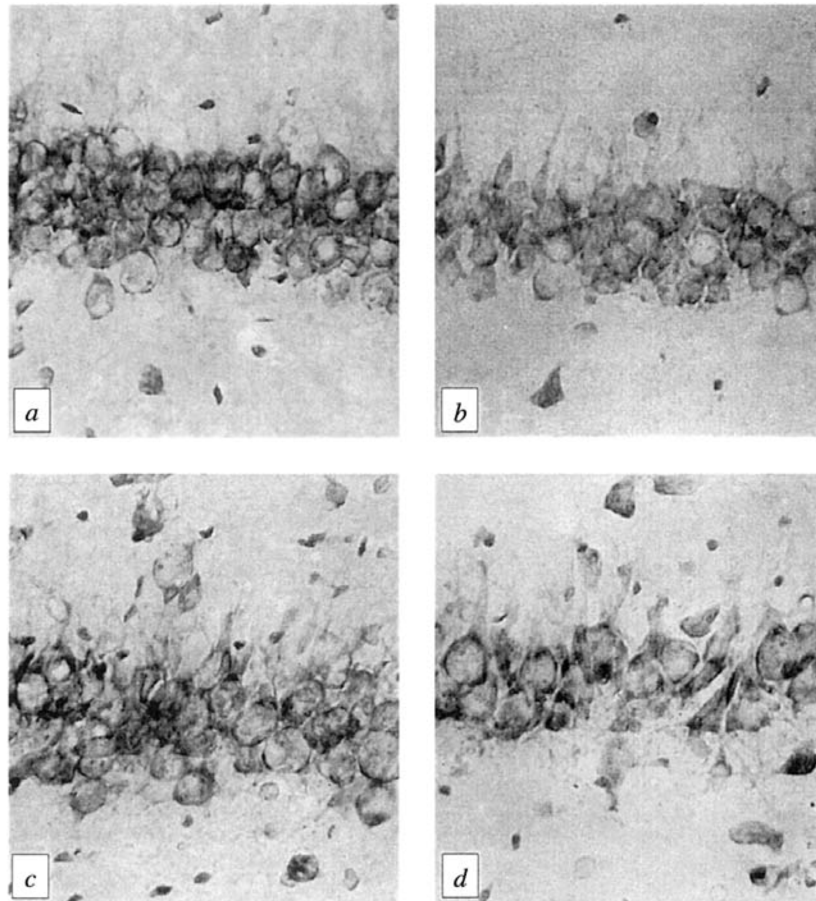

Fig. 2. Intrinsic hippocampal fields (Ammon's horn) in intact rats (*a*, *c*) and one month after kindling of the ventral hippocampus (*b*, *d*). *a*, *b*) Field CA1; *c*, *d*) field CA3. Stained with cresyl violet. Magnification ×400.

marginal layer decreased by 78% ($p = 0.001$), the number in the pyramidal layer decreased by 55% ($p = 0.006$), and the number in the radial layer decreased by 73% ($p = 0.01$); in CA3, the number in the marginal layer decreased by 68% ($p = 0.04$), the number in the pyramidal layer decreased by 67% ($p = 0.002$), and the number in the radial layer decreased by 77% ($p = 0.05$) (Fig. 1, *a*); in CA4, the number decreased by 12% ($p = 0.5$); in the hilus, the number decreased by 78% ($p = 0.03$); and in the dentate gyrus, the number decreased by 59% ($p = 0.04$) (see Fig. 1, *b*). In animals with implanted electrodes but without stimulation of the hippocampus, there were no significant changes in any of the fields.

The number of neurons in the hippocampus was also smaller than that in intact animals one month after electrical stimulation of the ventral hippocampus (see Fig. 1, *c*, *d*). In particular: in field CA1 (Fig. 2, *a*, *b*), the number in the marginal layer decreased by 55% ($p = 0.03$), the number in the pyramidal layer decreased by 59% ($p = 0.02$), and the number in the radial layer decreased by 77% ($p = 0.002$); in field CA3 (see Fig. 2, *c*, *d*), the number in the marginal layer decreased by 68% ($p = 0.05$), the number in the pyramidal layer decreased by 56% ($p = 0.03$), and the number in the radial layer decreased by 70% ($p = 0.03$) (see Fig. 1, *d*); in field CA4, the number decreased by 26% ($p = 0.08$); in the hilus, the number decreased by 68% ($p = 0.02$); in the dentate gyrus, the number decreased by 68% ($p = 0.02$) (see Fig. 1, *d*). In contrast, animals with implanted electrodes but without stimulation showed no significant changes in neuron counts.

DISCUSSION

Thus, fields CA1, CA3, the hilus, and the dentate gyrus showed significant reductions in the numbers of cells of different types two weeks after kindling. The death of interneurons in the hilus and field CA3 has been described in most forms of experimental epilepsy. This characteristic mediates the pathological innervation by mossy fibers of

neurons in the lower sublayer of the molecular layer of the dentate gyrus (one of the most important morphological signs of an absolute majority of types of epilepsy and epilepsy-like states), facilitating the formation of pathological excitatory states. In our material, significant reductions in cell counts were seen in the upper and lower layers in fields CA3 and CA1, where interneurons of different chemical assignment, structure, and function are concentrated, and in the pyramidal layer, which suggests significant losses of both intrinsic and main cells of the two major sections of the hippocampus. Thus, by just two weeks after kindling, there must be a significant reorganization of intrahippocampal circuits. At both two weeks and one month, cells in Ammon's horn, directly adjacent to the hilus (field CA4) were the most persistent – the decrease in the number of cells in this part of the brain was insignificant. However, data have been obtained from studies of specimens from patients and animals with the corresponding experimental models showing that some zones of the hippocampus undergo more marked rearrangements in receptors (primarily GABAergic) than cell death at certain stages of epileptogenesis [2, 3, 5]. It has also been demonstrated that at some stages of the development of the pathological process, the structure of surviving interneurons in tissues of the dentate gyrus and hilus in humans and animals undergo compensatory rearrangements directed to balancing the increased excitability of the inputs [7]. In this process, an important role must be played by cytokines and growth factors, including tumor necrosis factor, interleukin-1 receptors, and basic fibroblast growth factor, increased expression of which has been described in the hilus in several forms of experimental epilepsy: acting as trophic and protective factors, they facilitate cell survival without affecting convulsion parameters such as duration, intensity, etc. [8, 11]. It is also interesting that in the marginal layer of field CA1 one month after stimulation, the number of cells (interneurons) was significantly increased compared with the number at two weeks. It is known that neurogenesis (along with cell death, gliosis, and sprouting) is an important morphological manifestation of an absolute majority of types of clinical and experimental epilepsy. Developing as a result of convulsion-induced cell death, it has been described predominantly in the dentate gyrus [8, 11]. More recent studies, however, have demonstrated that newly formed cells from the subventricular zone migrate both to the dentate gyrus and to other damaged parts of the brain, including the striatum and neocortex in different pathological states; it was suggested that neurogenesis may also occur within these areas themselves [1, 4, 6]. For example, studies of a model of Alzheimer's disease showed increased neurogenesis in field CA1 [4].

There are two hypotheses explaining how convulsion-mediated cell death initiates the process of epileptogenesis. The first states that normal inhibition and excitation are maintained by "non-main" cells exposed to the causative influence; death of these cells inactivates inhibitory interneurons such that main cells are disinhibited and become hyperexcited. The second hypothesis holds that initial cell death stimulates main cells which did not previously contact each other to form aberrant recurrent excitatory connections. Additional influences include the kindling process, which gradually weakens postsynaptic inhibition, as well as changes in the intercellular space facilitating synaptic and ephaptic depolarization [10].

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