Study of the interactions between glucocorticoids and neurotrophins in physiological and neuropathological conditions

Tsimpolis Alexandros, PhD student

Home Lab: Regenerative Pharmacology Lab, Department of Medicine, University of Crete

Host Lab: Gene Regulation and Adaptive Behaviors, Department of Neuroscience, IBPS, University Pierre and Marie Curie

Host Lab Supervisor: Dr. Sheela Vyas

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During my short stay at the lab of Dr. Vyas, I had the opportunity to achieve both goals set by the team prior to my arrival: a) create a protocol to obtain Glucocorticoid Receptor-Knockout (GR-KO) astrocytes in order to study the implication of GR on the effects of Glucocorticoids (GCs) on Brain-derived Neurotrophic Factor (*bdnf*) expression and b) follow a training course in mice handling and performing behavioral tests.

In order to further validate results that we have obtained on my home lab regarding the effects of GCs on the expression of *bdnf* and being able to study the implication of GR in the mechanism, we initially seek to create primary astrocytic cultures that would be conditionally KO of the GR. For this reason, I used GR-floxed C57BL/6J mice, dissected their brain and obtained pure cortical astrocyte cultures. Then, I treated these cultures with an adeno-associated virus 2/9 (AAV2/9) vector, expressing the Cre recombinase. Expressed Cre, should locate the LoxP flanked DNA segment and recombine it, thus deleting the expression of GR in our astrocytic cultures. Indeed, as seen in Figure 1, GR expression was abolished by more than 70% in astrocytic cultures treated with the viral vector compared to the control, untreated floxed cultures.

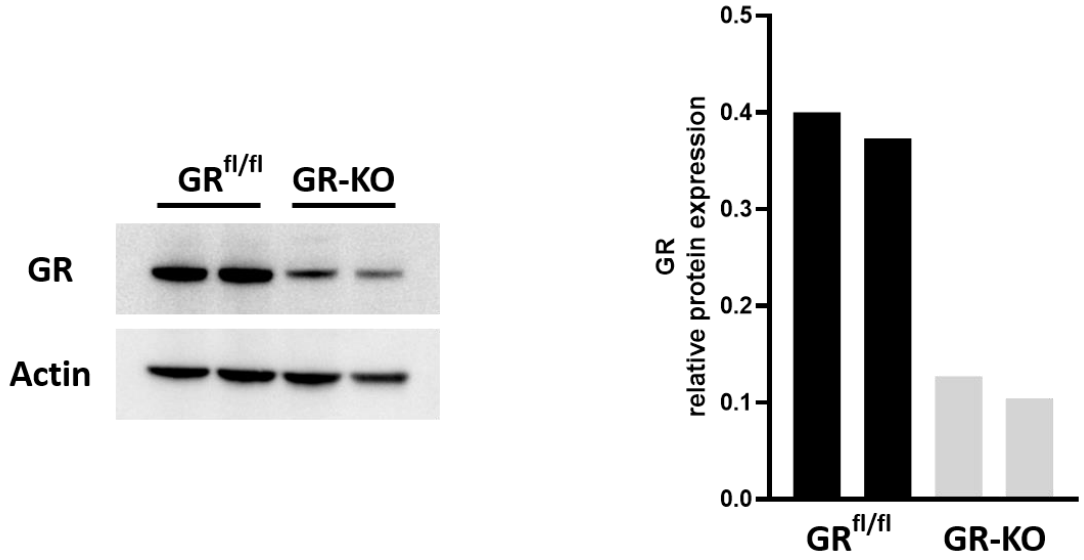

Figure 1: Western blot analysis of the GR expression levels in GR-KO astrocytic cultures compared to the floxed untreated ones. GR expression was reduced by at least 70% in the GR-KO cultures as depicted on the graph at the left. The relative GR expression in each condition was estimated by normalizing to Actin.

Using these GR-KO astrocytic cultures and control untreated ones, we performed a series of chronopharmacological experiments, where corticosterone (the natural murine glucocorticoid) was administered in both cultures in specific timepoints and *bdnf* expression was then measured

using real-time PCR (RT-PCR). In data that are not being demonstrated in this report, I was able to replicate in the control untreated cultures, the unique *bdnf* expression pattern that we have already characterized in my home lab. Interestingly, in the GR-KO astrocytic cultures, this expression pattern of *bdnf*, was completely abolished, highlighting the implication of GR in the mechanism, as initially hypothesized.

At the same time, under the supervision of Dr. Vyas and other collaborators in the lab, I underwent a small mice handling training session and later I was taught how to perform a few behavioral stress tests specifically used to evaluate anxiety and depression state in mice. Such tests, included the chronic unpredictable stress (CUS) protocol, the repeated social defeat stress protocol and the prepulse inhibition (PPI) test to name a few. This training will be of major significant for the continuation of my project, as it will allow me to transfer these new protocols in my home lab and exploit them in order to advance the results of my *in vitro* study to an *in vivo* model.

Overall, I evaluate my NENS Exchange experience as quite replete, as I was given the opportunity to further validate and significantly enrich my already existing results, get trained and acquire new research skills that will follow me for the entirety of my research career, meet and become friends with new colleagues/future collaborators and expose myself in a different lab culture and environment. In my opinion, it is a must-have experience for all young and passionate neurobiology researchers.