Effect of Acoustic Stimulation on Cell Composition of Auditory Brain Structures in Krushinskii—Molodkina Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 10, pp. 400-402, October, 2006
Original article submitted June 22, 2005

We studied the effect of acoustic stimulation on cell composition of auditory brain structures in Krushinskii—Molodkina rats. Significant structural changes in the inferior colliculi of rats with high seizure activity were revealed 1 month after acoustic stimulation. Therefore, this brain structure plays a role in the development of audiogenic epileptic activity.

Key Words: *Krushinskii-Molodkina rats; inferior colliculi of the quadrigeminal plate; auditory cortex; quantitative cell analysis*

Krushinskii—Molodkina rats are genetically predisposed to the development of seizure activity in response to acoustic stimulation. These animals are a natural model to study the mechanisms of hereditary and audiogenic epilepsy. The main cause of these disorders is a deficiency of GABAergic transmission [3-5]. Biochemical and electrophysiological studies showed that this deficiency accompanies the increase in excitation transmission and is most pronounced in auditory structures, particularly in the relay structure (inferior colliculi of the quadrigeminal plate) [4-6]. The number of basket and GABA-containing neurons is maximum in this structure. The presence of a considerable number of these cells should contribute to the formation of the epileptic focus [5,7]. It was hypothesized that rats with genetic predisposition to audiogenic seizures differ from healthy animals in the structure of $GABA_A$ergic receptors in auditory structures [5,6]. It remains unknown whether seizure activity induced by epileptogenic acoustic stimulation can cause structural changes in auditory brain structures of these rats.

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Neuronal death is a typical morphological sign of various forms of epilepsy. Quantitative analysis of main cells (interneurons and GABA-containing cells) in the central nucleus of the inferior colliculi and anterior and posterior regions of the auditory cortex in Krushinskii—Molodkin rats was performed 1 month after audiogenic epileptic activity induced by acoustic stimulation.

MATERIALS AND METHODS

Experiments were performed on 9 adult male Krushinskii—Molodkina rats (main group) and 7 male outbred albino rats (control group). The animals were placed in a chamber for acoustic isolation. An electric bell was fixed in the upper part of a Plexiglas box (80×80×80 cm). Acoustic stimulus was presented over 1 min. The motor component was evaluated by the Jobe scale with minor modifications. This component remained unchanged in control rats, while animals of the main group demonstrated epileptic activity of different degree. Further experiments were performed on rats of the main group with maximum seizure activity (limb clonus, rigidity of skeletal muscles, lateral posture, ataxia, and asphyxia) [8]. Animal behavior was studied daily at 9:30-12:30 and 16:00-19:00 over 1 month

0007-4888/06/1424-0419

after acoustic stimulation. Seizure activity was not observed during this period. One month after stimulation, the rats of both groups were perfused with 4% solution of paraformaldehyde in phosphate buffered saline (pH 7.2-7.4) through the carotid artery under sodium ethaminal anesthesia (40 mg/kg). The brain was postfixed in the same solution, cryoprotected, and frozen at 80°C. Serial coronary sections (10 μ) were prepared on a freezing microtome and fixed on polylysine-coated slide plates. They were treated with cresyl violet (Nissl's method) or subjected to immunohistochemical staining to detect GABA-containing cells. Polyclonal GAD-67 against glutamate decarboxylase served as the primary antibody. Antibodies (AB) were used as the detection system. Antibodies, AB complex, solutions, and buffers were manufactured by Santa Cruz Biotechnology. Staining was performed according to manufacturer recommendations. The preparations were intensified with osmium tetroxide. Stereological study of the total number of neurons, interneurons, and GABA-containing cells [8] in the central nucleus of the inferior colliculus of the quadrigeminal plate and all layers of the anterior and posterior regions of the auditory cortex was performed as described elsewhere [2]. During quantitative analysis, we took into account the cytoarchitectonics of examined structures in cresyl violet-stained preparations. Large (main cells) and small cells (interneurons) were counted in the central nucleus of the inferior colliculi. Pyramidal cells were counted in layers III-IV of the auditory cortex. Interneurons were counted in layers I, II, V, and VI. The total number of GABAergic cells was estimated in immunohistochemically stained preparations. Nerve cells were counted in each 5th section (10 sections from the animal) using an ocular morphometric grid (×800). The number of cells was calculated as follows: $N=Q^{-}/t$, where N is total cell number in the relative volume of dissected brain tissue; Q^{-} is cell number in the series of sections; and $t={}^{1}/_{5}$. The results were statistically analyzed by means of MINITAB software (Basic Study).

TABLE 1. Number of Various Cells in the Central Nucleus of Rat Auditory Cortex 1 Month after Acoustic Stimulation (Count of Neurons in the Relative Volume, *M*±*m*)

Parameter	Control group	Main group	*p*
Large cells (main cells)	1623±78	1155±140	0.02
Small cells (interneurons)	1408±93	1060±54	0.02
GABA-containing cells	737±73	533±5	0.04

RESULTS

The number of large (main cells) and small cells (interneurons) in the central nucleus of the inferior colliculi decreased by 29 and 25%, respectively, 1 month after acoustic stimulation (Table 1).

The number of various cells also decreased in the auditory cortex (statistically insignificant, Table 2). We revealed a decrease in the number of layer I interneurons (by 51%), layer II granular cells (by 8%), layer II pyramidal cells (by 18%), layer III pyramidal neurons (by 8%), layer IV small granular cells (by 8%), layer IV pyramidal cells (by 23%), layer V pyramidal cells (by 8%), and layer VI interneurons (by 6%) and total count of GABAergic cells (by 6%) in the anterior region. We also found a decrease in the number of layer I interneurons (by 24%), layer II granular cells (by 8%), layer II pyramidal cells (by 16%), layer III pyramidal cells (by 13%), layer IV granular cells (by 20%), layer IV pyramidal cells (by 10%), layer V pyramidal cells (by 15%), layer VI interneurons (by 5%), and GABAergic cells (by 9%) in the posterior region. A signi-

TABLE 2. Number of Various Cells in Rat Auditory Cortex 1 Month after Acoustic Stimulation (*M*±*m*)

Parameter	Anterior region of the auditory cortex			Posterior region of the auditory cortex		
	control	treatment	*p*	control	treatment	*p*
Layer I interneurons	565±15	275±15	0.01	445±35	340±40	0.04
Layer II granular cells	670±11	418±73	0.1	465±65	432±3	0.5
Layer II pyramidal cells	1755±105	1625±45	0.2	1315±75	1110±24	0.3
Layer III pyramidal cells	1005±32	930±11	0.3	1015±75	890±60	0.1
Layer IV granular cells	630±30	580±30	0.1	680±90	545±85	0.2
Layer IV pyramidal cells	1472±48	1290±50	0.02	1050±10	955±26	0.6
Layer V pyramidal cells	1335±20	1235±55	0.5	1225±65	1045±15	0.09
Layer VI interneurons	1345±135	1270±10	0.5	1330±40	1275±55	0.3

ficant change in the cytoarchitectonics of the inferior colliculus of the quadrigeminal plate (relay structure) was revealed 1 month after the incidence of seizure activity induced by acoustic stimulation. This conclusion was derived from the significant decrease in the number of main cells (exciting pyramidal cells, interneurons, and GABA-containing neurons). Our results provide support for published data that the inferior colliculus and GABAergic system of this structure play an important role in the development of audiogenic epileptic activity [3-5]. However, seizure activity practically does not modulate the cytoarchitectonics of the higher auditory region (auditory cortex). The observed features are mainly related to high compensatory properties of a phylogenetically new and highly organized nervous tissue. These data are consistent with the results of electrophysiological studies. It was shown that auditory epilepsy is accompanied by changes in subcortical auditory structures, but not in the neocortex [3,5]. Our previous experiments demonstrated a more significant decrease (by 50-80%) in the number of main cells, interneurons, and GABAergic cells in limbic structures (hippocampus and piriform cortex) under similar experimental conditions [1]. It can be hypothesized that audiogenic seizure activity significantly modulates the structure of emotiogenic zones, but has little effect on structural characteristics of the subcortical auditory region directly reacting to specific epileptogenic stimulation. GABAergic neurons of the quadrigeminal plate are heterogeneous (similarly to cells in other brain regions). It is important to estimate the subtypes of GABAergic cells in auditory regions that directly respond to epileptogenic stimulation.

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