



HIGH-RESOLUTION CORRELATIVE ELECTRON TOMOGRAPHY TO SOLVE BIOLOGICAL PROBLEMS

F Capani¹⁺, Ezequiel Saraceno¹, Maria Inés Herrera^{1,2}, Pablo Vázquez¹, Cecilia Quarracino¹, Rodolfo Kolliker Frers¹.

¹Inst. Inv. Cardiológicas Prof. Dr. Alberto C. Taquini, UBA-CONICET, Buenos Aires, Argentina; ² CIPP Universidad Católica Argentina Buenos Aires, Argentina. * e-mail:

franciscocapani@hotmail.com; fcapani@fmed.uba.ar

Perinatal asphyxia (PA) induced short and long term synaptic and cytoskeletal alterations that has been associated with neuronal cell death following hypoxia. The lack of knowledge about the mechanisms underlying this dysfunction prompted us to investigate the changes in the synapse and neuronal cytoskeleton and related structures.

For this study, we used a well-established murine model of PA. Full-term pregnant rats were rapidly decapitated and the uterus horns were replaced in a water bath at 37 °C for different time of asphyxia. When their physiological conditions improved, they were given to surrogate mothers. One month, 4, 6 and 18 months old after PA rats were included in this study. Modifications were analyzed using photo-oxidation with phalloidin-eosin, conventional electron microscopy (EM), immunocytochemistry and ethanolic phosphotungstic acid (E-PTA) staining combining with electron tomography and 3-D reconstruction techniques [1].

After one and two months of the PA insult, an increase in the F-actin staining in neostriatum and hippocampus synapses was observed using correlative fluorescent electron microscopy for phalloidin-eosin.[2] Mushroom-shaped spines showed the most consistent staining. Strong alterations in the dendrite and astroglial cytoskeleton organization were found at four months of PA. After six months of PA, post synaptic densities (PSDs) of the rat neostriatum are highly modified. We observed an increment of PSDs thickness related with the duration and severity of the hypoxic insult. In addition, PSDs showed an increase in the ubiquitination level. Using 3-d reconstruction and electron tomography we observed showed clear signs of damage in the asphyctic PSDs [1]. These changes are correlated with intense staining for ubiquitin. Finally, in 18 months old rat was observed a reduction in the number of synapses in the PA animals related with a decrease in BDNF staining. Overall, these results demonstrate that synaptic dysfunction following PA might be produced by early changes in the actin organization and long-term misfolding and aggregation of proteins in the PSDs [4].

Therefore, we hypothesize that the synaptic and neuronal cytoskeleton changes induced by PA in the rat CNS could lead to the cellular dysfunction and death. In addition, electron tomography and correlative light and electron microscopy contributed to dissect critical alterations that were not described using conventional microscopy.

References

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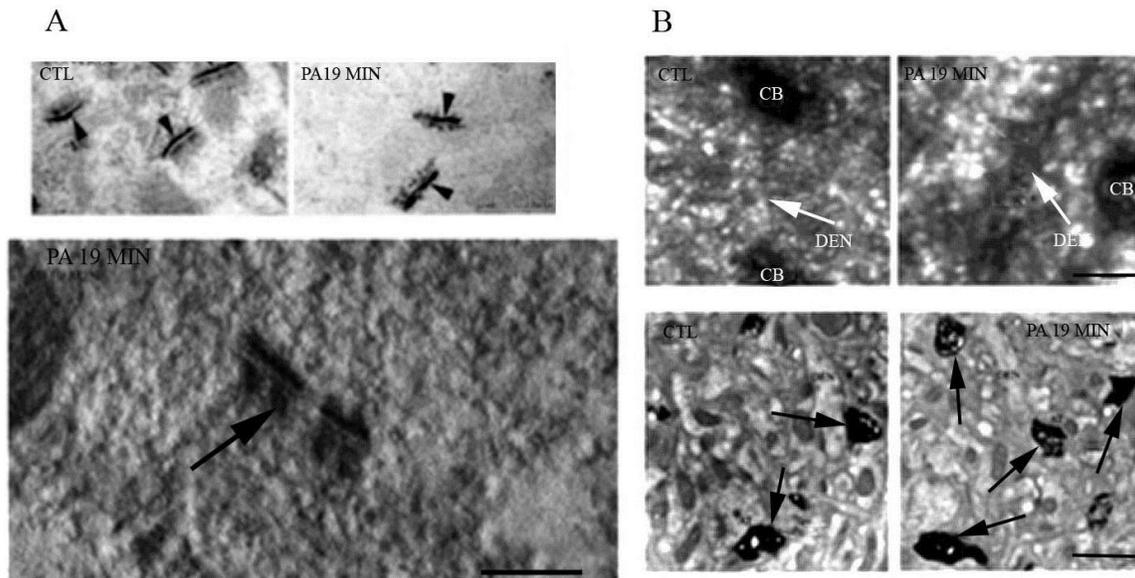


Figure 1. Electron microscopy images of the neostriatum in control and PA animal. (A) Micro photographs of E-PTA staining tissue showing alterations induced by PA in PSDs. Arrows pointed PSDs. Scale bar: 0.5 μ m. (B) Micro photographs of neostriatum tissue using correlative light and electron microscopy. Alterations in actin network dendritic spines were found after PA. Arrows showed correlatively dendrites spines that were photo-oxidized. Scale bar: 1 μ m.