THE INDUCTION PHASE OF THE ACTIVITY-BASED ANOREXIA (ABA) MODEL IS ASSOCIATED WITH THE ACTIVATION OF IL-6 "*CLASSICAL SIGNALING*"

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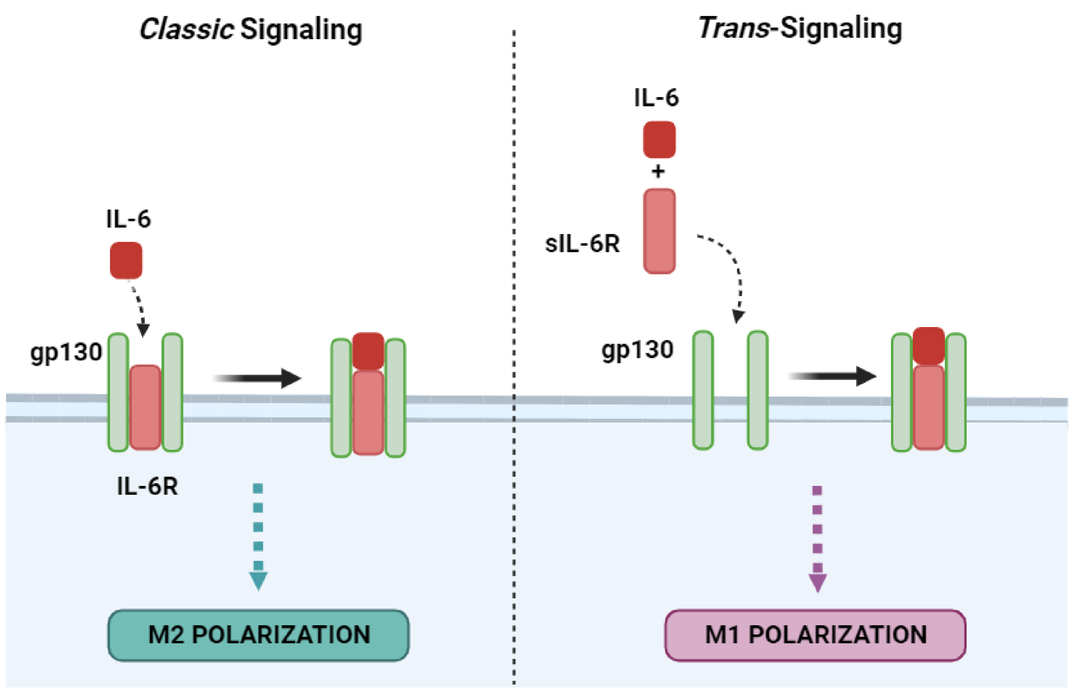
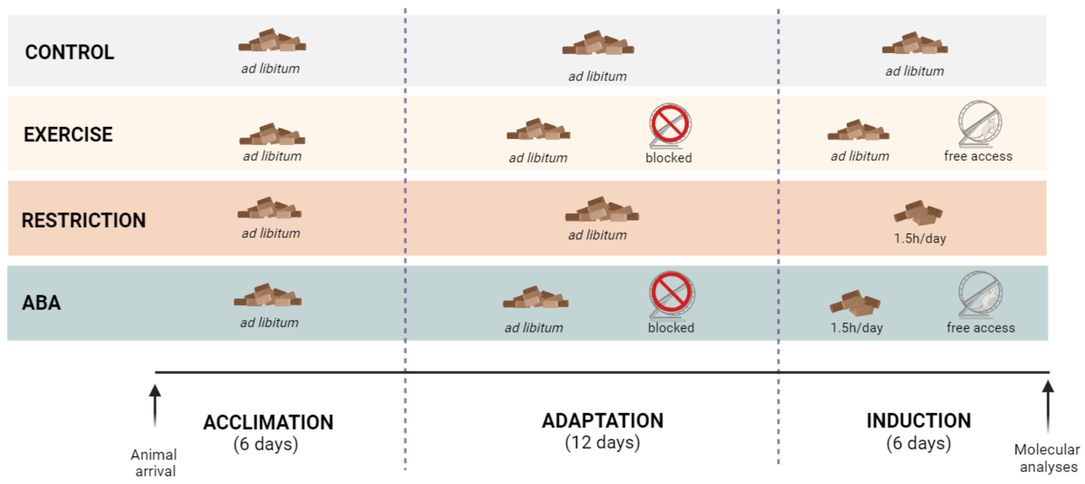
Background

Anorexia nervosa (AN) is a life-threatening psychiatric disorder characterized by the adoption of disturbed eating behaviors and excessive exercise activity. Reiteration of these habits progressively leads to severe consequences for general and mental health, making AN the psychiatric disorder with the highest mortality rate. To date, the exact molecular mechanisms underlying AN development have not been fully elucidated. Recently, a subset of research has focused on **neuroinflammation** as possible **contributor for eating disorders**, although the results are often contradictory. In this context, in a previous study, we employed the **Activity-Based Anorexia (ABA)** model to assess the gene expression levels of cardinal mediators of inflammation, observing an overall downregulation of pro-inflammatory cytokines that we ascribed to a possible **adaptive response to ABA induction** and representing an **initial phase** of AN. Only IL-6 showed an opposite trend as compared to the other cytokines. Interestingly, it is widely recognized the role of IL-6 as both anti- and pro-inflammatory mediator, being able to activate downstream either the "classical" or the "trans" signaling, respectively leading to the M2 microglial phenotype (neuroprotective) or the M1 phenotype (neuroinflammatory).
On these bases, the aim of our study was to explore IL-6 downstream signaling to better understand if this mediator contributed to the anti-inflammatory profile observed in the ABA group **in the induction phase.**

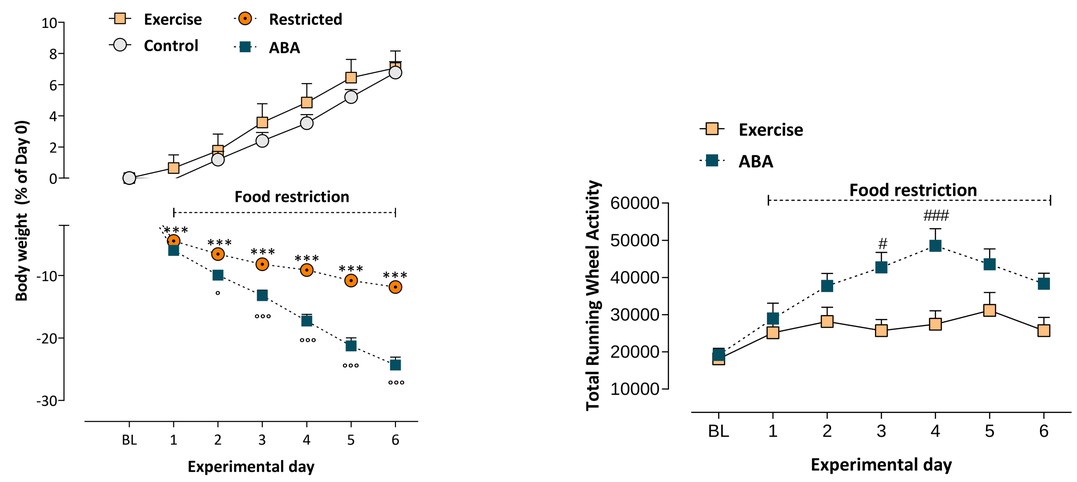


Adapted from Scheller et al, 2011 (doi:10.1016/j.bbamcr.2011.01.034)

Experimental design



For six days, depending on the belonging experimental group, adolescent Sprague-Dawley female rats were subjected to: (1) normal maintenance procedure (**Control group**), (2) voluntary running wheel activity (**Exercise group**), (3) imposed food restriction for 1,5h/day (**Restriction group**), (4) a combination of food restriction and wheel activity (**ABA group**).
At the end of the last day of induction, animals were euthanized, **dorsal hippocampus** dissected, and proteins extracted for the subsequent molecular analyses. Protein expression analyses of IL-6 and its downstream pathway mediators was performed through Western Blot.
The graphs below respectively represent the running wheel activity and animal body weight variations during the 6-days induction phase.



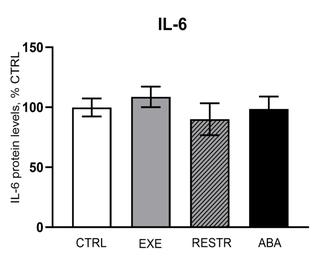
***P< 0,001 vs. CTRL.; #P<0,05 ###P<0,001 vs. EXE; °P<0,05 °°°P<0,001 vs. RESTR. Two-way ANOVA with repeated measures with Bonferroni post hoc test

Results

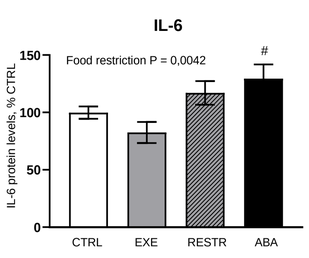
*Protein expression analysis in **dorsal hippocampus***

IL-6

Whole homogenate

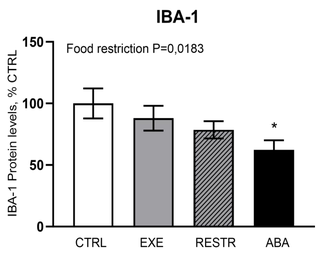


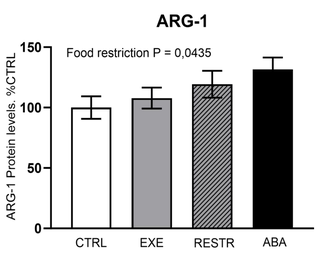
Membrane



Microglial markers

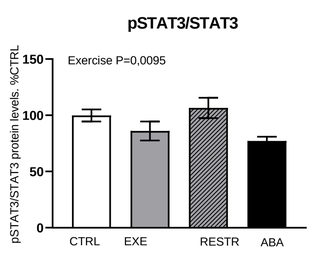
Membrane





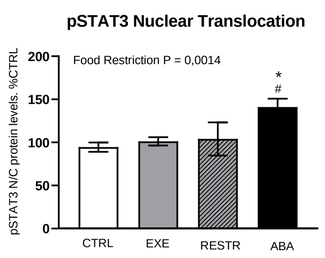
IL-6 downstream pathway

Cytosol

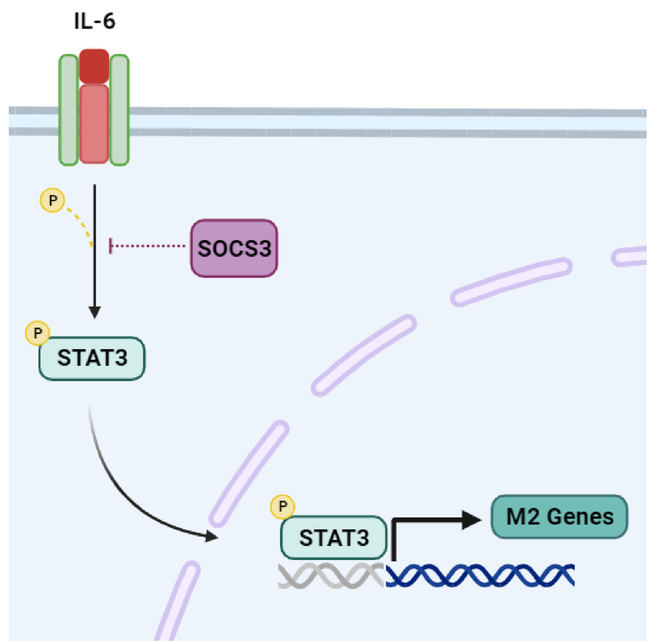


Nucleus

Nucleus



Cytosol



*P< 0,05 vs. CTRL.; #P<0,05 ##P<0,01 vs. EXE. Two-way ANOVA with Tukey post hoc test

Following ABA induction, we observed an increase in IL-6 in the membrane fraction, but not in the whole homogenate, suggesting the binding of the cytokine to its intramembrane receptor. This trend was paralleled by a tendency to increase of M2 marker ARG-1 and a significative downregulation of the M1 marker IBA-1 in the ABA group. In line with the microglial M2 shifting, we observed also an increase of the phosphorylated form of STAT3 in the nuclear subfraction, corresponding also to an increase in its nuclear translocation in the ABA group. Finally, in both ABA and RESTR group, we observed a downregulation of the suppressor of this pathway, SOCS3.

Conclusions

Our results suggest that IL-6 may participate in the overall **anti-inflammatory profile** previously observed after ABA induction by activating downstream its "*classical signaling*". This specific pathway, through the induction of several downstream mediators, is responsible of shifting of the microglial activation towards the "neuroprotective" M2 phenotype. In line with this hypothesis, we found increased IL-6 protein levels paralleled by a reduction in IBA-1 (M1 phenotype marker) and a tendency to increase in ARG-1 (M2 phenotype). Moreover, we found an increase in both protein levels and in the nuclear translocation of pSTAT3, a key mediator of this downstream signaling responsible for regulating the M1 to M2 switch. In support of this, we found a reduction in SOCS3 protein levels, an inhibitor of STAT3 activation.
Although a deeper understanding of IL-6 pathways is needed, our results clearly indicate an involvement of this cytokine and its fine downstream regulation in response to the ABA induction, possibly suggesting new interesting molecular targets that may be involved in the **early phases of AN**.

References

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