Studying stress in BV2 microglial cells: from model development to translational perspectives

B. Brusa[1], S. D'Amelio[1], D. F. Lattuada[1], J. Mingardi[1], R. Molteni[1]

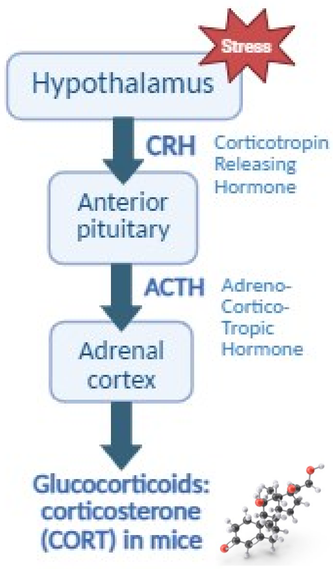
[1]Laboratory of Neuropsychopharmacology, Department of Medical Biotechnology and Translational Medicine, University of Milan, L.I.T.A, Milan

POSTER ID: 2.34
beatrice.brusa@unimi.it

BACKGROUND

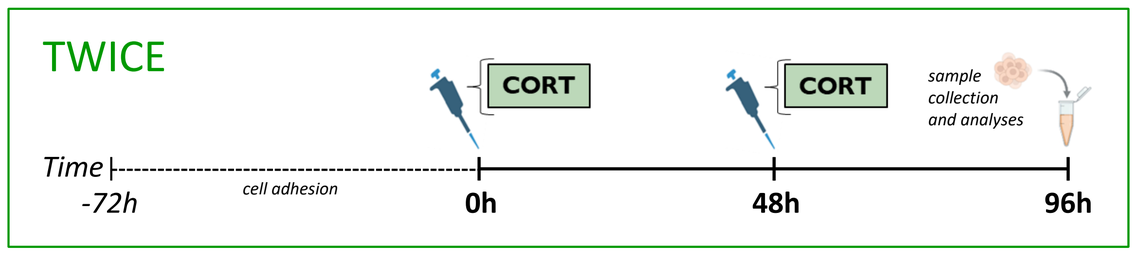
Chronic stress is a major risk factor for the development of neuropsychiatric disorders and is characterized by prolonged **activation of the hypothalamic–pituitary–adrenal (HPA) axis**, resulting in sustained glucocorticoid (GC) release. While acute GC signalling promotes adaptive responses through the coordinated activation of mineralocorticoid (MR) and glucocorticoid receptors (GR), prolonged exposure may lead to a dysregulated GR-driven state associated with altered cellular homeostasis.

Literature has frequently reported the **pivotal involvement of microglia in the pathophysiology of psychiatric disorders**; nevertheless, the underlying molecular mechanisms are not fully explained.



OBJECTIVES AND EXPERIMENTAL STRATEGY

The aim of our study was **to set up an *in vitro* protocol of chronic stress based on prolonged CORT exposure in BV2 murine microglial cell line** to investigate stress-dependent cellular and molecular consequences.



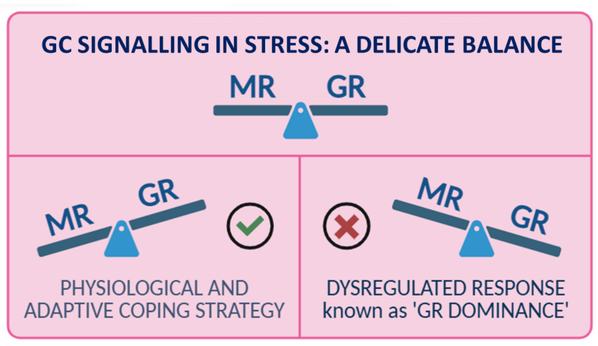
WITH VARYING CONCENTRATIONS:

Untreated CTRLs | CORT 0.05µM | CORT 0.25µM | CORT 0.50µM | CORT 1µM | CORT 5µM

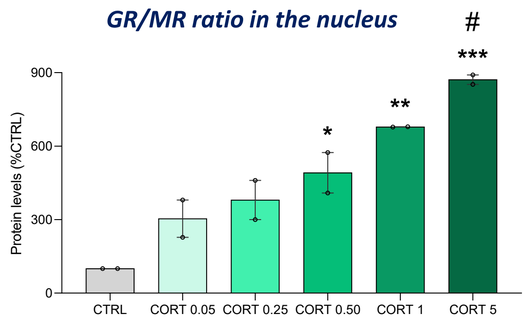
The color coding shown here is maintained in all subsequent graphs.
N=3 independent biological replicates

RESULTS

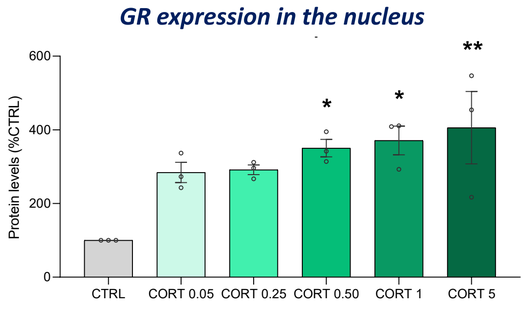
1. Impact of prolonged CORT treatment on GC signalling



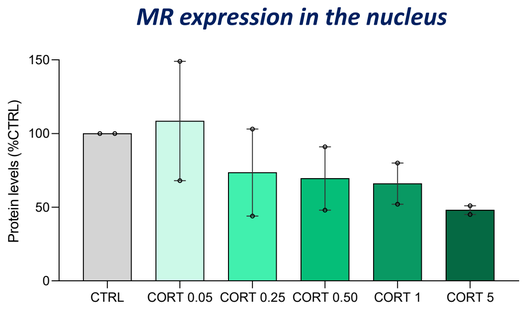
GR/MR ratio in the nucleus



GR expression in the nucleus

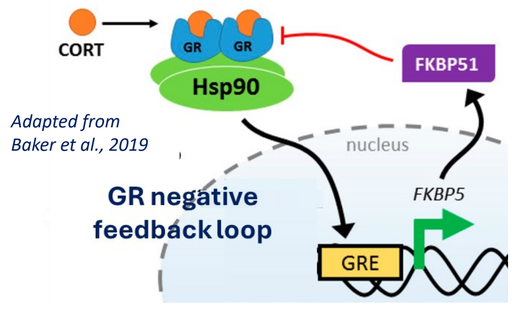


MR expression in the nucleus

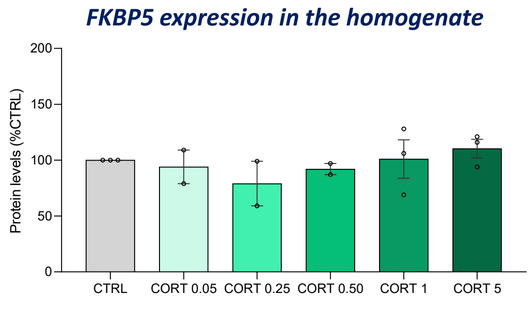


p<0.05 (*), p<0.01 (**) and p<0.001 (***) vs CTRL; p<0.05 (#) vs CORT 0.50; one-way ANOVA with Tukey's post hoc test

2. FKBP5 regulatory mechanism recruitment failure upon chronic exposure

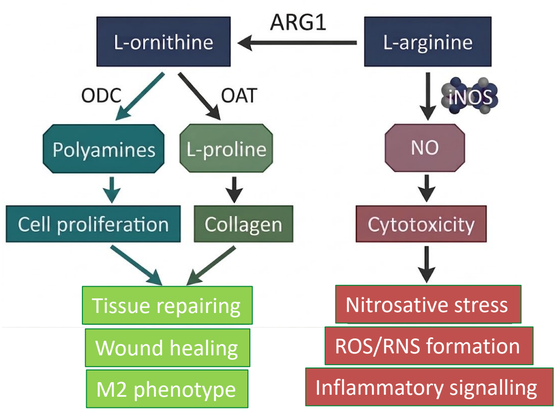


FKBP5 expression in the homogenate

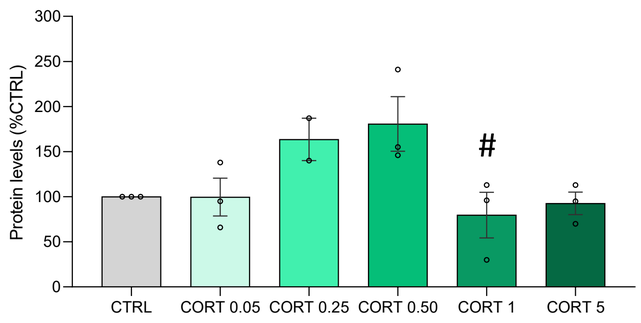


3. CORT-induced biphasic response of arginase 1 (ARG1)

Adapted from Yang & Ming, 2014

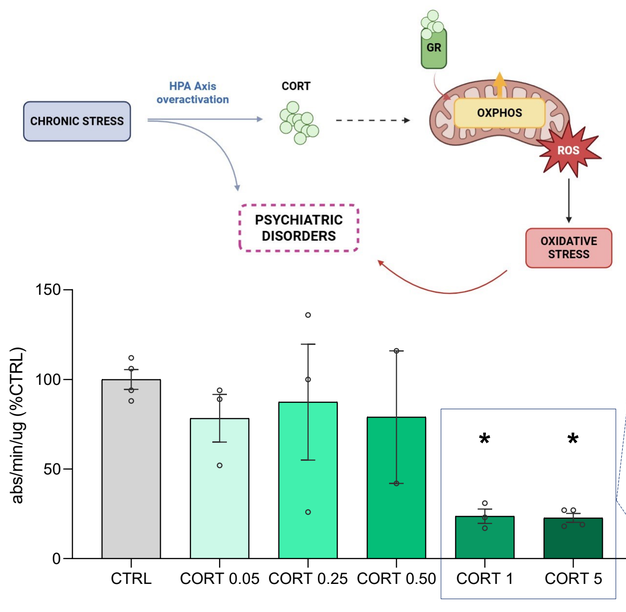


ARG1 expression in the homogenate

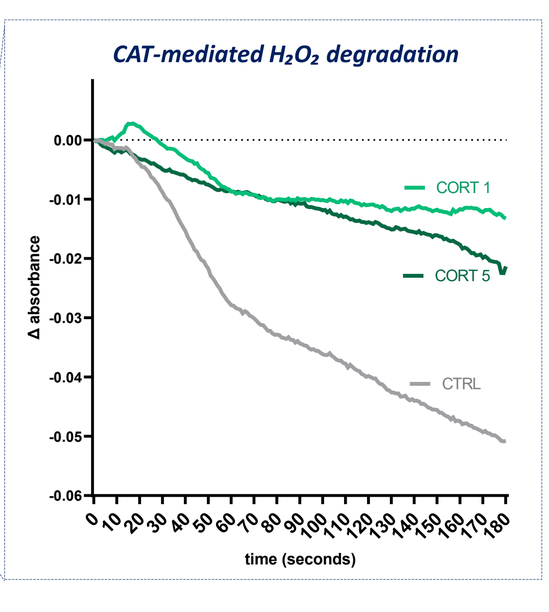


p<0.05 (#) vs CORT 0.50; one-way ANOVA with Tukey's post hoc test

4. Effects of sustained GC signalling on oxidative stress: a focus on catalase (CAT) activity



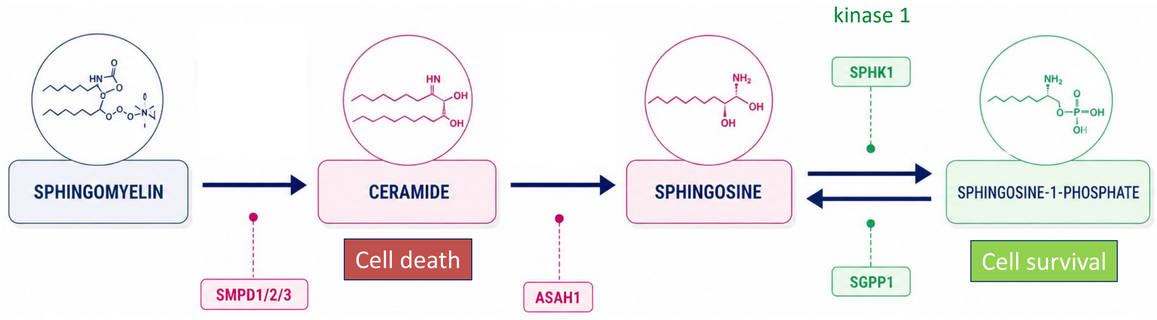
CAT-mediated H_2O_2 degradation



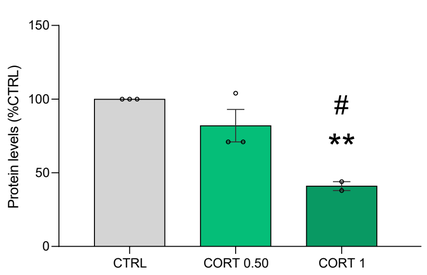
p<0.05 (*) vs CTRL; one-way ANOVA with Tukey's post hoc test

IN PROGRESS

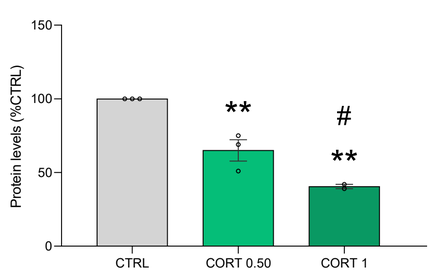
5. Sphingolipid rheostat dysregulation



SMPD2 expression in the homogenate



ASAH1 expression in the homogenate



p<0.01 (**) vs CTRL; p<0.05 (#) vs CORT 0.50; one-way ANOVA with Tukey's post hoc test

CONCLUSIONS

- Prolonged CORT treatment induced an increase in GR nuclear expression and an imbalance of the 'MR-GR switch' towards GR, coupled with an impairment of its FKBP5-mediated negative feedback mechanism.
- The non-linear modulation of ARG1 expression and CAT activity implies a shift in microglial response across increasing CORT levels and suggests the loss of coping capacity beyond a certain threshold.
- The model may provide valuable insights into stress-related molecular alterations in biological processes beyond direct GC signalling, such as oxidative stress and the sphingolipid rheostat, thereby offering a useful platform to evaluate potential therapies.

REFERENCES

(1) Kwon, D. (2022). Guardians of the brain: How a special immune system protects our grey matter. **Nature**.
(2) Bassil, K. *et al* (2023). In vitro modeling of the neurobiological effects of glucocorticoids: A review. **Neurobiology of Stress**, 23, 100530.
(3) Aebi, H. (1984). Catalase in vitro. **Methods in Enzymology**, 105, 121–126.
(4) de Kloet, E. R. *et al* (2005). Stress and the brain: From adaptation to disease. **Nature Reviews Neuroscience**, 6(6), 463–475.
(5) de Kloet, E. R. *et al* (2016). Stress and depression: A crucial role of the mineralocorticoid receptor. **Journal of Neuroendocrinology**, 28(8).
(6) de Kloet, E. R., & Joëls, M. (2023). The cortisol switch between vulnerability and resilience. **Molecular Psychiatry**.
(7) Hoertel, N. *et al* (2026). The role of sphingolipids in major depressive disorder and associated cognitive impairment: interactions with monoaminergic signaling, neuroinflammation, and neurogenesis. **Molecular Psychiatry**.
(8) Merkulov, V.M. *et al* (2017). Mechanisms of brain glucocorticoid resistance in stress-induced psychopathologies. **Biochemistry Moscow**, 82, 351–365.