Long-term transcriptomic changes in the ventral hippocampus and liver of adult rats exposed to prenatal stress

Ilari D'Aprile[1,2], Giulia Petrillo[1,2], Moira Marizzoni[1], Samantha Saleri[1] , Valentina Zonca[2], Monica Mazzelli[1], Marco A Riva[1,2] and Annamaria Cattaneo[1,2]

[1]Biological Psychiatry Unit, IRCCS Fatebenefratelli San Giovanni di Dio, Brescia, Italy;
[2]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy

Abstract

Exposure to early-life stress (ELS) has been identified as the primary risk factor contributing to enhanced vulnerability to the onset of mental and metabolic disorders. Previous studies showed that perinatal adversities lead to behavioral and neurobiological defects but there are no evidences regarding their impact on other biological systems since possible causative mechanisms linking exposure to ELS with psycho-metabolic comorbidity are still poorly investigated.

We have used the Prenatal Stress Model (PNS), where pregnant dams have been exposed to a restraint stress paradigm from gestational day 14 to 21. Animals have been then euthanized (PND84), liver and ventral hippocampus (VHIP) were collected. We have performed transcriptomic analyses using RNAseq technique on them to investigate pathways significantly modulated by stress. Next, the transcript-level differential expression was assessed using DESeq2 (v1.30.1) in R. We used the obtained lists of differentially expressed genes (DEGs) to perform pathway analyses by using Ingenuity Pathway Analysis (IPA) (Qiagen).

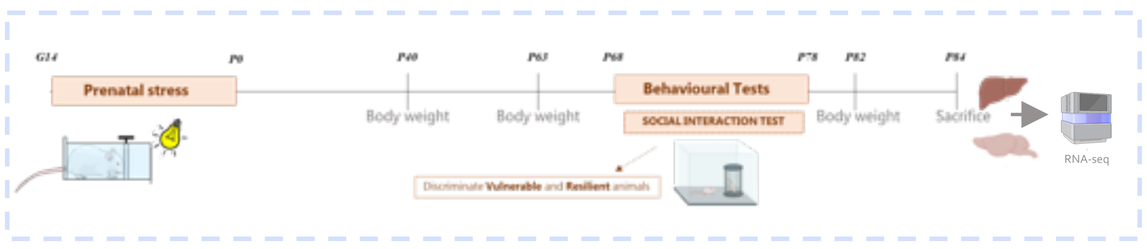
In the VHIP, we identified 527 DEGs with p-value < 0,05 and FClog < -0,263 or FClog > 0,263 in PNS adult males compared to control group. In liver pathway analysis, PNS adult males showed 610 DEGs with p-value < 0,05 and FClog <- 0,263 or FClog >0,263.

Keywords: prenatal stress, comorbidity, liver, VHIP, transcriptomic profiles

Methods

Pregnant Sprague-Dawley rats have been exposed to a PNS paradigm from gestational day 14 to 21.

• All animals have been euthanized at PND 84 by decapitation, brain and liver were collected and total RNA extracted.



•RNA-seq libraries were prepared using Illumina Stranded mRNA Prep, Ligation kit (Illumina) and sequenced (75bp, Illumina NextSeq 2000).

• The transcript-level differential expression was assessed using DESeq2 (v1.30.1) in R.

• On the obtained lists of differentially expressed genes (DEGs) we performed pathway analyses by using Ingenuity Pathway Analysis (IPA).

Results

1. PNS alters inflammatory response, metabolic and neuronal functions in the VHIP and liver of adult male animals

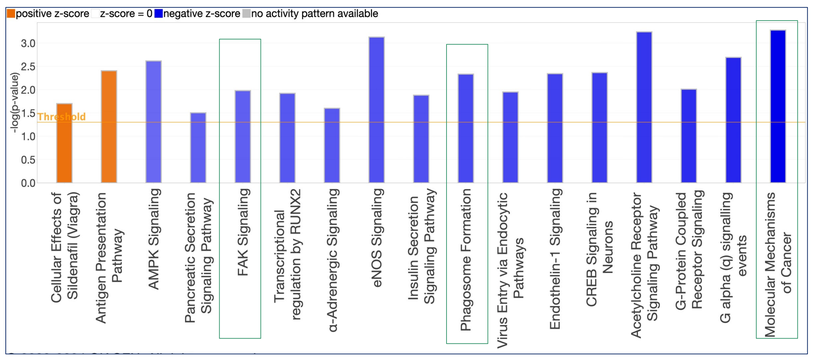


Figure 1: In the **VHIP** we identified two pathways significantly upregulated and 15 significantly downregulated in PNS exposed animals respect to controls (z-score > |2|, p<0,05)

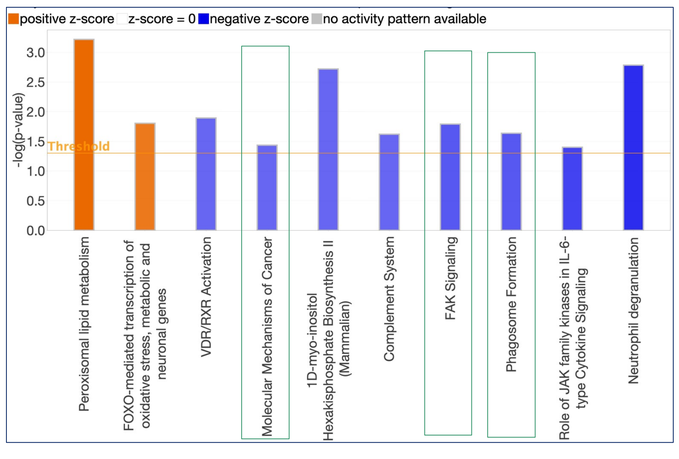
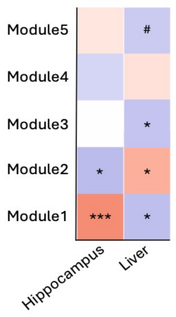


Figure 2: In the **liver**, we identified 2 pathways significantly upregulated and 8 significantly downregulated in PNS exposed animals respect to controls (z-score > |2|, p<0,05). Among them, three downregulated pathways are in common with the brain

2. PNS exposure leads to alterations common to both brain and liver



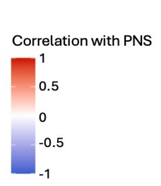


Figure 5: In Fig5 are shown consensus modules associated with PNS. Red indicates a positive, and blue means a negative correlation.

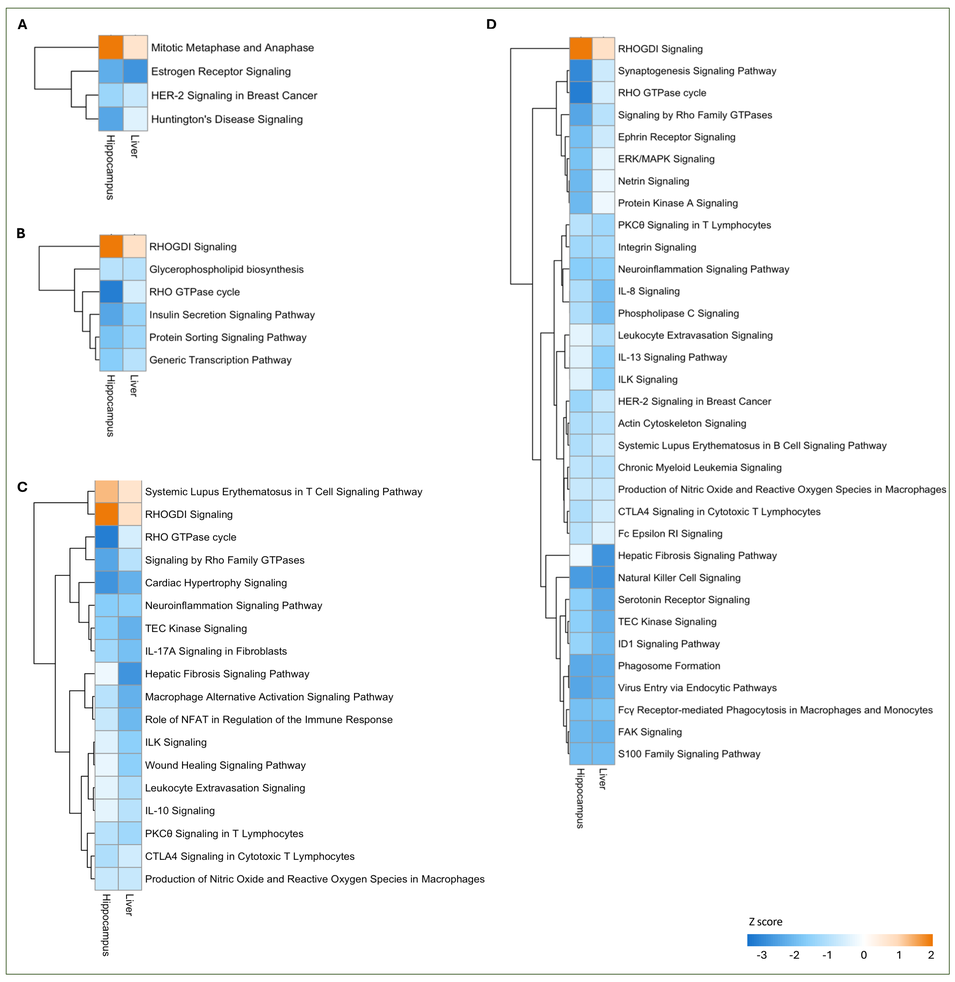


Figure 4: Modules identified between Ingenuity Pathway analysis and WGCNA consensus in both the hippocampus and the liver. Each panel shows the heatmap of the pathways with concordant z-scores found significantly enriched (p<0.05) in consensus in A) module 1, B) module 2, C) module 3 and D) module 5. Hierarchical clustering was used to group pathways. Blue bars: negative z-score; orange bars: positive z-score.

Conclusion

These findings underscore the intricate interplay between early-life stress, liver and brain functions, shedding light on potential mechanisms underlying the development of mental and metabolic disorders.

Acknowledgments: This work was supported by EarlyCause Project (grant no.848158), by PSR (Piano Sostegno Ricerca) from the University of Milan to A.C. The work was also supported by Italian Ministry of University & Research by M.A.R. (PRIN-grants number 2017AY8BP4).