MODIFICATIONS IN SYNAPSES AND RELATED STRUCTURE INDUCED BY PERINATAL ASPHYXIA

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Decrease in the oxygen levels during birth induced short and long term post- synaptic and cytoskeletal alterations that has been associated with neuronal cell death following hypoxia and several neurological diseases. 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 photooxidation 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. However, we also observed some filopodia indicating that at the beginning PA induced formation of new synapse. Glia, axons and dendrites did not show important modifications. At secondmonth of PA actin positive spines were less consistent associated with an increment of marker for neuronal and glial dysfunction such as GFAP, neurofilament and MAP-2.Strong alterations in the dendrite and astroglial cytoskeleton organization were found at four months of PA [3]. 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. In addition,these modifications produced the typical neuroglial reaction and disruption in intermediates filaments. Therefore, we hypothesize that the synaptic and neuronal cytoskeleton changes induced by PA in the rat CNS could lead with the increment of the age of the PA animal to the dramatic modifications in synapse and related structure that could triggerdysfunction and neuronal death. In addition, electron tomography and correlative light and electron microscopy contributed to dissect critical alterations that were not described using conventional microscopy.

References

[1] Capani F. et al. *Exp Neurol*. (2009) (219) (404).
[2] Saraceno GE. et al. *Exp Neurol*. (2016) (223) (615).
[3] Saraceno GE. et al. *Synapse*. (2012) (66) (9).

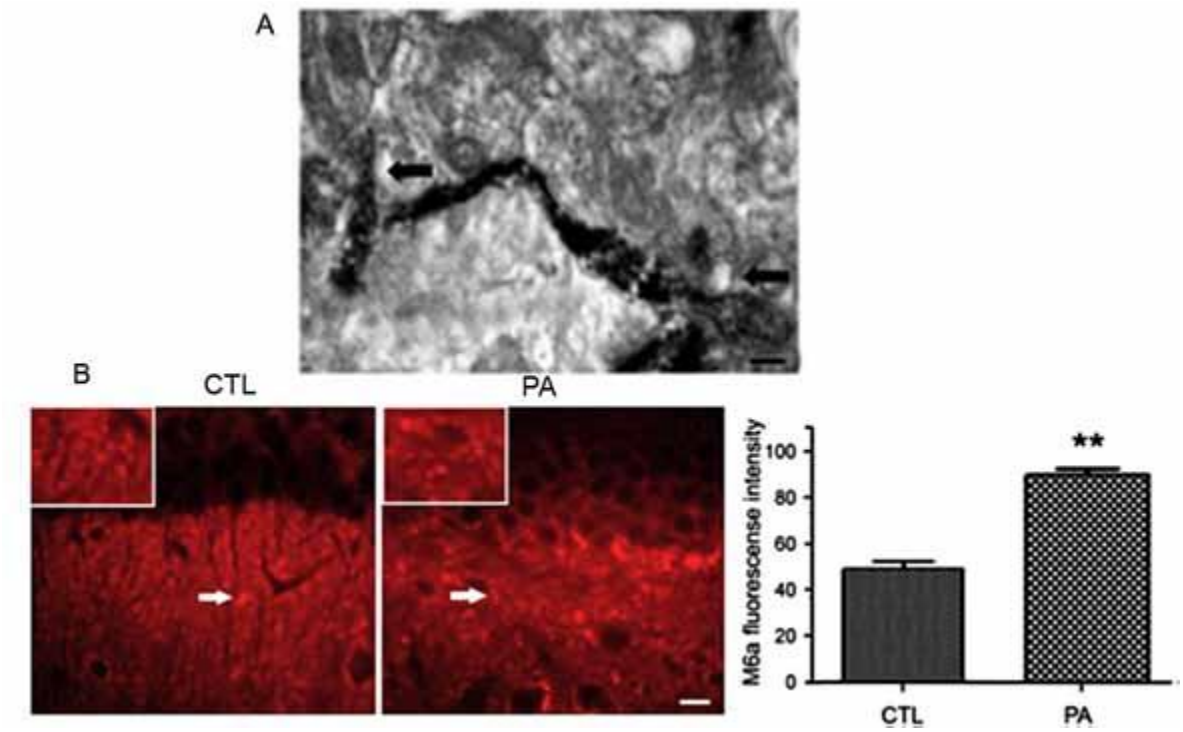

Fig. 1. Filopodium andM6a expression enhancement induced by PA. A) Electronmicrograph of filopodia. Themagnification of a filopodium allows us to observe its long structure and theparenteral dendrites (arrows). Scale bar: 1 μm. B) Fluorescence microscope images of M6a immunostaining from Stratum radiatum of CA1 hippocampal tissue from one-month-old control and PA rats (arrows).An increase in the punctate staining was observed in asphyctic animals (inset). **p b 0.01. Bars and error bars represent mean ± SEM. Statistical analyses were determined by Studentt-test. Scale bar: 10 μm.