Interneuronal Membrane Contacts and Syncytial Perforations in CA2 Hippocampal Area after Brain Trauma

N. M. Paramonova, O. S. Sotnikov, and T. V. Krasnova

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Membranes of pyramid neuron bodies located in CA2 hippocampal area were studied by electron microscopy after gunshot craniocerebral injury. In control group, asynaptic contacts and interneuronal syncytial perforations forming from tight junctions were observed. Contacts and perforations increased in size after trauma. Their number was maximum after severe gunshot injury. They reached their maximal size on days 5-7 after the injury.

Key Words: *membrane perforations; interneuronal syncytial network; gunshot craniocerebral injury*

Numerous modern investigations make a point of interneuronal and glial-neuronal contacts developing in various areas of the central and peripheral nervous system after changes in the physiological state, exposure to extreme factors, or during various pathologies [6,8]. Lability of theses connections appears to be a fine instrument of neuronal plasticity. Tight junctions and gap junctions are the most common and well studied connections [11,14]. There are also some recent reports on syncytial connections of neuronal cells [5,9]. Syncytial connections were found in brain hypoxia [7] and in intact brain [15]. They were also described in autonomic ganglia in Wallerian degeneration [8].

In experiments on the previously developed model of gunshot craniocerebral injury (GSCI) we studied pathogenetic and compensatory-reparative processes in CNS [4].

The objective of this study was to investigate interneuronal membrane relations. Exposure to blast wave at considerable distances from the epicenter, even in cases with non-penetrating GSCI without damage to skull bones and formation of the wound channel, is a peculiarity of gunshot injury. In the zone of molecular contusion, we studied the hippocampus playing the key role in processes of memory and integration of information flow in the brain [2].

Laboratory of Neuronfunctional Morphology and Physiology, I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg. Russia. ***Address for correspondence:*** sotnikov@kolt.infran.ru. O. S. Sotnikov

MATERIALS AND METHODS

The study was carried out on 14 mature Chinchilla rabbits weighing 1.5-2 kg. The animals were intraperitoneally anesthetized with sodium thiopental (5 ml 1% solution) and fixed in a special device in accordance with "Principles for studies with employment of experimental animals" (1977). Shots were performed at a certain angle to the skull from a distance of 1-2 cm from small-bore sport rifle using adapted bullets. The severity of injury was controlled by bullet velocity, which depended on the magnitude of powder charge. Bullet velocity of 130-150 m/sec caused severe fatal injury, while non-penetrating closed GSCI was produced at bullet velocity of 50-60 m/sec. Group 1 (control) comprised intact rabbits. Animals with severe injury comprised group 2. They were decapitated immediately after the injury. In group 3 rabbits, mild injury was modeled and they were followed up for 5-7 days. All animals were decapitated under narcosis (chloroform vapor). Then, 1-mm^3 fragments of CA2 hippocampal area were isolated, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 1.5 h and postfixed in cold 1% osmium tetroxide for 1 h. After dehydration in ascending alcohols,

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the specimens were embedded into aradite mixture. Ultrathin sections were made on LKB-5 ultratome and stained after Reynolds (triple staining). Visualization and photographing were carried out on a LEO 10 electron microscope at accelerating voltage of 80 kV. Negative slides were scanned in transillumination regimen using a UMAX Astra 4000V scanner. Positive images were analyzed by morphometry using Image J software.

RESULTS

The bodies of CA2 pyramidal neurons have a diameter ~20 μ and are arranged in a single layer. The absence of glial interlayer between them is not uncommon. Closely located membranes of adjacent cells in all groups of animals, including intact, have nonsynaptic contacts looking like tight and gap junctions, as well as syncytial intermembrane perforations connecting the cytoplasm of neighboring neurons (Fig. 1). Most commonly, perforations were a continuation of tight junctions, which suggests that perforations appear on the basis of tight junctions. Perforations have either linear (representing residues of broken tight junction) or rounded edges (merged membranes of neighboring neurons). Residual membrane bodies looking as oval structures or vesicles with the diameter comparable to the width of intercellular gap were found in the perforation lumen. In intact animals, single perforations were observed almost in half of all investigated pairs, 40% contacting cells had two perforations; the rest 10% neighboring neurons had 3 and more relatively small perforations.

The area of adjacency of closely located neurons having no glial insulation significantly increased in both experimental groups after GSCI as well as extension of contact areas between them (Table 1). Mean length of contacts and interneuronal perforations also increased. Moreover, in animals with severe brain injury (Fig. 2, *a*), contacting pairs of membranes 4 times more often (40%) have 3-6 syncytial perforations than

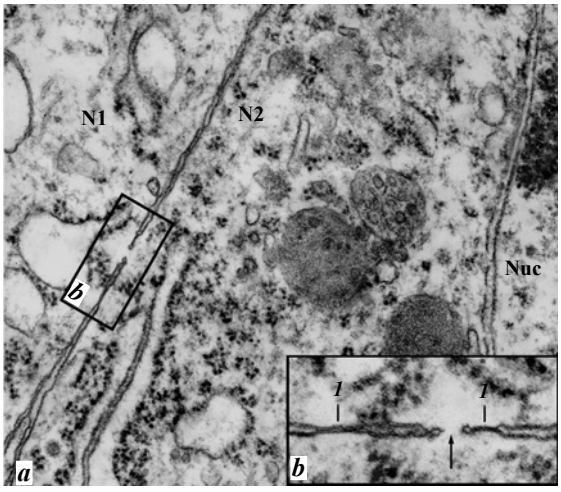

Fig. 1. Syncytial perforation of contacting external cell membranes of two neurons in CA2 hippocampal area in intact rabbit. *a*) general view of contacting neurons; *b*) magnified part of this image. *1*) tight junctions (one the basis of one of them the perforation appeared (arrow); on the periphery of perforations residual membrane bodies are being formed). N1, N2: neuronal bodies, Nuc: neuron nucleus. ×28,000 (*a*), ×56,000 (*b*).

in control group. However, the length of individual perforation was maximum on days 5-7 after mild GSCI (Fig. 2, *b*). In this case, 45% contacting neuronal membranes had 1 perforation, 35% had 2, and only one-fifth of all investigated contiguous neurons had 3 and more syncytial contacts. Apparently, the perforation length can increase due to integration of small defects of contiguous membranes. These findings indicate that the development of syncytial connections in injured animals takes a certain time. The ratio of total length of non-synaptic contacts and perforations to total length of adjacency of neighboring neuronal bodies and processes 1 week after the injury increased 2-fold in comparison with the control (Table). In group 3, the total length of tight junctions and their mean number on days 5-7 after the injury also decreased almost 3-fold in comparison with tight junctions in rabbits with severe brain injury, although these values

TABLE 1. Morphometric Parameters of Interneuronal Membrane Contacts and Syncytial Perforations in CA2 Hippocampal Area after Experimental GSCI in Rabbits

Group	Mean length of individual perforation, nm	Range of perforation length, nm	Total perforation length, nm	Mean tight junction length, nm	Total tight junction length, nm	Total length of contact area, including perforations (*l*), nm	Total length of contiguous neighboring membranes (*L*), nm	Ratio *l*:*L*
1 (intact)	63	11-181	13 212	31	3684	17 170	197 716	1:12
2 (severe injury)	72	13-359	19 576	145	8735	37 616	297 109	1:8
3 (mild injury)	194	19-995	174748	47	1420	205 929	13 243 90	1:6

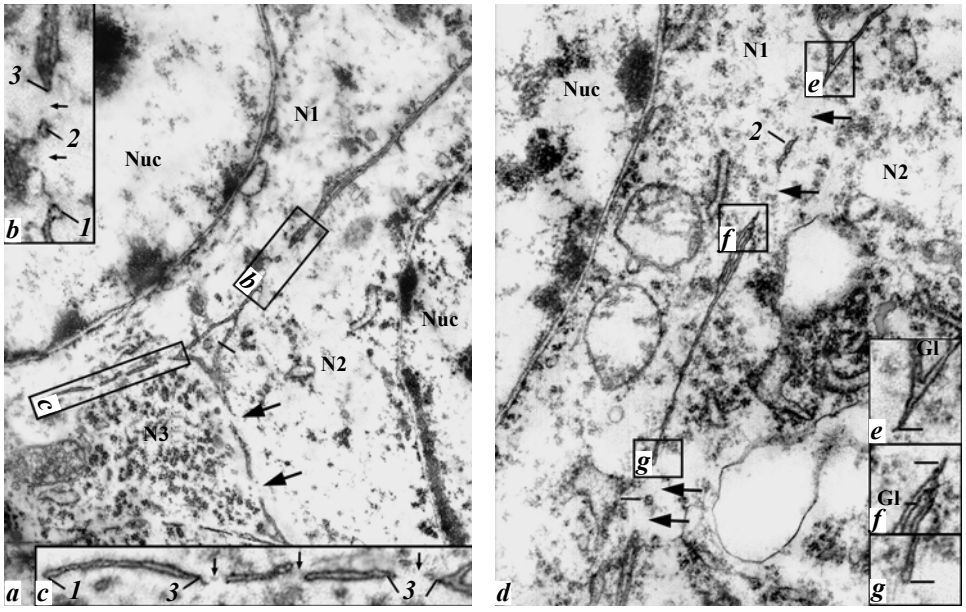

Fig. 2. Multiple interneuronal syncytial connections appeared in CA2 hippocampal region immediately after severe GSCI (*a-c*) and 5-7 days after mild GSCI (*d-g*). *a*) area of contacts for 3 neurons; *b*, *c*) magnified fragments of this image; *d*) adjacency area for two neurons; *e-g*) magnified fragments of this image. *1*) edges of syncytial interneuronal perforations sealed by fused membranes of two cells; 2) residual membrane bodies; *3*) tight junction residues at the periphery of intermembrane perforations. Arrow: intercellular syncytial perforations; Gl: glia, N1-N3: neuronal bodies, Nuc: neuronal nucleus. ×15,000 (*a*, *d*), ×38,000 (*b*), ×41,000 (*c*), ×25,000 (*e-g*).

exceed those in the control group. Simultaneously, the mean length of perforations and their total length sharply increased. It can be assumed that some tight junction appear to be perforated and a part of their length goes from group of contacts to the group of syncytial connections, *i.e.* formation of tight junction and intermembrane perforations appear to be different stages of one process.

Thus, gunshot injury and blast wave induced by it increase the area of adjacency of membranes of neighboring neurons without glial interlayer between them. It can be associated with an increase in the area of neuronal contacts and syncytial cytoplasmic junctions between them due to enlargement of perforations and their enhanced formation from tight junctions. The latter is more pronounced in severe brain injury. Tight junctions due to additional perforations are partially transformed into syncytial connections and form integrated neuronal cluster consisting of several neuronal bodies.

It should be additionally emphasized that the described serious changes in the hippocampus associated with molecular contusion of the brain are based, similarly to many other pathologies, on the processes existing under normal conditions. Membrane contacts and syncytial perforations were already described in hippocampus, cerebellum [5], and cortex of intact brain [15]. Formation of nano-sized pores in bilipid membranes under the influence of thermal molecular fluctuations is well known [1,3]. Conversion of these molecular pores into "superthreshold" pores, *i.e.* into microscopic membrane perforations requires only low energy expenditures. This process probably takes place between contacting neurons in the intact brain. In our

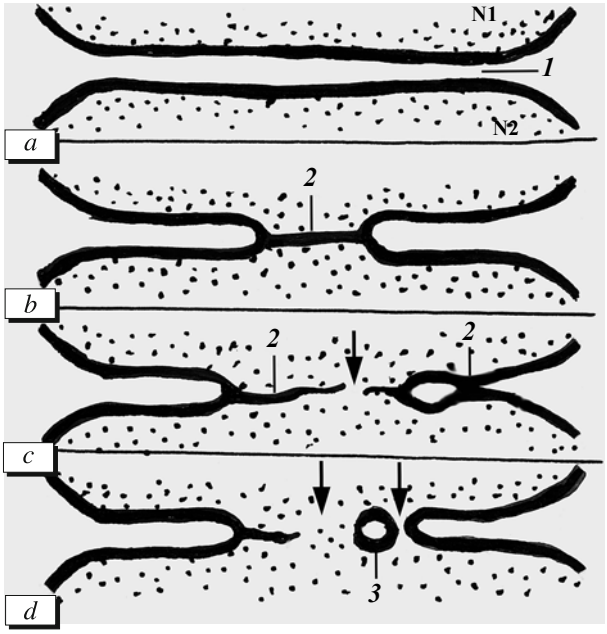

Fig. 3. Schematic representation of the formation of syncytial intercellular perforation of adjacent membranes of two contiguous neurons. *a*) adjacent membranes of 2 contiguous neurons and intercellular cleft between them; *b*) fusion of neuronal membranes with tight junction formation; *c*) formation of syncytial pore in tight junction; *d*) formation of syncytial perforations and residual membrane body between them. *1*) interneuronal cleft; *2*) tight junction; *3*) residual membrane body. Arrow: syncitial pore. N1, N2: neurons.

experiments we demonstrated pathological intensification of the pore formation in biological membranes.

Membrane fusion is a fundamental process in the functioning of eukaryotes [10,12,13]. Necessary condition for the membrane fusion is their close contact, which is provided by a number of specific transmembrane proteins, cadherines. Under these conditions, the outer layers of external cell membranes fuse with the formation of "tight junctions". This unstable transitional state is associated with periodical formation of transient labile pores, which can merge increasing in size and forming "conjugation pores" [10]. Enlarging perforations lead to the formation of syncytial connections between the cells (Fig. 3). It is possible that the result of these processes was observed by us in the hippocampus under conditions of high-energy influence of the bullet after gunshot injury.

REFERENCES

1. A. Ya. Valiakhmetov, *Biol. Membrany*, **25**, No. 2, 83-89 (2008).
2. O. S. Vinogradova, *Zh. Vyssh. Nerv. Deyat.*, **50**, 743-774 (2000).
3. R. Yu. Molotkovskiy and S. A. Akimov, *Biol. Membrany*, **26**, No. 2, 149-158 (2009).
4. A. P. Novozhilova, E. A. Dyskin, V. D. Isakov, and V. V. Kolkutin, *Morfologiya*, **109**, No. 2, 17-22 (1996).
5. N. M. Paramonova and O. S. Sotnikov, *Ibid*, **134**, No. 6, 13-17 (2008).
6. N. V. Samosudova, V. P. Reutov, N. P. Larionova, and L. M. Chaylakhyan, *Ibid*, **131**, No. 2, 53-58 (2007).
7. V. V. Semchenko, N. N. Bogolepov, and S. S. Stepanov, *Ibid*, **128**, No. 4, 76-81 (2005).
8. O. S. Sotnikov, L. I. Archakova, S. A. Novakovskaya, and I. A. Solov'eva, *Bull. Eksp. Biol. Med.*, **147**, No. 2, 207-210 (2009).
9. O. S. Sotnikov, N. N. Kamardin, G. I. Rybakova, and I. A. Solov'eva, *Zh. Evoluts. Biokhim. Fiziol.*, **45**, No. 2, 223-232 (2009).
10. G. Basanez, *Cell Mol. Life Sci.*, **59**, No. 9, 1478-1490 (2002).
11. J. Kirk, J. Plumb, M. Mirakhur, and S. McQuaid, *J. Pathol.*, **201**, No. 2, 319-327 (2003).
12. B. R. Lentz, *Eur. Biophys. J.*, **36**, Nos. 4-5, 315-326 (2007).
13. A. Mayer, *Annu. Rev. Cell Dev. Biol.*, **18**, 289-314 (2002).
14. A. Pienado, *J. Neurophysiol.*, **85**, No. 2, 620-629 (2001).
15. R. S. Santander, G. M. Cuadrado, and M. R. Sáez, *Acta Anat.*, **132**, 74-76 (1988).