

# Effect of Kindling on GABAergic Neurons of the Hippocampus and Piriform Cortex

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GABAergic neurons in different fields of the hippocampus and piriform cortex were examined 2 weeks and 1 month after electric stimulation of the ventral hippocampus. The counts of GABAergic neurons in the studied structures decreased significantly. The most pronounced shifts in the piriform cortex were found in the central compartment. Decreased number of inhibitory elements in the two major epileptogenic structures attests to appreciable restructuring in the functions of their neuronal circles.

**Key Words:** GABAergic cells; kindling; hippocampus; piriform cortex

Even a slight decrease in GABAergic synaptic inhibition modifies the function of CNS. It remains unclear whether decreased inhibition is an obligatory component of hyperexcitability, typical of epilepsy. In some acute models of epilepsy and epileptiform status cell loss in epileptogenic zones correlates with the decrease in synaptic inhibition [1]. This correlation is not obligatory for chronic experimental epilepsy: cell loss is not paralleled by disorders in GABAergic inhibition [11] or can take place even in the presence of increased inhibition (for example, enhanced release of the transmitter [11] or enhanced regulation of postsynaptic GABAergic receptors [6]). The data on the direction of changes in the GABAergic system of patients with epilepsy of the parietal lobe also attest to decreased, retained, or increased GABAergic synaptic inhibition [5]. Evaluation of the role of GABAergic system in epileptogenesis remains an important problem. In light of this, studies of GABA-containing neurons in structures involved in epileptogenesis in various forms, stages, and terms of experimental epilepsy are very promising.

Kindling (specific electric stimulation of the cerebral epileptogenic zones) is the most adequate method for studying chronic temporal epilepsy. We studied GABAergic neurons in different fields of the hippocampus and piriform cortex 2 weeks and 1 month after kindling of the ventral hippocampus.

## MATERIALS AND METHODS

The study was carried out on 15 outbred male laboratory rats. Control group ( $n=5$ ) consisted of animals kept under standard vivarium conditions. In experimental group electrodes were implanted into the ventral hippocampus [8] under intraperitoneal narcosis (4% chloralhydrate, 40 mg/kg); on day 7 after surgery this structure was electrically stimulated by the rapid kindling protocol [9]. Twenty-four hours after the last stimulation the animals received 5 test electric stimulations at 5-min intervals. The animals exhibiting stage 4 convulsions [9] 5 times in succession were examined. The brain was isolated 2 weeks and 1 month (5 animals per term) after test stimulations. All animals were perfused with 0.9% NaCl and then with 4% paraformaldehyde on phosphate buffer (pH 7.4) through the aorta under chloralhydrate (4% solution) narcosis. The brain was fixed in the same fixative and stored at  $-70^{\circ}\text{C}$ . Serial coronal sections (15  $\mu$ ) were made on a

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freezing microtome, washed in phosphate buffer, and fixed on polylysine-coated slides. Every 5th section was immunocytochemically stained for detecting GABA-containing cells with polyclonal antibodies (GAD-67) to glutamate decarboxylase (GAD) using ABC method (all antibodies, AB complex, solutions and buffers from Santa Cruz Biotech). Staining was carried out according to manufacturer's protocol, with subsequent intensification of the preparations with osmium tetroxide. Stereological analysis of GAD-immunopositive cells in hippocampal CA1 and CA3 fields and in the anterior, central, and posterior compartments of the pyriform cortex was carried out using a morphometrical grid (0.000625 mm;  $\times 800$ ) in 30 randomly selected visual fields. Cell counts in the hippocampal and hilus fields were estimated by the formula:  $N = Q \times 1/t$ , where  $N$  is the total cell count in an arbitrary volume of cerebral tissue from which the sections were made,  $Q$  is cell count in the given series of sections, and  $t$  is  $1/5$  [13]. The statistical significance of the data was evaluated using Basic Statistic software (Minitab).

## RESULTS

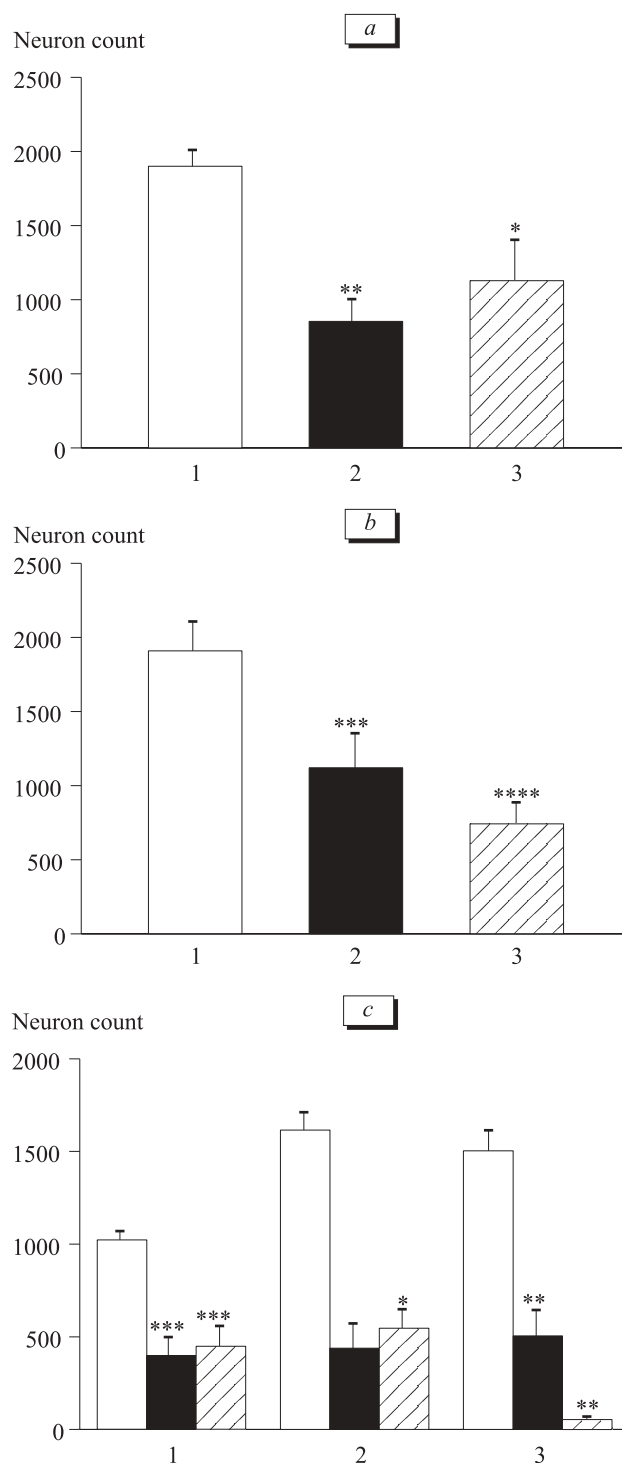
Kindling significantly decreased the number of GAD-positive cells in the hippocampal fields in comparison with the control: after 2 weeks their count in CA1 field decreased by 56% and after 1 month by 41%, in CA3 field it decreased by 42% after 2 weeks and by 61% after 1 month (Fig. 1). The differences between the values in different terms were also significant. The decrease in cell count in CA1 field was more pronounced after 2 weeks than after 1 month ( $p=0.03$ ), while in CA3 field prolongation of the experimental period led to a progressive "loss" of cells ( $p=0.007$ ).

The number of GAD-positive cells decreased after kindling in all compartments of the pyriform cortex. After 2 weeks this decrease in the anterior compartment was 59%, after 1 month 56%, in the central compartment 73% after 2 weeks and 66% after 1 month, in the posterior compartment 66% after 2 weeks and 63% after 1 month. No differences between the values in different terms were observed.

Changes in the count of GAD-positive neurons are expected to reflect the immediate effect of kindling. Decrease in the number of inhibitory structural elements indicates appreciable restructuring in the functioning (presumably, hyperexcitation) of the neuronal circles of the two major limbic structures: pyriform cortex and hippocampus.

The direction of shifts in the number of GAD-positive cells in different hippocampal fields was different: the changes in CA1 field were more pronounced after 1 month than after 2 weeks, while in CA3

field the number of GAD-positive cells decreased with prolongation of the experimental period. Neurogenesis, most probably of adaptation nature, was observed



**Fig. 1.** Cells loss in CA1 (a) CA3 (b) and pyriform cortex (c) after ventral hippocampus kindling. Anterior (1), central (2), and posterior (3) compartments of pyriform cortex. Ordinate: neuron count in arbitrary volume. Light bars: control; dark bars: kindling (2 weeks); cross-hatched bars: kindling (1 month). \* $p=0.001$ , \*\* $p=0.002$ , \*\*\* $p=0.01$ , \*\*\*\* $p=0.05$  compared to the control.

at different stages of epileptogenesis in many epileptogenic structures under the effects of cytokines and growth factors [2,12]. The possibility of migration of neuronal precursors from the supraventricular zone and rostral migration flow into ectopic zones in the dentate fascia, olfactory tubers, striatum, septum, visual tuber, and neocortex in some pathological conditions, including experimental epilepsy, is hypothesized [7,10].

According to our data, the central zone is most of all involved in epileptogenesis in kindling of the hippocampus: GABAergic neurons are notably lost in this compartment. These cells are most numerous in this compartment of the cortex; presumably, it is directly involved in generalization of convulsions [4].

Many specific inhibitory processes, essential for optimal operations, cannot be realized by strictly defined neuronal populations. GABA-containing neurons of the pyriform cortex and hippocampus are morphologically and biochemically heterogeneous and differ by the content of calcium-binding proteins, soma structure, dendritic and axonal processes, and molecular phenotype. Specific distribution of the dendritic tree is worthy of note from the functional viewpoint. Accurate definition of the nature of GABAergic neurons of the hippocampus and pyriform cortex, "suffering" during kindling, is needed.

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