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Morphofunctional State of Neurons in the Temporal Cortex and Hippocampus in Relation to the Level of Spatial Memory in Rats after Ablation of the Olfactory Bulbs

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Ablation of the olfactory bulbs (bulbectomy) in mice and guinea pigs evokes a neurodegenerative process which, in terms of its morphological, biochemical, and behavioral features, is similar to Alzheimer's disease. We report here studies of the long-term sequelae of bulbectomy in rats. One year after surgery, testing of spatial memory in bulbectomized rats (BER) allowed the animals to be divided into two groups – those with good memory (BER-gm) and those with poor memory (BER-pm). Quantitative analysis of the morphofunctional state of neurons showed that BER-pm, as compared with the BER-gm group, had more marked pathological lesions in neurons of the temporal cortex and hippocampus, with significant increases in the numbers of cells showing pyknosis, karyolysis, cytolysis, and vacuolization. Both groups showed decreases in the distribution density of cells in the cortex. In terms of the level of brain β-amyloid, the study groups fell in the order: BER-pm > BER-gm > control sham-operated rats. These results provide evidence of the long-term nature of changes in the morphofunctional state of neurons in the brains of BER, correlating with their levels of spatial memory.

KEY WORDS: bulbectomy, spatial memory, temporal cortex, hippocampus, neurodegeneration.

Previous studies have demonstrated that ablation of the olfactory bulbs (bulbectomy; BE) in mice initiates a neurodegenerative process in those brain structures with which the olfactory bulbs are connected by direct and indirect pathways: the hippocampus, the temporal cortex, and the basal acetylcholine-synthesizing magnocellular midbrain nucleus [2, 4, 8]. Deterioration of the morphofunctional state of these structures occurs on the background of increases in the content of brain β-amyloid and is accompanied by sharp impairments in spatial memory as tested in the Morris water maze [1]. Furthermore, β-amyloid deposition in the form of plaques has been observed in the cortex and hippocampus in guinea pigs at different stages of the post-operative period [7]. These data led to the suggestion that olfactory bulb lesions have a role in the genesis of Alzheimer's disease. This allowed bulbectomized animals (BER) to be regarded as a valid model of the sporadic form of this disease. However, the question of the duration of morphological, behavioral, and biochemical changes evoked by BE remains unanswered. The aim of the present work was therefore to study the effects of ablation of the olfactory bulbs on these characteristics.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n = = 21). Animals were kept in cages in groups of 5–7 individuals with free access to water and food, natural illumination, at a temperature of 22–23°C. Rats underwent BE at age eight months in sterile conditions under Nembutal anesthesia (40 mg/kg, i.p.), with s.c. 0.5% novocaine for local

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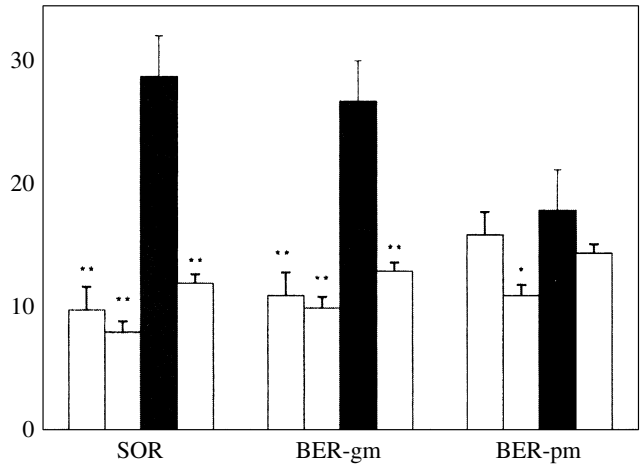

Fig. 1. Spatial memory in rats after ablation of the olfactory bulbs. Light columns show indifferent sectors; dark columns show the training sector; the horizontal axis shows SOR (sham-operated animals, controls), BER-gm, bulbectomized animals with good memory, and BER-pm, bulbectomized animals with poor memory; the ordinate shows the time spent in each sector (sec). Significant differences compared with controls: $*p < 0.05$; $**p < 0.001$. Vertical bars show standard errors.

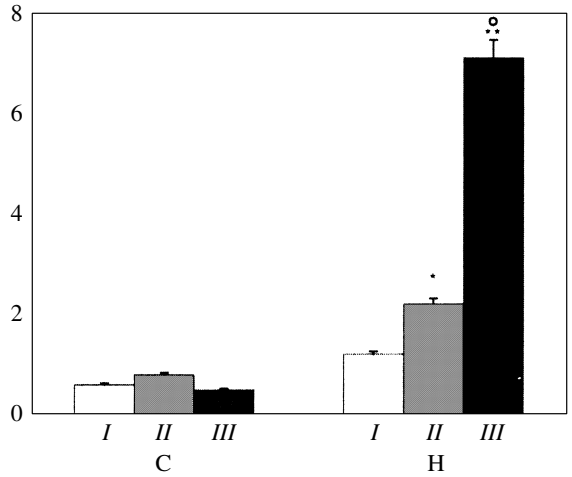

Fig. 2. β-Amyloid levels in the temporal cortex (C) and hippocampus (H) in rats after ablation of the olfactory bulbs (bulbectomy). The horizontal axis shows: *I*) sham-operated animals (controls); *II*) bulbectomized rats with good memory; *III*) bulbectomized rats with poor memory; the ordinate shows β-amyloid levels (μg/g of tissue); Significant differences compared with controls: $*p < 0.01$; $**p < 0.001$; °significant differences between measures in groups *II* and *III* at $p < 0.001$. Vertical bars show standard errors.

anesthesia during scalping. Bilateral BE was performed by aspiration of the olfactory bulbs through a trepanned opening. Controls consisted of sham-operated rats (SOR), subjected to an analogous procedure but without ablation of the olfactory bulbs. One year after surgery, rats were trained to a navigational reflex in a Morris water maze [10]. For this, rats were trained over a period of six days (four sessions per day) to find a rescue platform submerged beneath the water surface in one of the sectors of the basin. Spatial memory in trained animals was tested in 1-min trials in the basin without the rescue platform. The level of spatial memory, i.e., the ability to locate the training sector, was assessed in terms of the time spent by the rat in each of the four sectors of the water maze. All experiments were performed in accord with the "Regulations for Studies with Experimental Animals" (Decree of the Ministry of Health of the USSR, August 12, 1997, No. 755).

After memory testing, animals were decapitated under ether anesthesia. Brains were fixed in 4% paraformaldehyde solution in phosphate buffer pH 7.4 for one week, which was followed by washing for one day in tap water. A rotating freezing microtome with a selenium rectifier (Reichert, Austria) was used to cut sections of thickness 10 μm. Frozen brain sections were stained with cresyl violet (Kodak, USA) in 0.1 M acetate buffer pH 3.5 at a ratio of 1:10 using a modified Nissl method. Staining was performed for 20 min, after which sections were placed in acetate buffer for 1–2 min, washed, and dehydrated in ethanol concentrations gradually increasing to 100°, rinsed in xylene, and embedded in Damarlak. Morphofunctional analysis was performed on every fifth section of temporal cortex (the superior and inferior areas) and hippocampal fields CA1 and CA3. Neuron shape, size, and staining intensity were assessed, along with nuclear and cytoplasmic integrity; numbers of cells with pyknosis, karyolysis, cytolysis, and vacuolization were counted. This analysis was based on 1000 cells in 10 microscope fields for each structure (objective ×40, ocular ×10 with an embedded grid). In addition, neuron density per mm^2 was determined in each structure. The state of cellular structures was identified using a ×100 immersion objective.

Levels of β-amyloid in the temporal cortex and hippocampus were estimated using a modified immuno-DOT method with anti-β-amyloid monoclonal antibody 4G8 (Sigma, USA) [1].

The significance of differences between measures in control and experimental rats was determined using Student's *t* test.

RESULTS

Testing of memory in animals trained to the navigational skill at one year post-BE showed that the experimental group was heterogeneous in terms of the ability to locate the training sector (Fig. 1). These results provide evidence that only half the trained animals showed impairment of spatial memory, which was apparent as the inability to

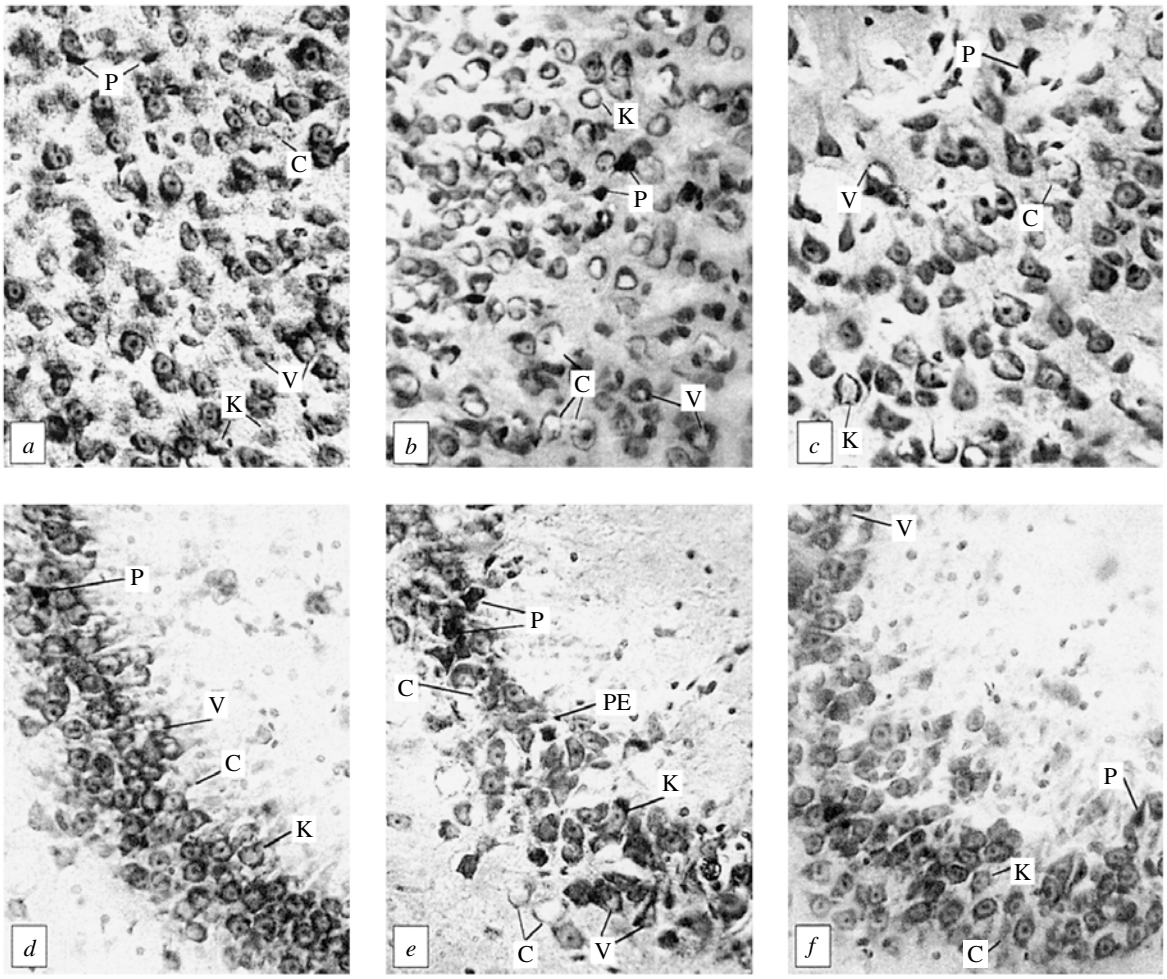

Fig. 3. Temporal cortex neurons (*a–c*) and hippocampal field CA3 neurons (*d–f*) in rats. *a*, *d*) Sham-operated rats; *b*, *e*) bulbectomized rats with poor memory; *c*, *f*) bulbectomized rats with good memory. P = pyknosis; C = cytolysis; K = karyolysis; V = vacuolization; PE = perinuclear edema. Stained with cresyl violet. *a–c*) objective ×40, ocular ×10; *d–f*) objective ×20, ocular ×10.

locate the sector in which the rescue platform had been located during training. These data allowed the BER group to be divided into two subgroups, with good spatial memory (BER-gm) and poor spatial memory (BER-pm).

Analysis of β-amyloid contents in the temporal cortex and hippocampus in BER showed that there was a significant increase in the hippocampus of BER-pm (Fig. 2). A significant increase in β-amyloid content in this structure was also seen in BER-gm as compared with controls, though the increase was significantly smaller than that in BER-pm. In the temporal cortex of BER, there was no increase in β-amyloid content as compared with controls.

The completeness of ablation of the olfactory bulbs in BER was verified macroscopically. Morphological analysis of brain sections from these animals showed that the temporal cortex and hippocampus contained normally functioning neurons with an intermediate cytoplasmic staining intensity and uniformly distributed chromatophilic material, a centrally located nucleus, and a clearly distinguishable nucleolus; there were also cells with pathological changes including pyknosis, cytolysis, karyolysis, and vacuolization (Fig. 3). Pyknomorphic neurons were characterized by having irregular, angular, and sharply extended shapes. Cells with karyolysis showed sharp distortion of nuclear shape with displacement of the nucleus to the periphery of the cell and partial lysis. Cytolysis of neurons in these brain structures was indicated by ectopic positioning of the nucleus, partial disappearance of the cell profile, lysed chromatophilic material, fragmentation and coagulation of chromatin clumps, and displacement of clumps to one pole of the cell. The animals' brains contained vacuolized neurons with hypertrophic nuclei and some vacuoles in the cytoplasm, which sometimes fused into large lacunae. This was most characteristic for cells in the temporal cortex of BER,

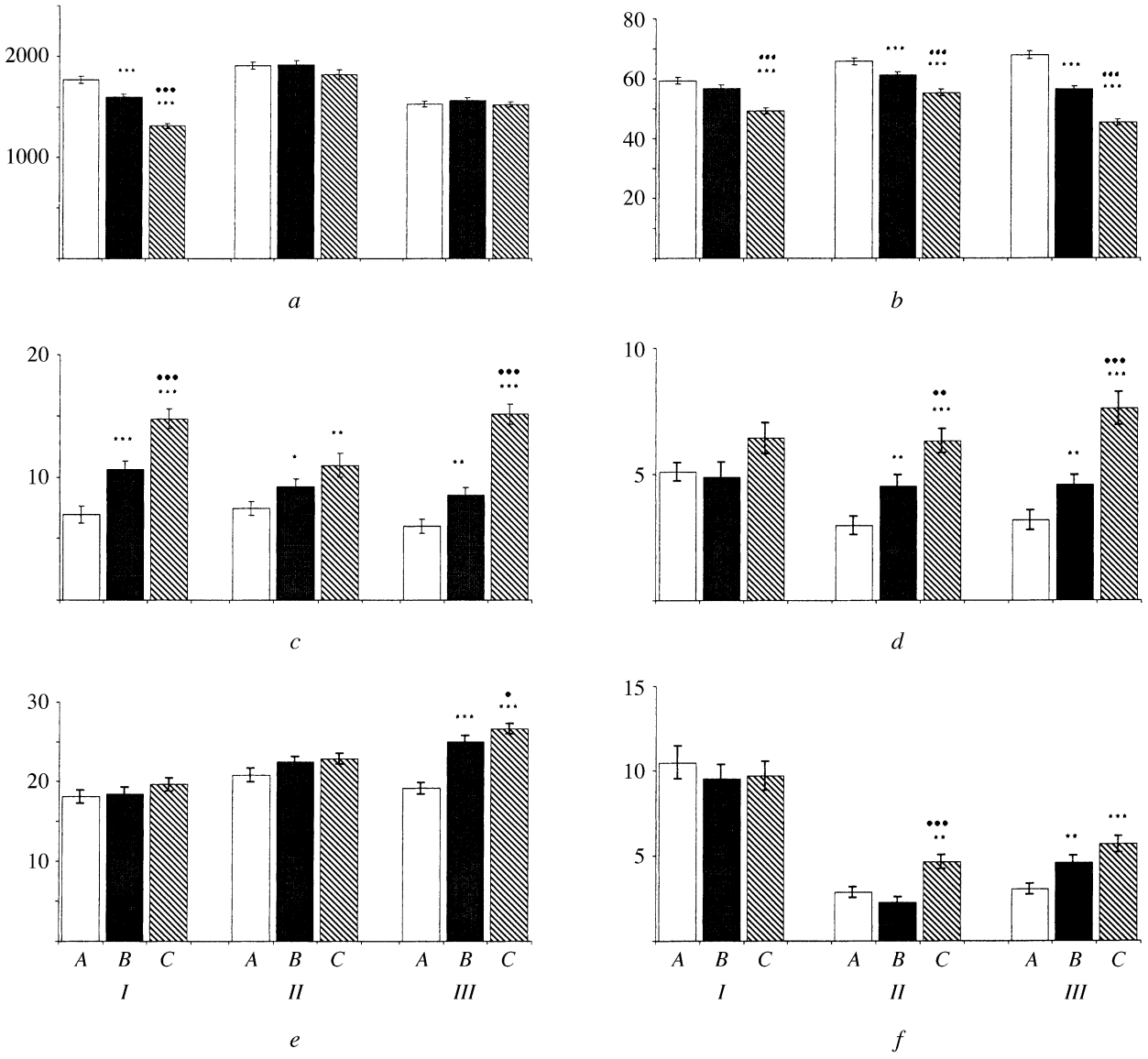

Fig. 4. Quantitative analysis of morphological changes in neurons in the temporal cortex and hippocampus (fields CA1 and CA3) in rats after ablation of the olfactory bulbs (bulbectomy – BE). Abscissa: *I*) temporal cortex; *II*) hippocampal field CA1; *III*) hippocampal field CA3; *A*) sham-operated rats; *B*) bulbectomized rats with good memory (BER-gm); *C*) bulbectomized rats with poor memory (BER-pm); ordinate: *a*) number of neurons per 1 mm^2; *b–f*) relative neuron contents: *b*) normal; *c*) pyknotic; *d*) with karyolysis; *e*) with cytolysis; *f*) with vacuolization. Significant differences in the BER-pm group compared with controls: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Significant differences in the BER-pm group compared with the BER-gm group: •$p < 0.05$; ••$p < 0.01$; •••$p < 0.001$. Vertical bars show standard errors.

where perivascular and pericellular hydropic lacunae were also seen close to small vessels. These changes were often found in layers II, III, and V of the temporal cortex of BER-pm and were accompanied by destruction of cytoarchitectonics, which led to erasure of the boundaries between layers with appearance of foci of cellular depletion. This resulted in a decrease in neuron density (Fig. 4, *a*). In the hippocampus, differences in this factor between groups were insignificant.

Quantitative analysis of morphological changes to neurons in hippocampal fields CA1 and CA3 in BER demonstrated a significant reduction in the proportion of normally functioning cells as compared with controls (see Fig. 4, *b*). In the temporal cortex, a decrease in the number of normal cells was seen only in BER-pm, which also showed a significant increase in pyknomorphic neurons. The reduction in the number of normal cells in the hippocampus of BER was accompanied by an increase in pyknotic cells and cells with

karyolysis. Hippocampal field CA3 of BER showed an increase in the number of neurons with dystrophic changes such as cytolysis and a form of hydropic dystrophy, i.e., vacuolization. In the BER-pm group, all of these changes were more marked, as shown by the significant differences between the BER-gm and BER-pm groups. Thus, more severe derangements to the morphofunctional state of neurons in the temporal cortex and hippocampus were seen in BER-pm, while BER-gm occupied an intermediate position between BER-pm and control animals.

DISCUSSION

The results presented here provide evidence that BE in rats evokes long-lasting changes in brain activity, which is predominantly associated with impairments in the morphofunctional state of neurons in the cortex and hippocampus. The most marked pathological reactions in these structures were seen in the poor-learning group of bulbectomized rats, where they correlated with a deficiency in spatial memory and high levels of β-amyloid in the hippocampus. Behavioral testing showed that some BER retained spatial memory. In terms of the severity of pathological changes in neurons of the temporal cortex and hippocampus and β-amyloid content, BER-gm occupied an intermediate position between BER-pm and control animals. The data presented here suggest that endogenous mechanisms temporarily compensating for the developing neurodegenerative process are activated in the brains of BER. These inadequately studied mechanisms also appear to be responsible for the prolonged period of asymptomatic development of neurodegeneration typical of Alzheimer's disease [3].

As shown above, all measures in BER were compared with analogous characteristics in SOR, which also showed some changes in the morphofunctional state of neurons, evidently due to the animals' age. The most marked morphological and biochemical changes appeared in the hippocampus of all BER, as evidenced by the greater effect of BE on this structure, which is known to have leading significance in learning and memory processes and to be the major site of abnormalities in Alzheimer's disease. Neuron dysfunction in the hippocampus at long post-BE periods, mainly due to the neurotoxic effects of increased β-amyloid levels and most marked in BER-pm, appears also to have led to memory impairment. However, a significant reduction in cell density accompanying cytoarchitectonic degradation was seen only in the temporal cortex, and not in the hippocampus of BER. This suggests that recovery of cell density in this structure occurred as a result of neurogenesis, which, as demonstrated previously, is initiated in the hippocampus after BE [3], and, according to data reported by other authors, is activated in cognitive deficit [5]. Activation of proliferative activity in the dentate gyrus and hippocampal fields has also been noted in Alzheimer's disease [9], though the functional role of this remains uncertain. The most widespread pathological reaction of neurons in BER was karyolysis. This may be associated with the fact that the death of cortical neurons in the brain occurs mainly as a result of apoptosis, which is apparent morphologically as chromatin fragmentation and degradation of the nuclear envelope [6].

Thus, the data obtained here provide evidence of the long-term nature of morphological, biochemical, and behavioral changes induced by BE, which resemble the manifestations of the degenerative process of the Alzheimer's type. This supports the validity of our BER model of the sporadic type of Alzheimer's disease.

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