Establishment and characterization of an *in vitro* model of chronic stress in BV2 microglial cells

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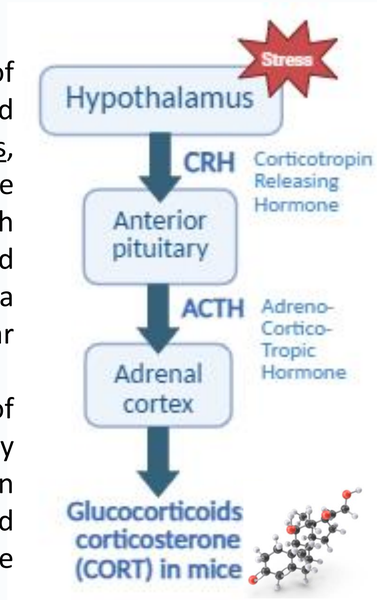
BACKGROUND

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

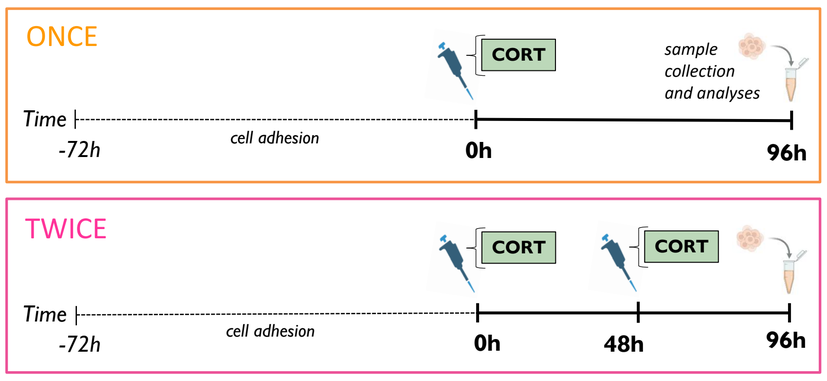
Literature has frequently reported the pivotal involvement of microglia in the pathophysiology of psychiatric disorders, as they are highly sensitive to stress hormones and play a key role in resulting alterations. Understanding how chronic glucocorticoid exposure affects microglial function is critical for modeling the core mechanisms underlying stress-related conditions *in vitro*.



OBJECTIVES AND EXPERIMENTAL STRATEGY

The aim of our study was stimulating chronic stress conditions in vitro by exposing the BV2 murine microglial cell line to prolonged corticosterone stimulation in order to investigate its effects on cellular and molecular physiology. To achieve this, cells were treated:

UNDER DIFFERENT TIMELINES



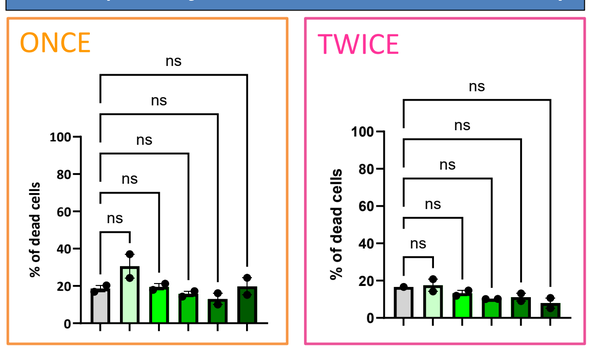
WITH VARYING CONCENTRATIONS

- Untreated controls
- 0.05µm corticosterone
- 0.25µm corticosterone
- 0.50µm corticosterone
- 1µm corticosterone
- 5µm corticosterone

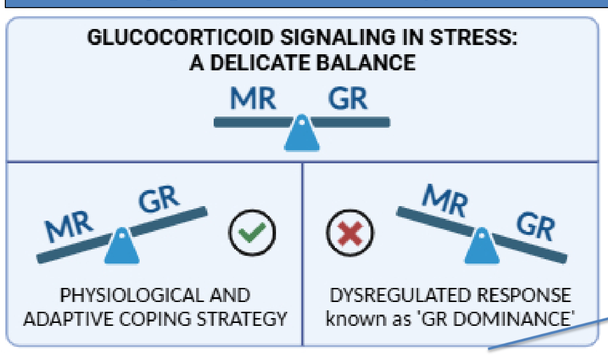
The color coding shown here is maintained in all subsequent graphs

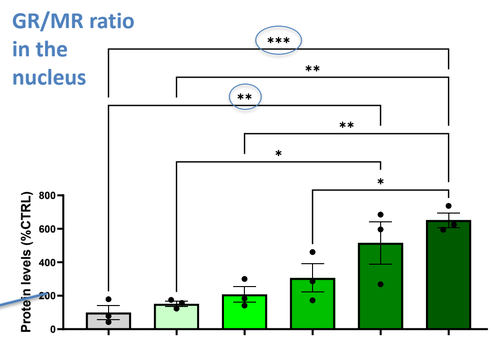
RESULTS

The impact of CORT treatment on vitality



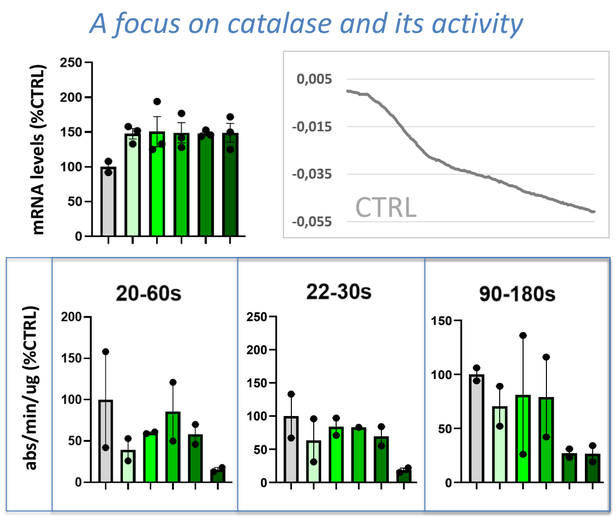
The role of glucocorticoid receptors in the regulation of chronic stress responses





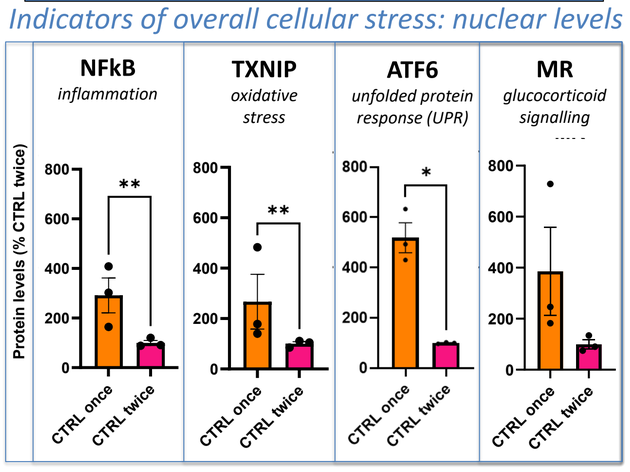
The involvement of oxidative stress

A focus on catalase and its activity



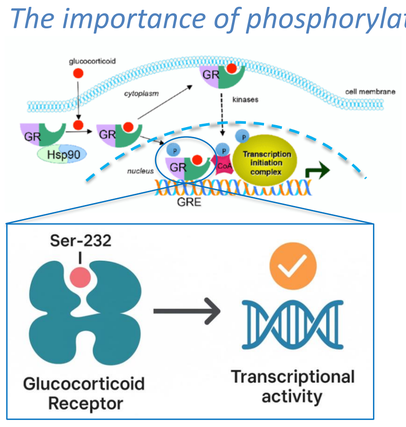
TIMELINE SELECTION: ONCE VS TWICE

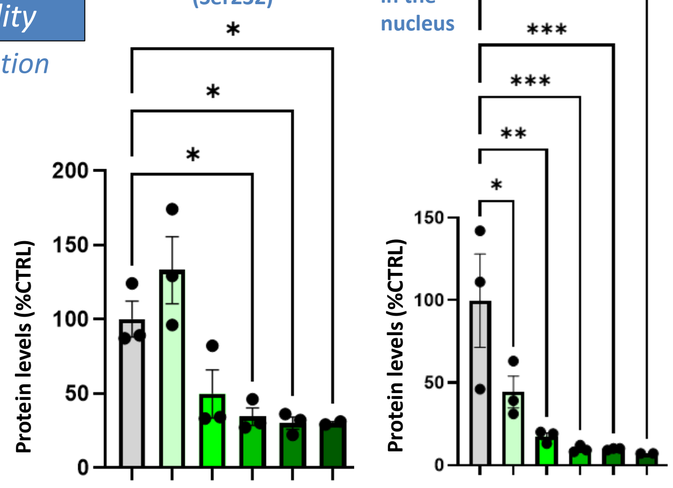
Indicators of overall cellular stress: nuclear levels

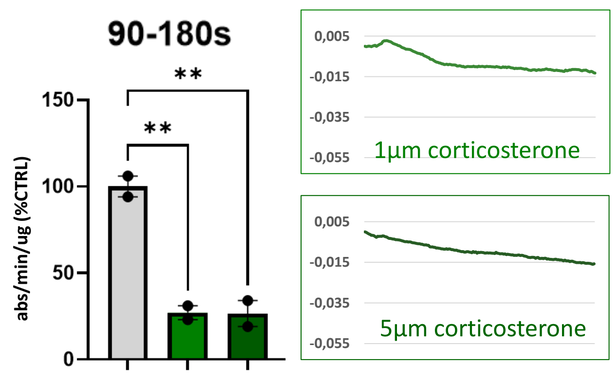


The effects of prolonged GR dominance on its functionality

The importance of phosphorylation





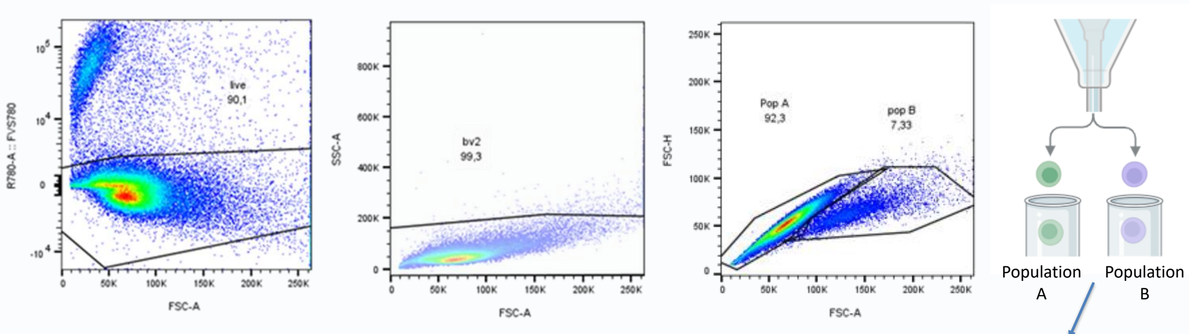


IN PROGRESS

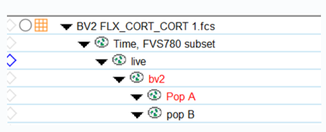
Previous insights: marked separation of BV2 microglial cells into two morphologically different populations following treatment, as observed upon flow cytometric analysis.

Preliminary data (to be validated): difference in responsiveness between the two populations based on higher mean fluorescence intensity (MFI) and percentage of positive cells for all assessed markers of activation or inflammation such as CD11b.

Future perspective: cell sorting to investigate the two populations and their differential response to chronic corticosterone treatment separately



GATING STRATEGY:



Downstream analyses: protein, mRNA and catalase activity levels + morphological studies (immunofluorescence)

CONCLUSIONS

These data demonstrate that, while prolonged treatment with corticosterone is not toxic for cells, the choice of the experimental strategy is crucial to cellular homeostasis: indeed, results made it clear that the single stimulus paradigm, named **ONCE**, leads to a generalized increase in basal levels of stress even in untreated controls, probably due to the lack of medium change for 96h when the studied BV2 cell line is usually exposed to fresh medium every 48h. The other timeline, defined as **TWICE**, respects this criterium and was thus preferred, proving that sustained CORT treatment induces in microglia a significative state of GR dominance and dysfunction, consistent with a chronic stress model, notably involving oxidative stress. Interestingly, some cells seem to exhibit varying degrees of responsiveness, thus opening the possibility to investigate the underlying factors that drive this differential cellular response.

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