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Morphological Types of Activated Microglial Cells in the Hippocampus Present after Transient Total Cerebral Ischemia

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The aim of the present work was to use immunocytochemical methods (detection of Iba-1 protein) to study the morphological characteristics of hippocampal microgliocytes in rats (n = 15) and describe their cell types in the situation of responses to single episodes of total cerebral ischemia lasting 12 min. The results obtained here provided evidence for the existence of several morphological types of microgliocytes in intact hippocampus and the appearance of new forms in responses to ischemia-induced neuron damage. These changes in the overall hippocampal microgliocyte population were associated with functional activation. During the postischemic period (3, 7, and 14 days), microgliocyte processes were characterized by the appearance of multiple microprocesses, pointing to increased interactions between these and the surrounding cellular elements, which may be an indication of a process eliminating damaged synaptic structures from the neuropil.

Keywords: hippocampus, microglia, Iba-1 protein, cerebral ischemia.

The microglia form a special population of cells of bone-marrow origin which in nervous tissues perform the functions of resident macrophages and are a key component in the development of local inflammatory processes [6]. Classical studies have demonstrated a multiplicity of morphological types of microgliocytes seen in different areas of the brain both in normal conditions and in pathology [1]. However, imperfections in the previously used impregnation methods introduce doubts that all the cell types found are in fact microgliocytes. Highly specific selective staining methods have now been developed for microgliocytes; these have high reproducibility in standard paraffin sections [5] and lack the disadvantages associated with classical impregnation methods, opening new perspectives for studies of the microglial brain cell population.

The aim of the present work was to study the morphological characteristics of rat hippocampal microgliocytes and describe the microglial cell types appearing during responses to single episodes of total cerebral ischemia.

Materials and Methods

The brains of 15 adult male rats (Sprague–Dawley) were studied. Animal keeping and all experimental manipulations were performed in compliance with the "Regulations for Studies Using Experimental Animals." Following a previously used version of standard methods [4, 8], rats (n = 12) were anesthetized and cerebral ischemia was induced by clamping both common carotid arteries for 12 min with subsequent reperfusion of the brain. Three animals served as controls. Rats were decapitated under anesthesia

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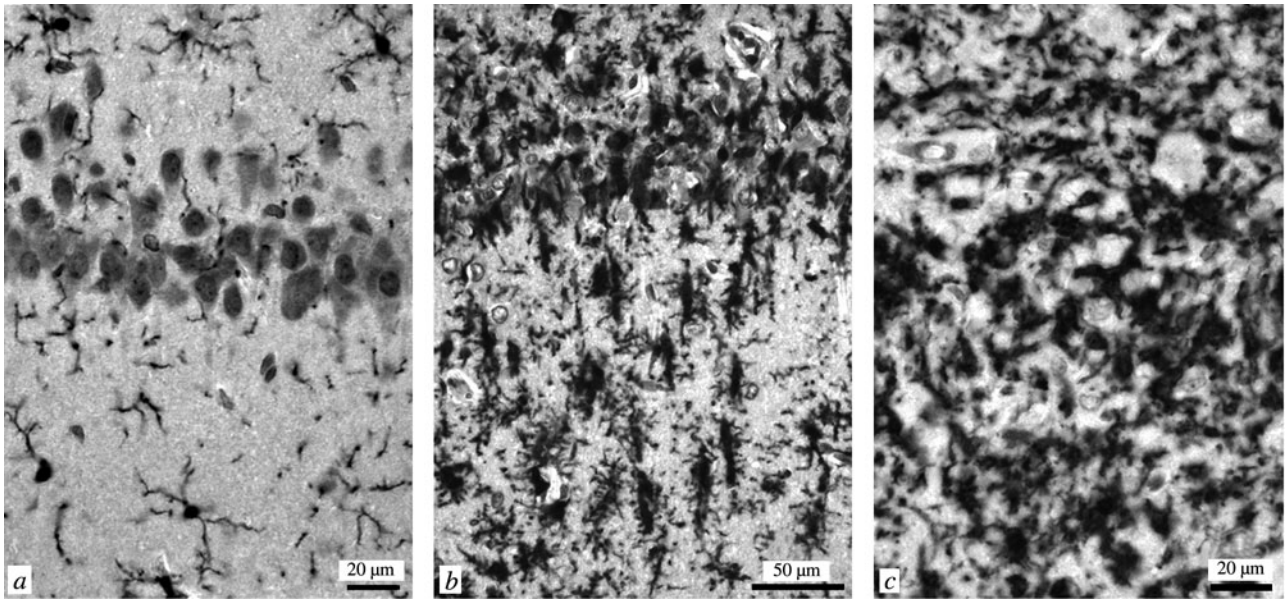

Fig. 1. General view of rat hippocampal field CA1. *a*) Control; *b*) 7 days after ischemia; *c*) 14 days after ischemia. Immunocytochemical reaction for the microglial marker Iba-1 protein, counterstained with hematoxylin.

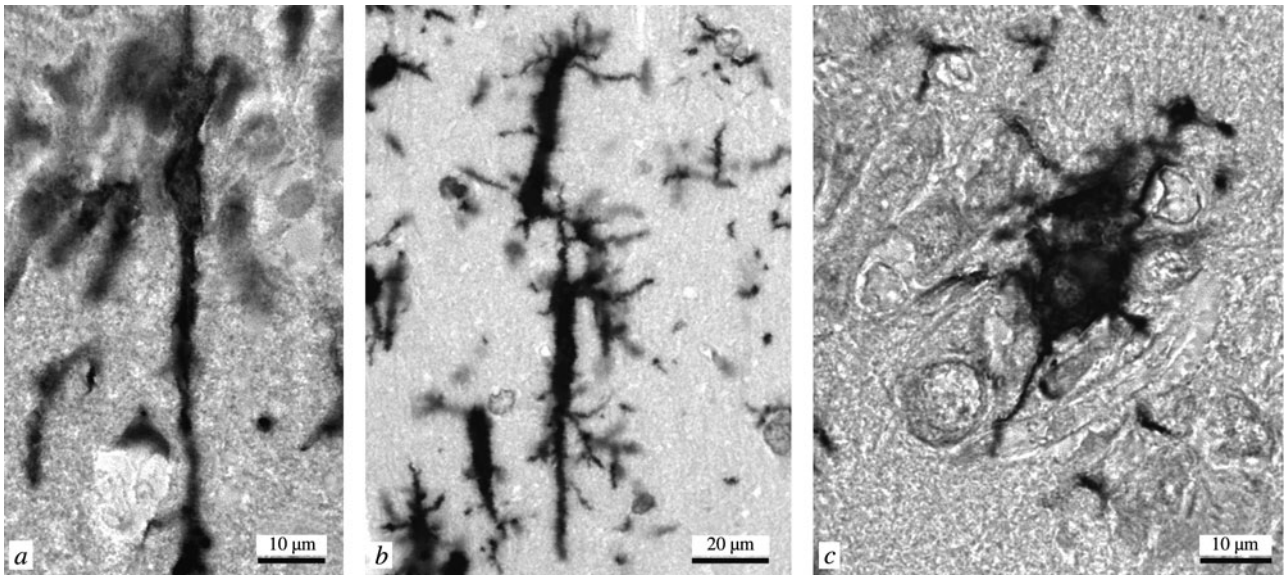

Fig. 2. Different microglial cell types in the rat hippocampus seen three (*a*) and seven (*b*, *c*) days after ischemia. Immunocytochemical reaction for microglial marker Iba-1 protein. *a*, *b*) Counterstained with hematoxylin; *c*) without counterstaining.

three ($n = 4$), seven ($n = 4$), and 14 ($n = 4$) days after surgery. Brains were fixed in ethanol-formaldehyde and zinc-ethanol-formaldehyde [2] and embedded in paraffin using standard methods. Serial sections were stained with toluidine blue by the Nissl method. Microgliocytes were detected immunocytochemically using goat polyclonal antibodies to Iba-1 antigen (AbCam, UK, catalog No. ab5076) [5]. Standard deparaffination and rehydration were followed by thermal antigen demasking in modified citrate buffer (S1700, Dako, Denmark). Incubation with primary antibod-

ies to Iba-1 (diluted 1:200) was for 80 min at 40°C. Antigen-antibody complexes were detected using an LSAB+ reagent kit (Dako, Denmark) and the product of the immunocytochemical reaction was detected with chromogen DAB+ (Dako, Denmark). Some sections were counterstained with alum hematoxylin. Controls for immunocytochemical reactions were performed in accordance with the reagent manufacturer's recommendations.

Results

Reactions for Iba-1 protein in control animals selectively stained microglial cells both in the hippocampus and other parts of the brain. All microglial cells in the intact hippocampus had small, round or oval nuclei and extremely small volumes of perinuclear cytoplasm. Most cells were characterized by numerous fine branching processes (Fig. 1, *a*), penetrating the whole of the hippocampal neuropil and entering groups of compactly distributed neurons (the pyramidal layer of the hippocampus itself, the granular layer of the dentate gyrus). Occasional microgliocytes were located in the pyramidal layer of the hippocampus. Their bodies intercalated with the neurons contacting them and were irregular in shape. On visual evaluation, no more than 20% of microgliocytes were located alongside capillaries. Despite the fact that most microglial cells in the intact hippocampus could be identified as a single morphological type of stellate, microgliocytes with fine processes, occasional extended cells were also found close to blood vessels, these having larger processes, which emphasized their spindle shape.

Degeneration of some neurons in the pyramidal layer of the hippocampus occurred after ischemia. Three days after reperfusion, field CA1 contained shrunken pyramidal neurons. After seven days, some neurons died, though groups of damaged neurons persisted; at two weeks, neuron were not found in some areas of field CA1. Gradual activation of microgliocytes occurred, with changes in their shape in areas of neuron destruction and significant increases in their population (see Figs. 1, 2).

The appearance of shrunken pyramidal neurons was associated with two tendencies to changes in microgliocyte location and shape. The first consisted of the appearance of numerous Iba-1-immunopositive cells in the pyramidal layer (see Fig. 1, *b*), some of which had shortened processes. The second related to the organization of microgliocytes in the radial layer into linear structures consisting of one or more cells lacking lateral processes and located along the main dendrites of hippocampal pyramidal neurons (see Fig. 2). Some microgliocytes in the marginal and lacunar-molecular layers retained their stellate shape with multiple processes typical of the intact hippocampus, and though they showed increases in the sizes of nuclei and the perinuclear cytoplasm, the processes themselves ceased to appear smooth as in intact animals, because of the large number of microprocesses. Occasional "giant" microgliocytes with large, extended processed appeared. Microgliocytes with typical curved perinuclear parts and lacking processes in the curved area sometimes appeared. In most cases, the identity of the structures in the neuropil responsible for the curvature of these cells remained unclear (no adjacent blood vessels or neurons). Microgliocytes were often seen in pairs. Interactions between their processes pointed either to tight contacts or indicated that these were incompletely divided cells.

As post-ischemic neuron degeneration and microglial reactions increased (seven days), the number of fusiform microgliocytes in the radial layer increased, these acquiring numerous short microprocesses running from the perinuclear area and the bases of processes, mainly at right angles. The number of typical ameboid microgliocytes increased. Outside areas of neurodegeneration, microgliocytes retained the shape typical of the intact hippocampus.

At the final stage of the response to post-ischemic neurodegeneration (14 days), typical bipolar microgliocytes disappeared (see Fig. 1). All layers in the damaged area of the hippocampus were characterized by an extremely dense distribution of similarly sized microgliocytes of irregular shape and with quite short and thick processes (compared with controls). In undamaged areas of the hippocampus, microgliocytes retained their stellate shape and had fine, branching processes.

Discussion

The results obtained here provide evidence of the existence of several morphological types of microgliocyte in the intact hippocampus and the appearance of new types in response to neuron damage induced by transient total cerebral ischemia. Undoubtedly, these changes in the overall hippocampal microgliocyte population are associated with changes in their functional state [7, 9]. The fact that microgliocyte activation leads to the disappearance of processes and their acquisition of a rounded, ameboid shape is well known [1], though typical ameboid microgliocytes constitute a small proportion of the ischemia-activated microglial cells of the hippocampus, distinguishing reactive changes in the hippocampal microglia from postischemic changes in the microglia of the striatum [3]. Although mitotically dividing microgliocytes in the study preparations were not seen, the distribution of some microglial cells in pairs at three days of reperfusion may point to preceding division. In the case of post-ischemic changes in microgliocyte process structure, the appearance of multiple microprocesses was characteristic, suggesting increases in their interaction with surrounding cellular elements, which may be a sign of the elimination of damaged synaptic structures from the neuropil [10].

Thus, the population of ischemia-activated hippocampal microglial cells contained, along with cells with multiple processes, giant microgliocytes, fusiform microgliocytes in the radial layer of the hippocampus, microgliocytes of irregular shape with short processes, and typical ameboid microgliocytes. These types are not permanent and reflect

both the functional state of cells and the characteristics of neuropil organization in different hippocampal layers.

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