Chronic ethanol treatment differentially affects the neuronal circuits in immature and mature organotypic hippocampal slice cultures

E. Gerace[1], E. Landucci[1], T. Scartabelli[1], F. Moroni[2], G. Mannaioni[2], D.E. Pellegrini-Giampietro[1]

[1]Dept. of Health Sciences, Clinical Pharmacology and Oncology Unit, University of Florence, Italy
[2]Dept. of Neuroscience, Psycology, Drug Research and Child Health (NeuroFarBa), Section of Pharmacology and Toxicology, University of Florence, Italy

Chronic ethanol consumption causes persistent molecular alterations in brain cells and affects the maturation of neuronal circuits by mechanisms that are not fully understood. We investigated the mechanisms of ethanol dependence by exposing either immature (2 days *in vitro*) or mature (10 days *in vitro*) rat organotypic hippocampal slice cultures to ethanol (100-300 mM) for 7 days, after which ethanol was withdrawn for 24 h. Our results show that ethanol withdrawal led to a dose-dependent CA1 pyramidal cell injury in mature but not in immature slices. To comprehend the mechanisms by which ethanol withdrawal induced cell death in mature neurons, we analyzed the expression levels of presynaptic (vGlut1, vGlut2, CB1 receptor, synaptophysin) and postsynaptic (GluA1, GluA2, NR2A, NR2B) proteins in immature and mature organotypic slices after chronic treatment or after ethanol withdrawal. We observed no changes in the expression levels of vGlut1, vGlut2, CB1 receptor proteins neither in mature nor immature slices. On the other hand, we observed a decrease in GluA1 and synaptophysin expression levels in immature slices and a significant increase in GluA1 expression in mature slices, suggesting an impairment in synaptic transmission that may underlie increased toxicity to glutamate. To confirm the latter hypothesis, whole cell voltage-clamp recordings from CA1 pyramidal cells of immature or mature organotypic slices were performed. We measured the frequency and the amplitude of spontaneous excitatory post synaptic currents (sEPSCs) 7 days after exposure to ethanol and 24 h after ethanol withdrawal. Our results show a significant reduction in the frequency but not amplitude of sEPSCs in immmature slices, on the contrary, a significant increase in the amplitude but not in the frequency of sEPSCs was observed in mature slices. These findings suggest that chronic ethanol treatment may promote abnormal synaptic transmission in immature hippocampal neurons and that ethanol withdrawal leads to cell death of mature CA1 pyramidal cells that may be important in neurodevelopmental and neurological disorders.