Distribution of Bax protein in the rat hippocampus

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Bcl-2 family proteins are the regulators of apoptosis, but also have other functions [1]. The Bax protein belonging to this family is involved in mechanisms of synaptic depression [2]. Though, the induction of long term potentiation in the synapses of the hippocampal CA1 field leads to increased Bax expression in the CA1 field homogenates [3]. If this increase occurs in neurons, this may indicate that the long term potentiation of synaptic connections is accompanied by the activation of a negative feedback, which limits growth of the strength of excitatory glutamatergic transmission. To address this question, we tried to assess cellular specificity of Bax distribution in the hippocampus. Bax immunoreactivity is visually detected mainly in the layer of pyramidal neurons (Figures 1-2) and is rarely detected in S100B-positive glial cells (Figures 3). However, a quantitative assessment of Bax colocalization with glial and neuronal markers (S100B and NeuN, respectively) showed that although the Bax content in S100B-positive glial cells is really low, the Bax neuronal somatic pool is not the main source of Bax protein in the hippocampus.

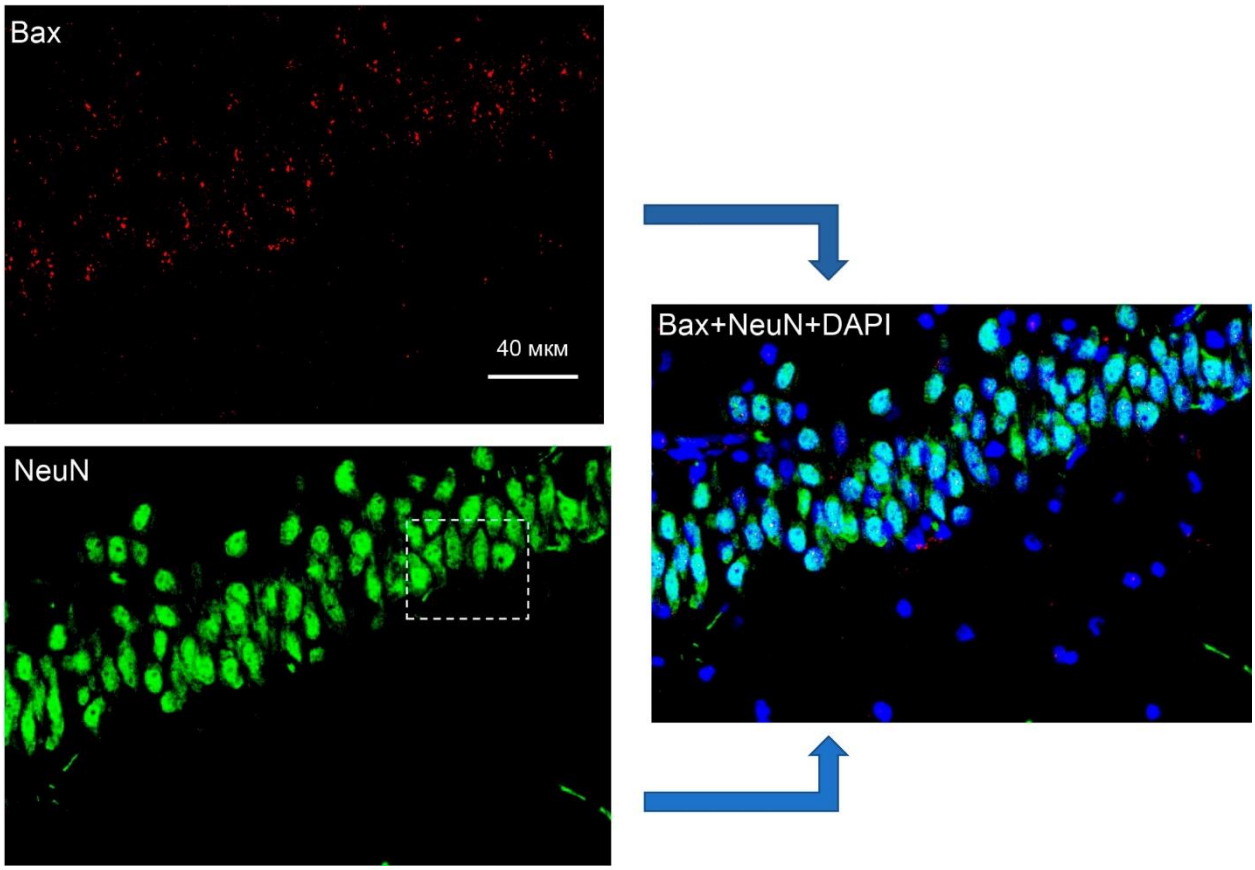

Figure 1. **Bax-immunoreactivity in rat hippocampal field CA1.** All images show the same fragment of an optical slice 0.67 μm thick. Bax (red) is predominantly detected in neurons (green), but is also found near nuclei of other types of cells (stained blue, but without neural marker NeuN). The dashed line marks the region presented in more detail in Figure 2.

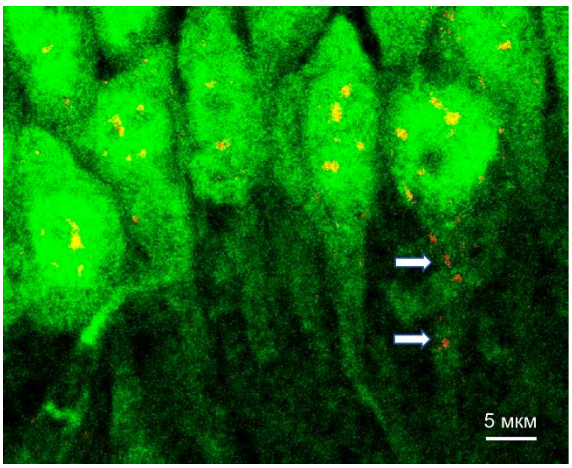

Figure 2. Colocalization of Bax (red) and the neural marker NeuN (green) in the neurons of the hippocampal CA1 pyramidal layer. Neuron marker NeuN is expressed primarily in the nuclei of neurons. In smaller quantities, it was also present in the cytoplasm of the soma and proximal dendrites. Pixels in which both colors are present appear yellow. In clusters of yellow pixels, red inclusions are visible in neuronal somata, i.e. with the spatial resolution used (voxel sizes 0.12 × 0.12 × 0.67 μm), not all Bax detected in neuronal somata is colocalized with NeuN. In addition, Bax is present in the processes of neurons, where the level of NeuN is very low (indicated by arrows). Thus, the colocalization of Bax and NeuN underestimates the degree of neuron specificity of the distribution of Bax immunoreactivity in the hippocampus. This fragment is marked in Figure 1 with the dashed line.

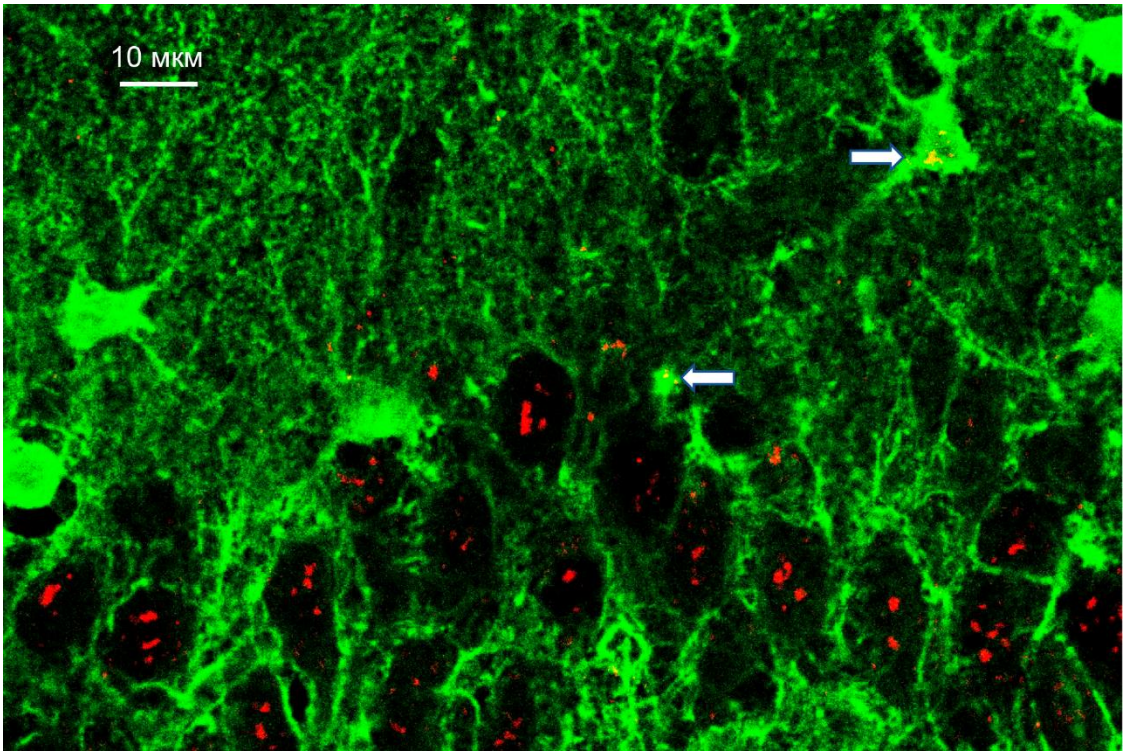

Figure 3. Colocalization of Bax (red) and the glial marker S100B (green) in the hippocampal field CA1. Pixels in which both colors are present appear yellow (indicated by arrows). Bax is detected in S100B-positive cells much less frequently than in neurons (dark ovals in the lower part of the figure).

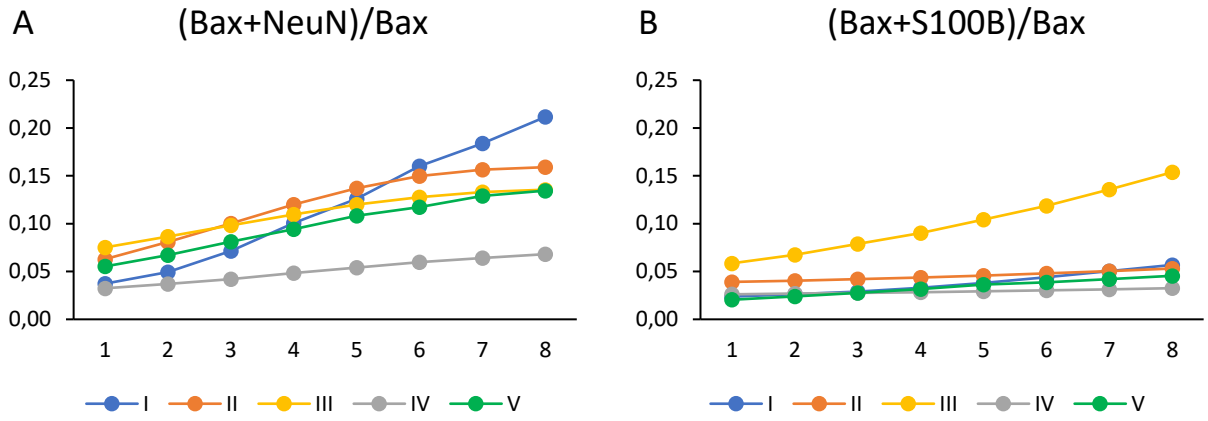

Figure 4. Colocalization coefficients of Bax with NeuN (A) and S100B (B) in the hippocampi of five rats (I-V) at different levels of Bax immunofluorescence (1-8). As a minimum estimate of the background fluorescence level, a MIN value was taken such that at least 99.99% of the voxels in the scan area outside the sample had a fluorescence level less than MIN. Since determination of the true background Bax signal is a challenging task, the colocalization coefficients of Bax with cell markers were calculated with threshold Bax levels Ln, which were determined as follows: Ln = MIN + n(MAX – MIN)/8. Here n = 0, 1, ..., 7, and MAX is such that no more than 0.1% of all voxels in the scan area had a fluorescence level greater than MAX.

The colocalization coefficient of Bax (the ratio of the number of colocalized voxels to the total number of Bax-positive voxels) with the glial cytoplasmic marker S100B, in most cases, did not depend on the threshold Bax level and ranged from 0.02 to 0.05 (Figure 4, B).

Although visually the degree of colocalization of Bax and the neuronal marker NeuN seems rather high (Figures 1-2), the coefficient of colocalization of Bax with NeuN turned out to be small – no more than 0.3 (Figure 4, A). Obviously, the colocalization coefficient of Bax with NeuN underestimates the Bax content in neurons. Perhaps a significant part of Bax is located in the processes of cells, where it exists mainly in the form of small inclusions, which are hardly perceived visually. However, although the content of Bax in S100B-positive glial cells is low, it cannot be ruled out that a significant Bax amount is present in cells of other types, for example, in blood vessels.

Conclusion

A visual analysis of the distribution of Bax in the hippocampal cells is insufficient to conclude that Bax is predominantly neuronal. To analyze cell specificity and neuronal activity-dependent dynamics of Bax expression, it may be appropriate to use animals with neuron-specific cytoplasmic expression of fluorescent markers.

References

[1] J.M. Hardwick and L. Soane, "Multiple functions of BCL-2 family proteins," Cold Spring Harb. Perspect. Biol., 2013. V. 5: a008722.
[2] Z. Li and M. Sheng, "Caspases in synaptic plasticity," Mol. Brain. 2012. V. 5: 15.
[3] V.O. Pustylnyak et al. "Expression of p53 target genes in the early phase of long-term potentiation in the rat hippocampal CA1 area," Neural Plast. 2015. V. 2015: 242158.