Early disruption of blood brain barrier integrity precedes NOX2 elevation in the social isolation rat model

S. Schiavone[1], E. Mhillaj [2], P. Tucci[1], M.G. Morgese[1], M. Bove[2], V. Cuomo[2], L. Trabace[1]

[1]Dept. of Clinical and Experimental Medicine, University of Foggia, Italy
[2]Dept. of Physiology and Pharmacology 'V.Erspamer', University of Rome 'Sapienza', Italy

The social isolation rearing of young adult rats is defined as a model of psychosocial stress and provides a non-pharmacological tool to study long-term alterations reminiscent of several symptoms observed in psychosis (Leng et al. 2004; King et al. 2009). Data from animal studies, as well as human evidence, have reported a key role of oxidative stress in the pathogenesis of this mental disorder. Our research group has previously demonstrated that social isolation leads to NADPH oxidase 2 (NOX2) enzyme elevations in the brain, which in turn contributes to the development of behavioural and neuropathological alterations in isolated rats (Schiavone et al. 2012). However, primary neuropathological events underlying early NOX2 elevations in the brain have not been established yet. The aim of this study was to investigate early genomic and molecular alterations leading to NOX2 increase in the brain. To this purpose, male pups were separated from their mothers (postnatal day 21), and were exposed to a short period of social isolation (1 week). The microarray technology was used in order to study the genome-wide gene expression profile in control and isolated rats. We focused our attention on genes, which were up-regulated after 1 week of social isolation, and their expression was further investigated by using real-time PCR and immunohistochemistry techniques. One week of social isolation increases the expression of brain vascular morphogenesis markers, such as oxidized low density lipoprotein receptors (ORLs), ischemia-related factors 21 and 16 (Vof21 and Vof16), leukocyte receptor cluster member 8 (Leng8), vomeronasal receptor 1 (Vnr1) and tetratricopeptide repeat and ankyrin repeat containing 1 (Trank1). The expression of specific genes involved on blood-brain barrier (BBB) integrity, such as matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), occludin-1 and plasmalemmal vesicle associated protein-1 (PV-1) are also significantly altered in isolated rats compared to controls. Moreover, blood brain barrier (BBB) permeability is significantly increased in isolated rats, as showed by Evans-blue dye assay. Conversely, no differences in NOX2 levels are detected at this time point. Our data demonstrate that early disruption of BBB precedes the NOX2 elevations in the brain, and might be its leading cause. Moreover, the up-regulation of genes that are considered as markers of BBB's integrity and function represent a very early event, which might also precede the appearance of psychotic-like symptoms in animals. In conclusion, these results provide a better understanding of the mechanisms linking psychosocial stress, early oxidative stress in the brain, disruption of the BBB and the development of mental disorders.

Leng et al. (2004). *Pharmacol Biochem Behav* 77,371-9
King et al. (2009). *Synapse* 63,476–83
Schiavone et al. (2012). *Transl Psychiatry* 2(5),e111