STRUCTURAL PLASTICITY OF NEURONAL CELLS OF CA1 HIPPOCAMPAL AREA AFTER LONG-TERM SYNAPTIC POTENTIATION

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***Halyna Skibo**, Professor, D.Sc., Bogomoletz prize laureate, Honored Science and Engineering Worker of Ukraine, Head of Cytolology Department of Bogomoletz Institute of Physiology. H. Skibo started her scientific activity in 1963 in the laboratory of nerve cell physiology and biophysics of Bogomoletz Institute of Physiology under the guidance of P.G. Kostuyk. To date H. Skibo is a distinguished neuromorphologist who has gained the recognition of international scientific community. Fundamental investigations of Prof. H. Skibo are aimed at elucidating the structure and function of nervous and glial cells in the brain in health and disease, as well as to search for efficient neuroprotective approaches. She is well-known for her expertise in many modern methods of cell biology, neurocytology, immunohistochemistry, image analysis, etc. Various models of neurological diseases have been established in the Department of Cytology headed by Prof. H. Skibo, allowing her to study the cellular and molecular mechanisms of brain pathology and neuroprotection and processes related to brain plasticity as well. Recently Prof. H. Skibo has started very promising cell research dealing with the effect of stem cell transplantation on brain pathology.*

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The photo shows a student of P.G. Kostyuk, Prof. H.G. Skibo

Excitatory dendritic spine synapses are extremely dynamic structures which change their functioning and morphology with activity and under pathological conditions (Yuste and Bonhoeffer, 2004; Lippman and Dunaevsky, 2005; Bourne and Harris, 2007). Induction of the long-term potentiation (LTP) of synaptic trans-

mission was shown to correlate with dynamic modifications in synapse morphology, and particularly with enlargement of the spine head, as well as with the changes in the proportion of different synaptic types (Desmond and Levy, 1986; Toni et al., 1999; 2001; Geinisman, 2000; Matsuzaki et al., 2004; Stewart et al., 2005; Park et al., 2006). The same kind of ultrastructural rearrangements was also observed after brief episodes of oxygen-glucose deprivation (OGD) which do not cause immediate cell damage but induce a lasting increase in excitatory postsynaptic potentials (EPSP) similar to the classic LTP (Jourdain et al., 2002; Kovalenko et al., 2006). These morphological changes are thought to contribute to the activity-related modifications of synaptic efficacy. However, most of these plasticity-associated morphological changes have been characterized at the level of postsynaptic spines, and little is known about the presynaptic partner or even less about the third component of the synaptic complex, namely the astroglial processes. Recent data suggest that astrocytes may control the local synaptic environment, participate in neuronal signaling, regulate synaptic activity and plasticity and thus contribute to coordinate synaptic network functions. This has led to the concept of tripartite synapse consisting of a synaptic contact and its associated glia (Araque et al., 1999; Haydon, 2001; Bushong et al., 2002; Schipke and Kettenmann, 2004; Volterra and Meldolesi, 2005; Slezak et al., 2006). Consistent with this, confocal imaging experiments have revealed highly dynamic aspects of glial processes and their possible involvement in synaptic plasticity (Hirrlinger et al., 2004; Haber et al., 2006; for review see Theodosis et al., 2008). However, precise details of this interaction and electron microscopic (EM) analyzes of the morphological plasticity of astroglia related to synaptic activity are scarce. The aim of the present study was to analyze, using 3D EM reconstructions of excitatory synapses, ultrastructural modifications of all three components of a synaptic complex (dendritic spine, presynaptic terminal, and associated astroglial processes) under plasticity-inducing conditions, such as brief OGD or theta-burst LTP induction protocol.

Hippocampal organotypic slice cultures from seven-day old rat pups were used in the experiments (Stoppini et al., 1991). Tetanic LTP was induced as described in (Toni et al., 1999). Oxygen-glucose deprivation paradigm was used to induce the so-called anoxic LTP (Hsu and Huang, 1997; Jourdain et al., 2002). CA1 synapses and glia were examined with electron microscopy. The synapse profiles were reconstructed and analyzed using software Reconstruct developed by J.C. Fiala and K.M. Harris (Boston University, Boston, MA; http://synapses.bu.edu/).

In CA1 stratum radiatum of cultured hippocampal slices, spine synapses were determined by the presence of a clear postsynaptic density (PSD) facing at least 2-3 presynaptic vesicles. Astroglial processes were identified by their morphological characteristics such as relatively lucid cytoplasm, irregular shape, presence of glycogen granules, and typical bundles of intermediate filaments in thicker processes. Three elements of synaptic complexes (dendritic spine, presynaptic terminal, and associated astroglial processes) were followed through serial sec-

tions, and their morphological properties were quantified through 3D reconstruction. In this study, we specifically analyzed two categories of spine synapses, chosen randomly within the neuropil: i) synapses with macular, or simple, PSDs referred to simple spine synapses (Fig. 1, *b*, *d*) and ii) complex, or perforated synapses, exhibiting a clear discontinuity in their PSD (Geinisman et al., 1987; Fig. 1, *b*, *e*). Dendritic spines with simple PSD had on average much smaller heads than those with complex PSD (0.026 ± 0.002 μm^3 versus 0.08 ± 0.006 μm^3, $p < 0.05$). The mean area of complex PSDs was significantly larger than that of simple ones (0.095 ± 0.005 μm^2 and 0.036 ± 0.002 μm^2, respectively, $p < 0.05$ (Fig. 1, *a*, *c*)).

Astrocytic processes occupied $4.6 \pm 0.4\%$ of the total neuropil volume. Simple synapses had astrocytic coverage in 77.8% of cases, while 96.6% of complex synapses were enveloped by glia. Coverage in these analyses was determined by the presence of a direct membrane apposition between the glial process and the postsynaptic spine or presynaptic terminal. On average, a simple synapse was contacted via 1.22 ± 0.08 glial processes, while a complex one had contacts with 1.68 ± 0.08 processes. In simple synapses, the mean surface of astrocytic contact on spine head and presynaptic terminal was respectively 0.087 ± 0.014 μm^2 and 0.053 ± 0.007 μm^2, thus larger than the size of the PSD. In larger complex synapses, the surfaces of glial coverage were also enlarged (0.127 ± 0.017 μm^2 and 0.132 ± 0.012 μm^2 contact surface for spine head and terminal, respectively), proportionally to the increase in the synaptic element size.

Previous works have shown that calcium precipitation protocol makes it possible to identify a subset of labeled spine profiles likely to represent stimulated spine synapses (Buchs et al., 1994; Buchs and Muller, 1996; Toni et al., 1999; 2001). Using the same approach here, we randomly selected spines containing calcium precipitates on at least one of the serial sections and analyzed their parameters and glial coverage. We again distinguished between simple spine synapses and synapses with complex PSD.

As shown in Fig. 1, *a*, the population of simple spine demonstrates a tendency to the increase of PSD area. The changes were more pronounced in complex synapses than in simple ones. Tetanic LTP induced a pronounced rearrangement of glial processes. Only 3.3% of simple synapses were still devoid of glial contacts 30 min after LTP, whereas all complex ones had glial coverage. The surface of glial contacts increased very markedly, especially on synapses with complex PSDs. This effect concerned both the postsynaptic spine and the presynaptic terminals (Fig. 1, *f*), representing 2.15 times increase of the glial coverage on the whole synapse at 30 min. The increase in the number of glial processes contacting synapses was one of the constituents of the glial coverage enlargement.

Our previous studies have shown that short OGD episodes which induced anoxic LTP produced no damage to the CA1 hippocampal tissue within the first hours after the insult (Jourdain et al., 2002). In the presented experiments, the neuronal tissue ultrastructure was also well preserved and there was no sign of neuronal damage or death during the first hour after OGD. As for brief tetanic

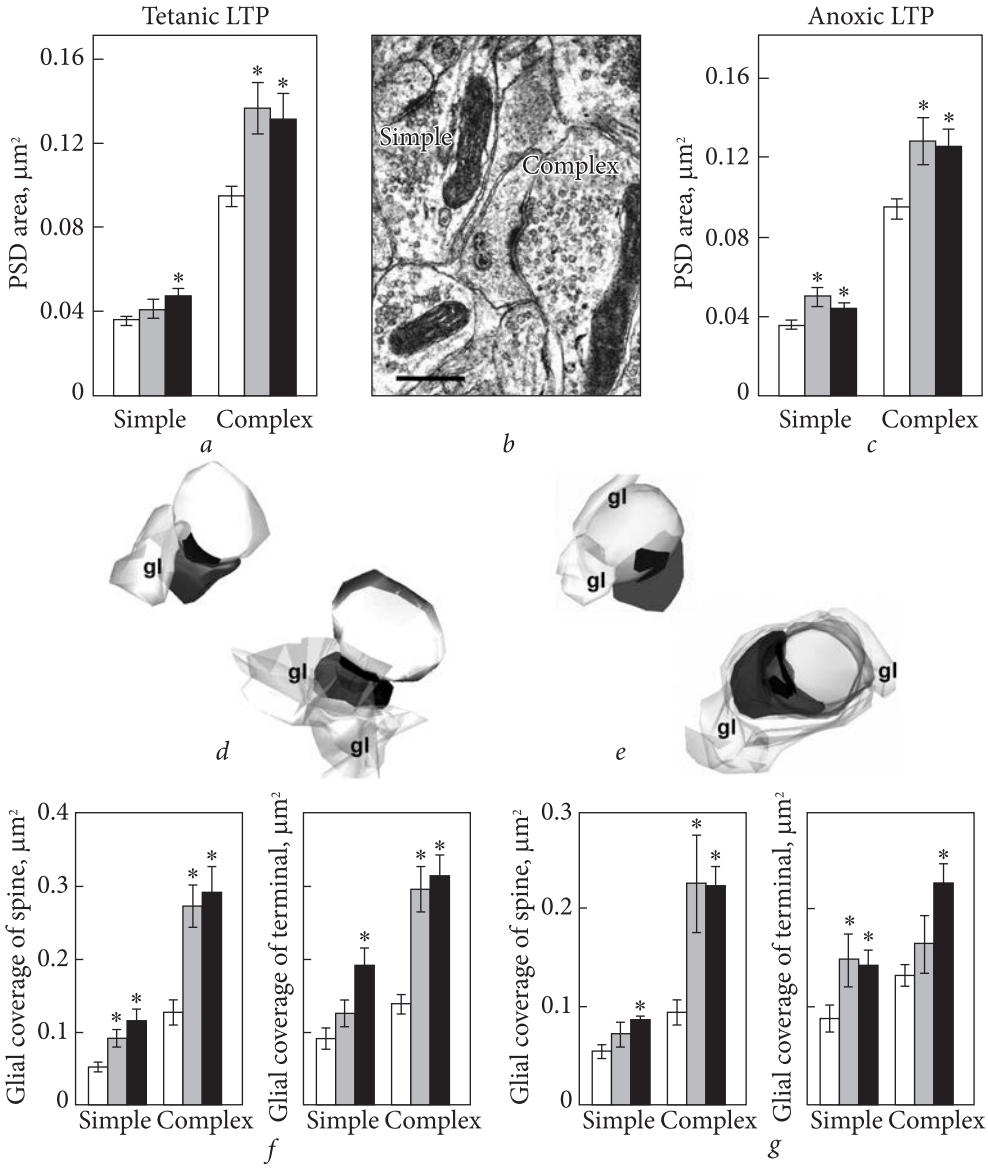

Fig. 1. Synapses with simple and complex PSDs and perisynaptic astroglia after induction of LTP by theta-birst stimulation and brief OGD episode: *a*, *c* — PSD area in simple (left group of bars) and complex (right group of bars) synapses under control conditions (white bars), 30 min after (gray bars) and 60 min (black bars) after theta-birst stimulation and brief OGD episode, respectively. *b* — electron microscopic illustration of a synapse with simple (macular) and complex (perforated) PSD. Bar: 0.5 μm. *d*, *e* — Examples of 3D reconstructions of simple (left) and complex (right) synapses with perisynaptic astroglia under control conditions (top) and 60 min after LTP stimulation (bottom); *f*, *g* — glial coverage in synapses with simple (left group of bars) and complex (right group of bars) PSDs under control conditions (white bars), 30 min (gray bars) and 60 min (black bars) after theta-birst stimulation and brief OGD episode, respectively. (*$p < 0.05$)

stimulation, brief OGD episode induced important modifications in the spines and terminal with simple and complex PSD. The increase in the PSD area is shown in Fig. 1, *c*. This stimulation protocol also induced marked rearrangements of glia. First of all, the proportion of synapses devoid of glial contacts significantly diminished (only 6% of simple synapses without glial coverage 30 min after OGD and all of them contacted by glia at 60 min, versus 22.2% in control situation). The surface of glial contacts also increased, both on spine head and presynaptic terminal (Fig. 1, *f*), e.g., 61.1% increase in the total glial contact on the synapse at 60 min. This enlargement of the glial coverage was, at least partly, due to the increase in the number of glial processes contacting a synapse.

Similar changes were induced by the brief OGD in the population of large spine synapses with complex PSDs, which included a gradual increase in the spine head volume, a further increase in the PSD size. With regard to glial coverage, all synapses with complex PSDs bore glial contacts yet at 30 min after OGD. Also, as for simple spine synapses, the surface of glial contact increased on both spine heads and presynaptic terminals, and the proportion of the synaptic surface covered by glia enlarged dramatically reaching 22% of the synaptic membranes covered by glia at 60 min after OGD. This increase was mainly due to the enlargement of the existing glial contacts.

Activity dependence of the glial rearrangement was further confirmed in the experiments where a group of slices was treated with a specific NMDA receptor antagonist D-AP5 for 15 min before and 10 min OGD to prevent anoxia-induced synaptic potentiation. Under such conditions, the parameters of all synaptic elements (spine volume, PSD area, terminal volume) remained unchanged with regard to non-stimulated controls or slices treated with D-AP5 only. The increase in the synaptic glial coverage observed after OGD was also prevented. The surface of contact between glial processes and spine head or presynaptic terminal and the number of glial processes per synapse did not significantly change. Thus activity-induced glial rearrangement observed with OGD required activation of NMDA receptors.

The results show that induction of synaptic potentiation under the conditions used promotes important modifications of pre- and postsynaptic elements, but also point at very significant changes in the glial coverage of activated synapses. These findings are consistent with the idea of an active participation of glial processes in activity-induced structural plasticity.

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