Set-up and molecular characterization of an *in vitro* model of chronic stress in BV2 microglial cells

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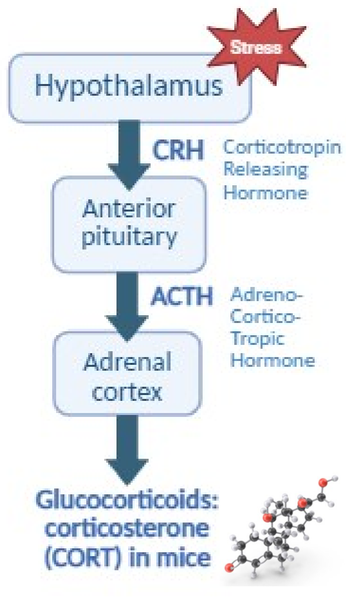
BACKGROUND

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.

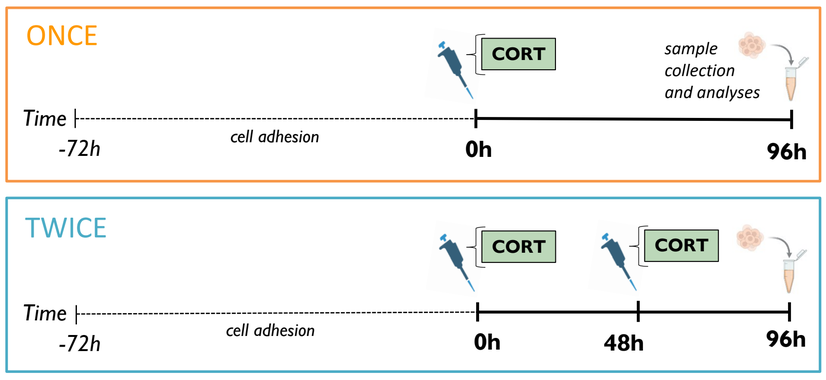
Literature has frequently reported the pivotal involvement of microglia in the pathophysiology of psychiatric disorders, as they are highly sensitive to stress hormones and play a key role in resulting alterations. Understanding how chronic glucocorticoid exposure affects microglial function is critical 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:



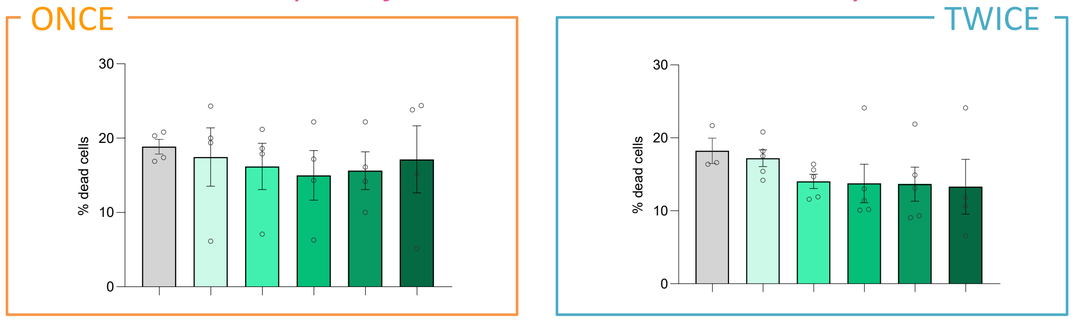
WITH VARYING CONCENTRATIONS:

- Untreated CTRLs
- CORT 5μM
- CORT 0.25μM
- CORT 0.50μM
- CORT 1μM
- CORT 5μM

The color coding shown here is maintained in all subsequent graphs.

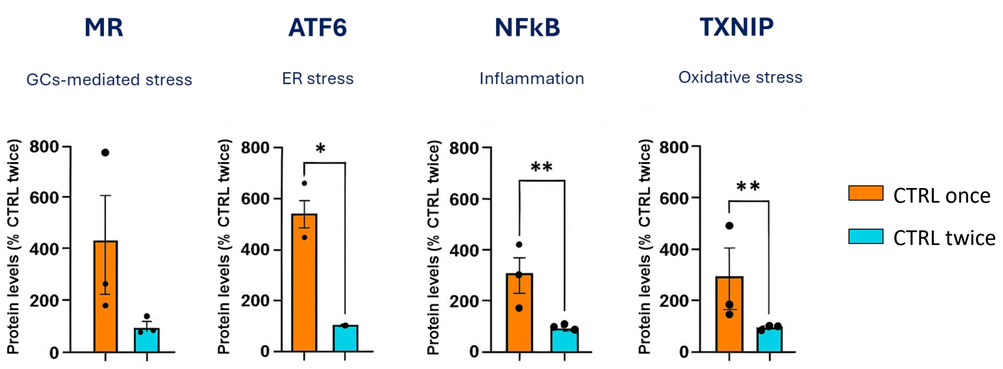
RESULTS

The impact of CORT treatment on cell viability

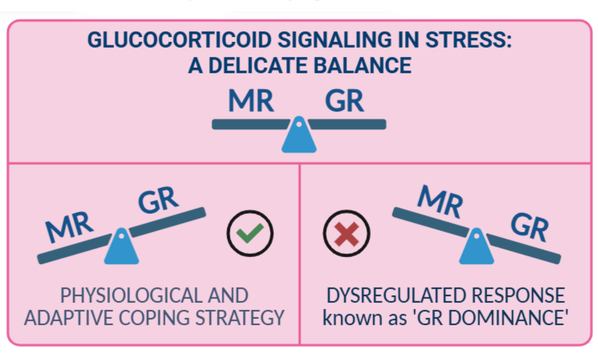


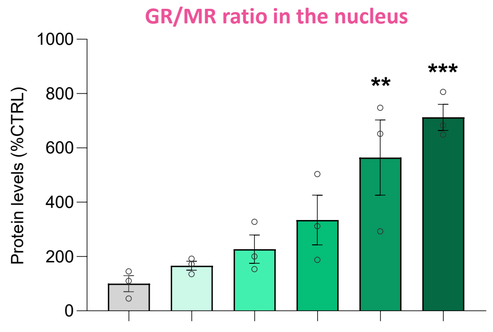
TIMELINE SELECTION: ONCE VS TWICE

Indicators of basal activation in untreated CTRLs: nuclear levels

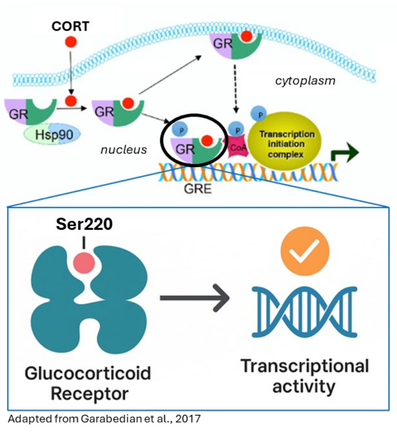


The receptors of glucocorticoids in the regulation of chronic stress



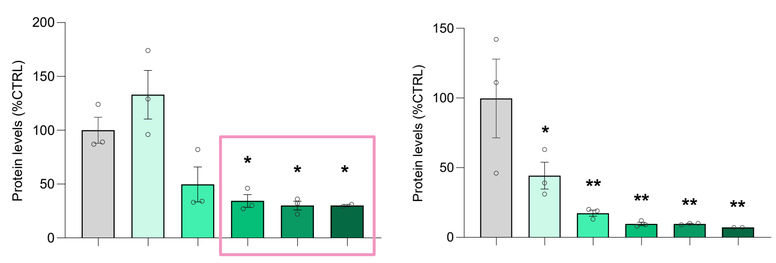


The effects of prolonged GR dominance on its functionality

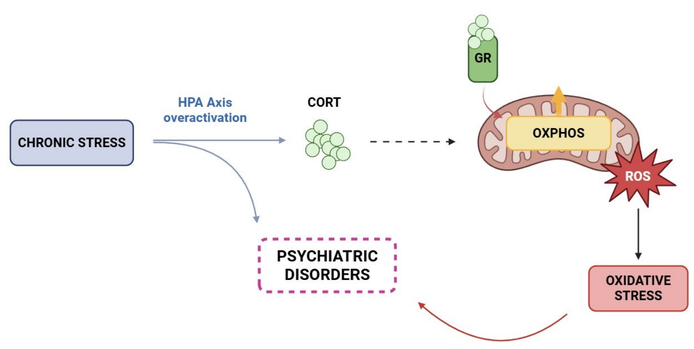


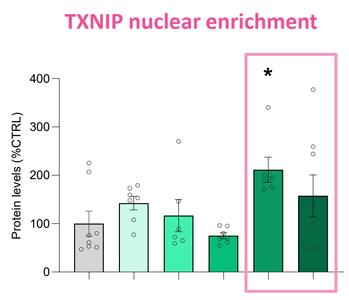
Adapted from Garabedian et al., 2017

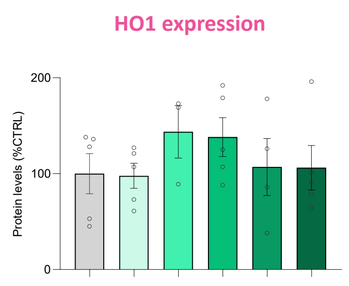
The importance of phosphorylation



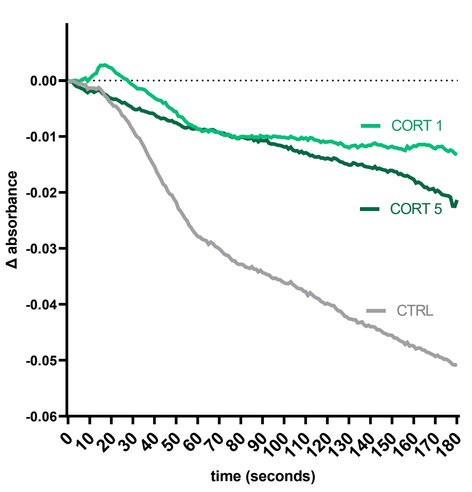
The involvement of oxidative stress







A focus on catalase activity



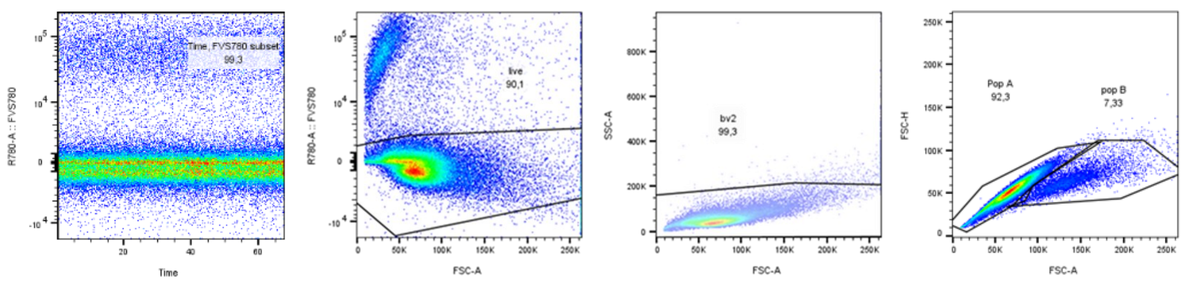
p<0.05 (*), p<0.01(**), p<0.001 (***) vs CTRL;
unpaired two-tailed Welch's t-test or one-way ANOVA with Dunnett's post hoc test

IN PROGRESS

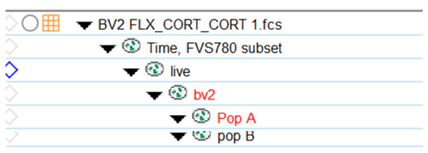
Previous insights: separation of BV2 microglial cells into two morphologically different populations even in untreated controls, as observed upon flow cytometric analysis.

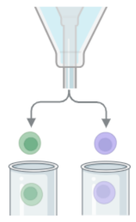
Preliminary data: difference in responsiveness between the two populations based on higher mean fluorescence intensity (MFI) for the marker of activation CD11b.

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

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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