Differential role of ionotropic and metabotropic glutamate receptors in immature and mature rat hippocampal slices exposed to ethanol dependence and withdrawal

D.E. Pellegrini-Giampietro[1], E. Gerace[1], E. Landucci[1], A. Totti[1], D. Bani[2], F. Moroni[3], G. Mannaioni[3]

[1]Dept. of Health Sciences, Section of Clinical Pharmacology and Oncology
[2]Dept. of Experimental and Clinical Medicine, Section of Anatomy and Histology
[3]Dept. of Neuroscience, Psychology, Drug Research and Child Health (NeuroFarBa), Section of Pharmacology and Toxicology, University of Florence, Italy

Chronic ethanol consumption causes persistent molecular alterations of neuronal circuits by mechanisms that are not yet fully understood. It has been demonstrated that the developing and adult brain display a differential sensitivity to ethanol toxicity, and that cerebral structure and function are diversely impaired according to the stage of synaptic maturation. To explore the mechanisms of ethanol toxicity in the developing and adult hippocampus, rat organotypic hippocampal slice cultures were exposed for 7 days to ethanol (100-300 mM) after 2 days (immature) or 10 days (mature) of culture in vitro; ethanol was then removed from the medium and 24 h later slices were examined for cell death. We observed that ethanol withdrawal led to a dose-dependent CA1 pyramidal cell injury in mature but not in immature slices. A significant increase in the expression of pre- and post-synaptic proteins in mature slices revealed that slice maturation is presumably associated with the development of new synapses. To study the mechanisms for this differential response to ethanol, we analyzed the expression levels of presynaptic (vGlut1, vGlut2, CB1 receptor, synaptophysin) and postsynaptic (GluA1, GluA2, NR2A, NR2B) proteins in immature and mature slices after chronic incubation or after ethanol withdrawal. Under both conditions, we observed a decrease in GluA1, GluA2 and synaptophysin expression levels in immature slices and a significant increase in the GluA1/GluA2 ratio in mature slices. Using whole cell voltage-clamp recordings from CA1 pyramidal cells of immature or mature slices, we measured the frequency and the amplitude of sEPSCs 7 days after exposure to ethanol and 24 h after ethanol withdrawal. Our electrophysiological results show a reduction in the frequency of sEPSCs in immature slices and a significant increase in the amplitude of sEPSCs in mature slices. Electron microscopy revealed disorganization of dendritic microtubuli in immature slices and signs of apoptotic cell death in mature slices. These results indicate that in immature slices ethanol induces an impairment of excitatory synaptic transmission similarly to what observed in fetal alcohol syndrome. In mature slices ethanol withdrawal leads to CA1 pyramidal cell death possibly due to expression of Ca2+-permeable AMPA receptors.