The interplay between insula and habenula in the vulnerability or resilience to prenatal stress

Valentina Zonca[1], Moira Marizzoni[2], Samantha Saleri[2], Monica Mazzelli[2], Giulia Petrillo[1,2], Maria Grazia di Benedetto[1,2], Floriana de Cillis[1,2], Marco Andrea Riva[1,2] and Annamaria Cattaneo[1,2]

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

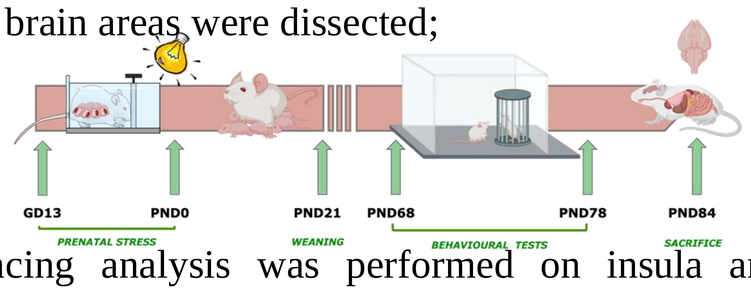
Introduction

Stress experienced early in life can have profound effects on brain development and has long-lasting effects on physical and mental health, potentially leading to the development of psychopathology later in life.
Clinical and preclinical studies have shown that exposure to stress during gestation or early postnatally can produce different behavioural alterations, although not in all the exposed individuals.
Recently habenula and insula have been suggested as new brain regions underlying the development of vulnerability or resilience to prenatal stress exposure.
The aims of this study are:

- Elucidate the biological mechanisms underlying the association between stress early in life and the onset of a vulnerable phenotype occurring in habenula and insula;
- Identify possible depressive-like phenotype taking advantage of a preclinical model of prenatal stress (PNS).

Methods

- Sprague Dawley pregnant rats underwent a prenatal stress experimental paradigm (PNS) during the last week of gestation.
- After birth, male offspring (n=48) were left undisturbed until early adulthood (PND68) when the social interaction test was performed to assess to sociability domain. One week later, animals were euthanized, and different brain areas were dissected;



- RNA-Sequencing analysis was performed on insula and habenula, differentially expressed genes were identified by using DeSEq2 (q-value <0.1, FC >|1.2|), and subsequently, pathways and weighted correlation network analysis (WGCNA) has been performed (p<0.05)

Results

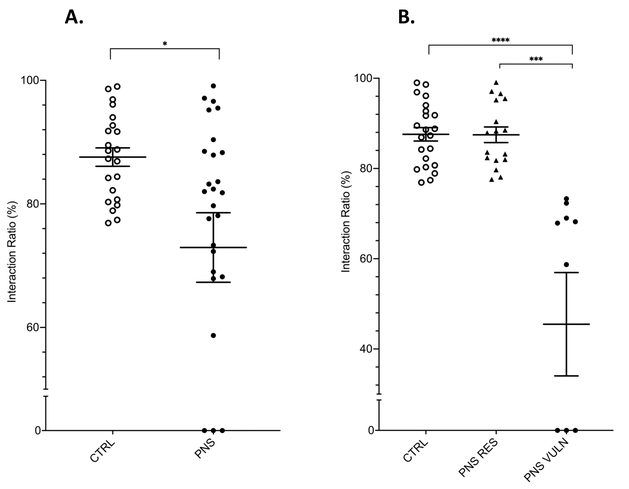


Figure 1. Results of the **Social Interaction test.** The social behaviour is here expressed by using the percentage of social interaction ratio (time spent interaction with the novel animal / time spent with the novel animal + time spent interacting with the empty cage). **(A)** PNS rats spent less time interacting with the novel animal (72.04% ± 5.8) compared with control animals (87.58 % ± 1.5) (p=0.019). **(B)** When diving the PNS rats in vulnerable and resilient, a significant difference was observed in the SI ratio % in PNS vulnerable (45.49% ± 11.4) compared with PNS resilient rats (87.47% ± 1.7)(p<0.0001) and CTRL animals (87.58% ± 1.48) (p<0.0001). Data are presented as Mean ± SEM; Mann-Whitney, *p<0.05; Kruskal-Wallis ***p<0.001, **** p<0.00001.

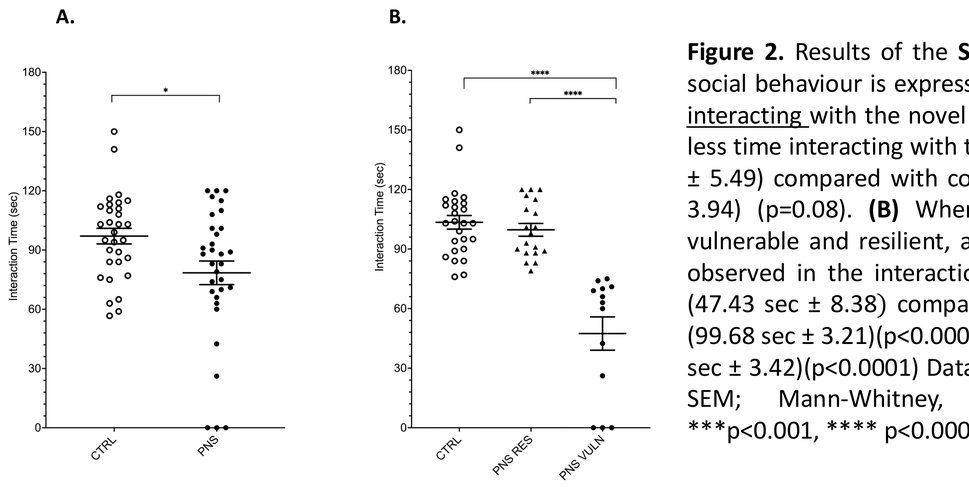


Figure 2. Results of the **Social Interaction test**. The social behaviour is expressed by the total time spent interacting with the novel animal. **(A)** PNS rats spent less time interacting with the novel animal (82.82 sec ± 5.49) compared with control animals (97.05 sec ± 3.94) (p=0.08). **(B)** When diving the PNS rats in vulnerable and resilient, a significant difference was observed in the interaction time in PNS vulnerable (47.43 sec ± 8.38) compared with PNS resilient rats (99.68 sec ± 3.21)(p<0.0001) and CTRL animals (103.5 sec ± 3.42)(p<0.0001) Data are presented as Mean ± SEM; Mann-Whitney, *p<0.05; Kruskal-Wallis ***p<0.001, **** p<0.00001.

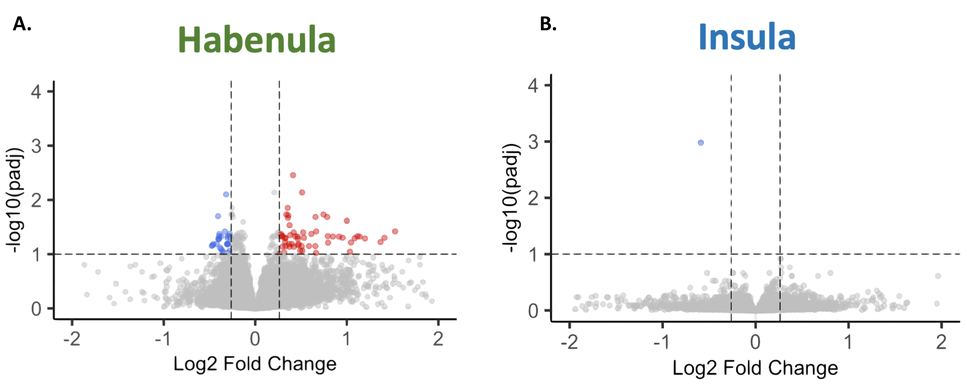


Figure 3. Transcriptomic profile of vulnerable animals in Habenula and Insula. (A) In the habenula brain region, 94 differentially expressed transcripts were identified as significantly modulated in vulnerable animals compared to controls. **(B)** In the insula brain region, 1 transcripts was differently modulated considering the q <0.1 cut-off. These results suggests that the vulnerable phenotype induce a modulation of the transcriptomic profile mostly in the habenula.
DeSeq2 analysis cutoffs: q-value<0.1, FC ± 1.2

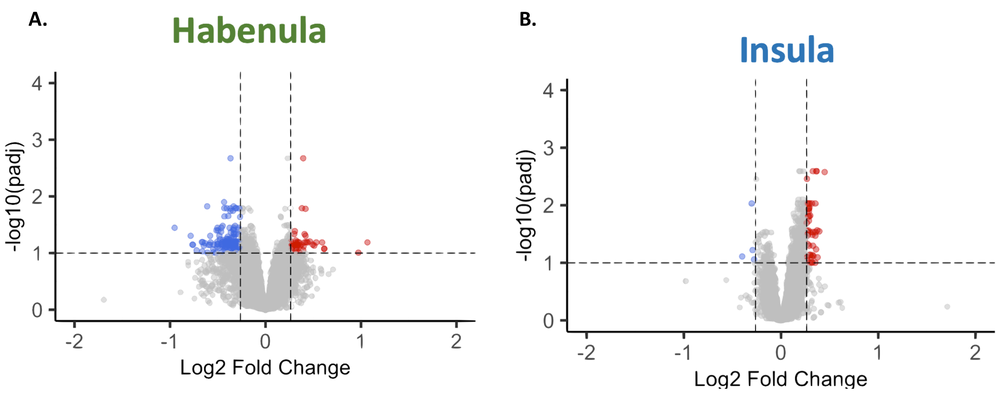


Figure 4. Transcriptomic profile of resilient animals in Habenula and Insula. (A). In the habenula brain region, 179 differentially expressed transcripts were identified as modulated in resilient animals compared to controls. **(B)** In the insula brain region, 53 transcripts was differently modulated in resilient animals compared with controls. These results suggest that the resilient phenotype is associated with more pronounced transcriptomic changes compared to vulnerable animals, indicating that resilience is an active, rather than a resting, condition. Furthermore, similar to vulnerable animals, the habenula exhibits greater transcriptomic alterations compared to the insula. DeSeq2 analysis cutoffs: q-value<0.1, FC ± 1.2

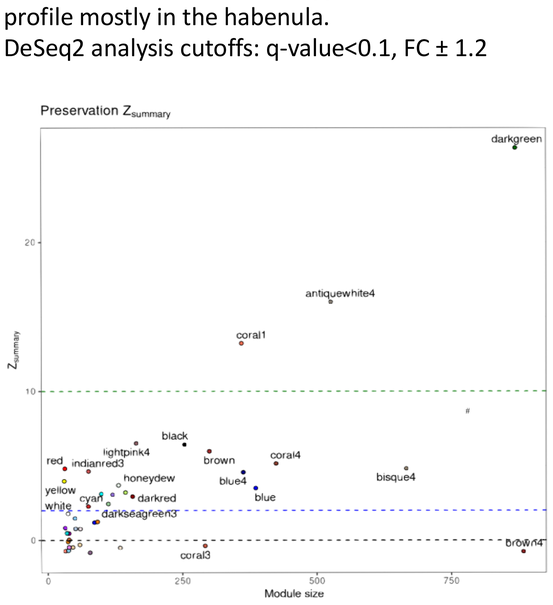


Figure 5. Co-expression module preservation using WGCNA in vulnerable animals. The preservation analysis in vulnerable animals identified 23 modules detected in the habenula. Specifically, 20 were not preserved whereas 3 were preserved in the insula, suggesting an overall low preservation in vulnerable animals. Specifically, the plum4 (Zsummary -0.82) and the brown4 (Zsummary -0.76) were the less preserved, whereas the only preserved modules were the darkgreen (Zsmmary 26.34), the antiquewhite4 (Zsummary 15.99) and the coral1 (Zsummary 13.21).

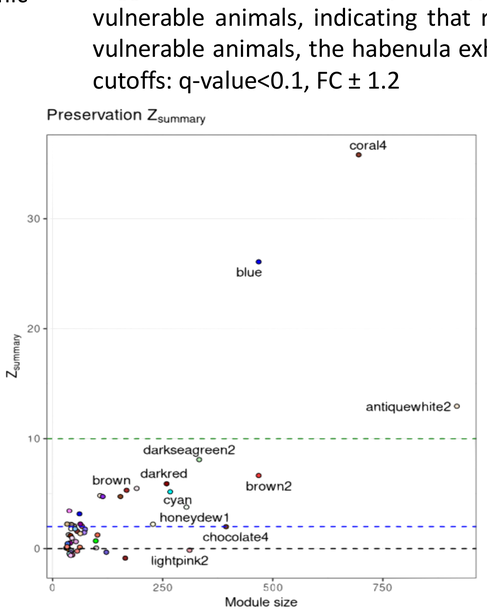


Figure 6. Co-expression module preservation using WGCNA in resilient animals. The preservation analysis in vulnerable animals identified 34 modules in the habenula. Specifically, that 31 out of 34 modules are not preserved in the insula, such as the brown (Zsummary -0.86) and plum1 (Zsummary -0.62). On the other hand, only 3 are preserved in the insula and are the coral4 (Zsummary 35.81), the blue (Zsummary 26.07) and the antiquewhite1 (Zsummary 12.94) modules .

Further pathways analysis performed by using Ingenuity Pathways Analysis software show that the preserved modules in resilient animals account for the highest number of genes, suggesting a strong activity in habenula and insula of resilient male animals. Specifically, both coral4 and blue modules are particularly enriched in neuronal processes, such as formation of dendrites, axonogenesis and synapsis organization

Conclusions

Exposure to prenatal stress resulted in impairments within the sociability domain in adult male rats. RNA Sequencing analysis revealed that resilient animals exhibit more transcriptomic alterations compared to vulnerable animals in both the insula and habenula regions, indicating that the resilient condition is characterized by active processes rather than being a resting condition. Moreover, some transcriptomic modulations of habenula are preserved in the insula in both vulnerable and resilient animals.

Acknowledgments: This work was supported by Ricerca Corrente (Italian Ministry of Health, MoH) and by the PSR (Piano Sostegno Ricerca) from the University of Milan (to Annamaria Cattaneo). The work has been also supported by to Marco Andrea Riva (PRIN – grants number 2015SKN9YT and 2017AY8BP4 and PON “Ricerca e Innovazione” PerMedNet -project ARS01_01226).