



Medicinski fakultet Osijek
Sveučilište J.J. Strossmayera u Osijeku

Stress affects survival of adrenal gland stem cells through leptin in Sprague Dawley rats

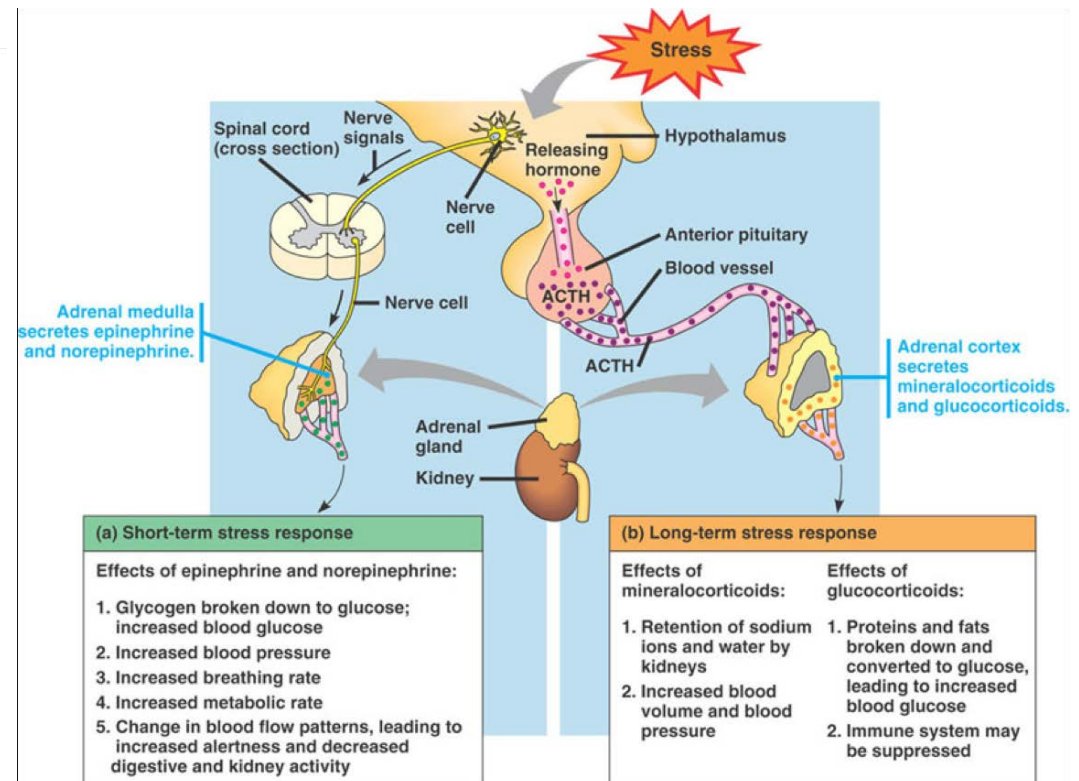
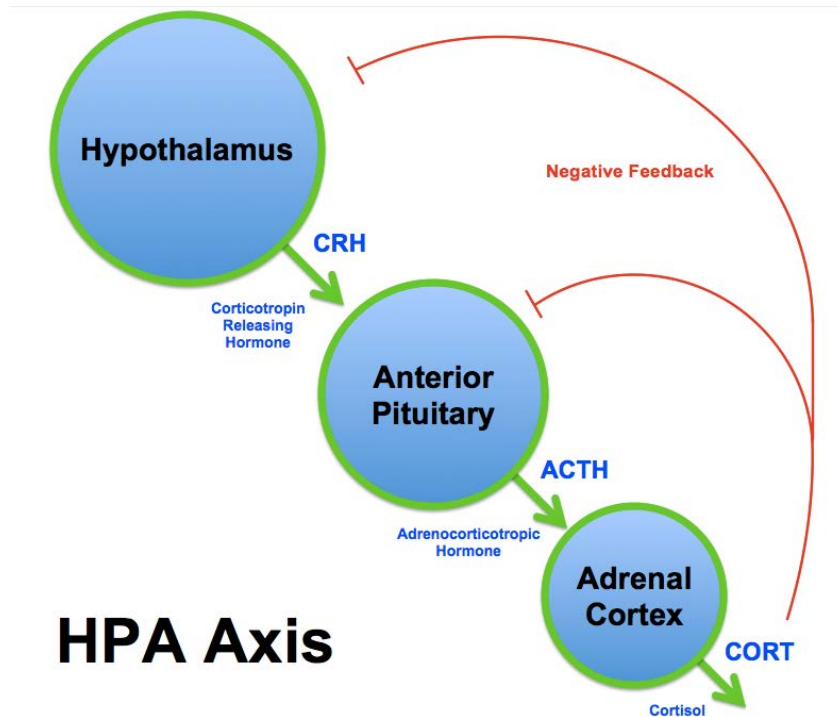
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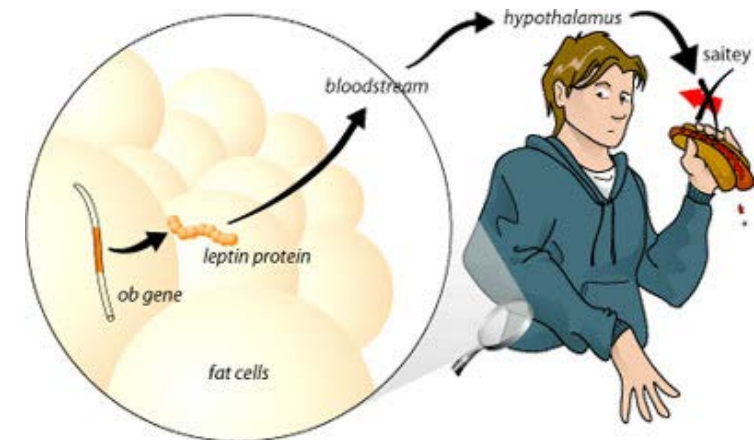
Background: Adrenal gland involvement in stress



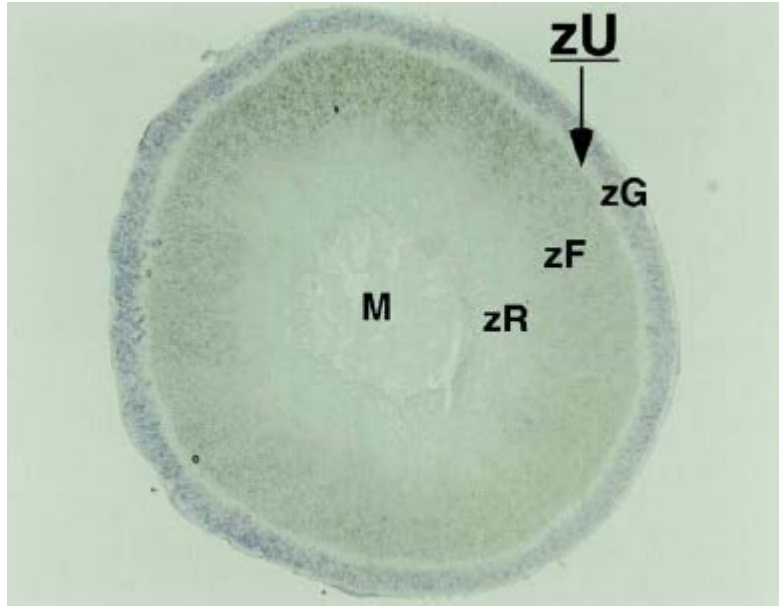
Introduction:

Stress and leptin

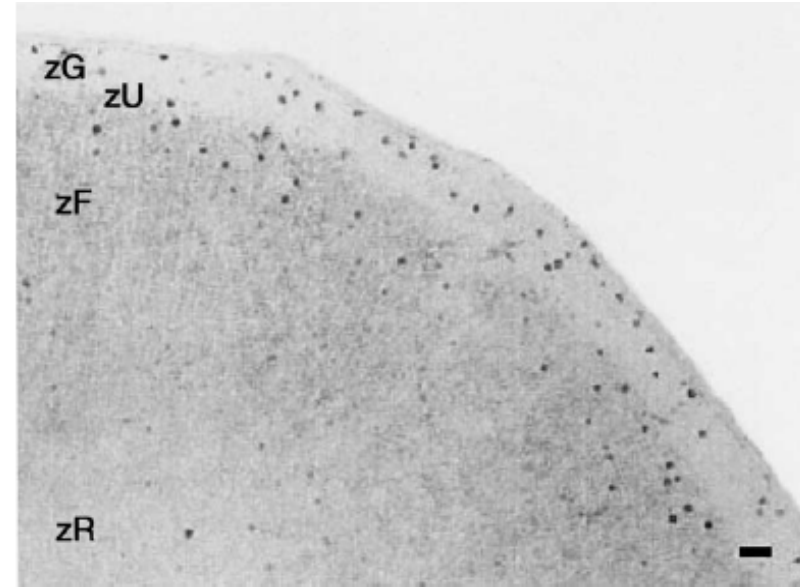
- The leptin is a hormonal product of *ob* gene which acts through leptin receptor (Ob-R)
- Secreted by adipose-tissue
- Leptin receptor is concentrated in the hypothalamus (but widespread through the brain)
- **Satiety**
- **Ob-R is present in adrenal gland (glucocorticoid suppression during stress)**



Adrenal gland structure



Functional zonation of adrenal gland:
zona glomerulosa (zG), *zona fasciculata* (zF), *zona reticularis* (zR),
undifferentiated cells zone (zU) and
medulla(M). Mitani et al. 2003.



Spatial distribution of BrdU labeled
cells in the adrenal gland cortex.
Mitani et al. 2014.

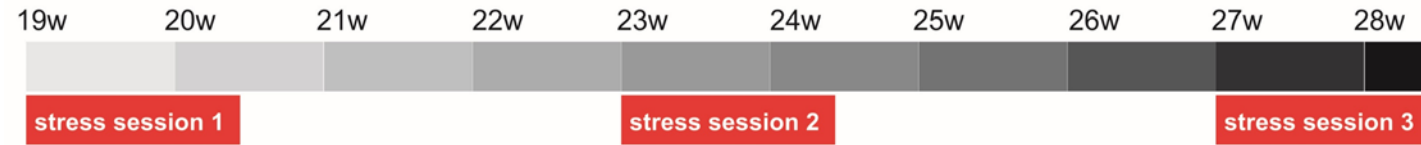
Aim of the Study

- To assess the changes of Ob-R expression during stress in Sprague Dawley rats
- In this study we hypothesize that leptin plays an important role in adrenal gland stem cells survival through its receptor (Ob-R) upon acute and chronic stress



Methods

- 22 male, 24 female (NON-OVX) and 24 ovariectomized female (OVX) of 19 weeks Sprague Dawley rats were used in the study. Animals were further divided in control (sham), acute and chronic stress groups
- Acute stress was performed by 1 hour cold restraint in metal tubes at +4°C



STRESS SESSION 1	
Day	Stressor
1	Food deprivation overnight (14-18h in total)
2	GTT test (baseline measurement)
3	60 minutes of restraint in metal tubes at +4°C and food deprivation
4	GTT test (acute stress reference)
5	Overnight lights on
6	60 minutes of cage rotation
7	3 minutes of forced swimming
8	Overnight lights on with irregular noise
9	Exposure to cat's odor and food deprivation
10	GTT test (chronic stress reference)

STRESS SESSION 2 and STRESS SESSION 3	
Day	Stressor
1	Food deprivation overnight (14-18h in total)
2	60 minutes of restraint in metal tubes at +4°C
3	Overnight lights on
4	60 minutes of cage rotation
5	3 minutes of forced swimming
6	Overnight lights on with irregular noise
7	Exposure to cat's odor and food deprivation
8	60 minutes of restraint in metal tubes at +4°C
9	3 minutes of forced swimming
10	GTT test (prolonged chronic stress reference)

Protocol of chronic stress

Examples of stressors



Methods

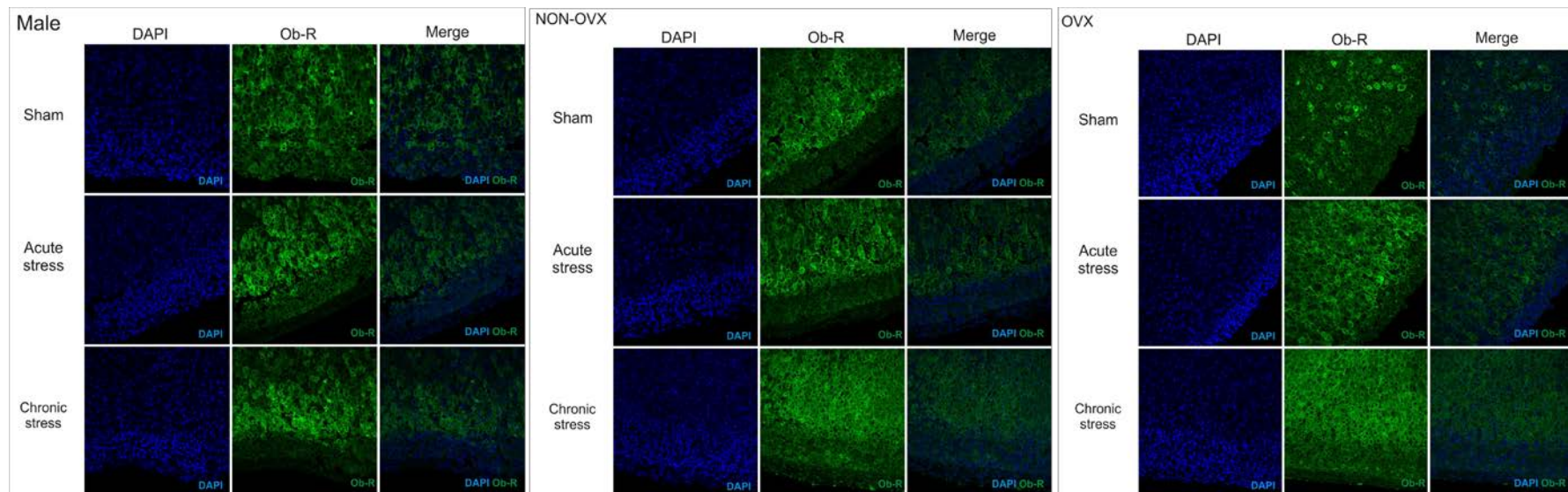
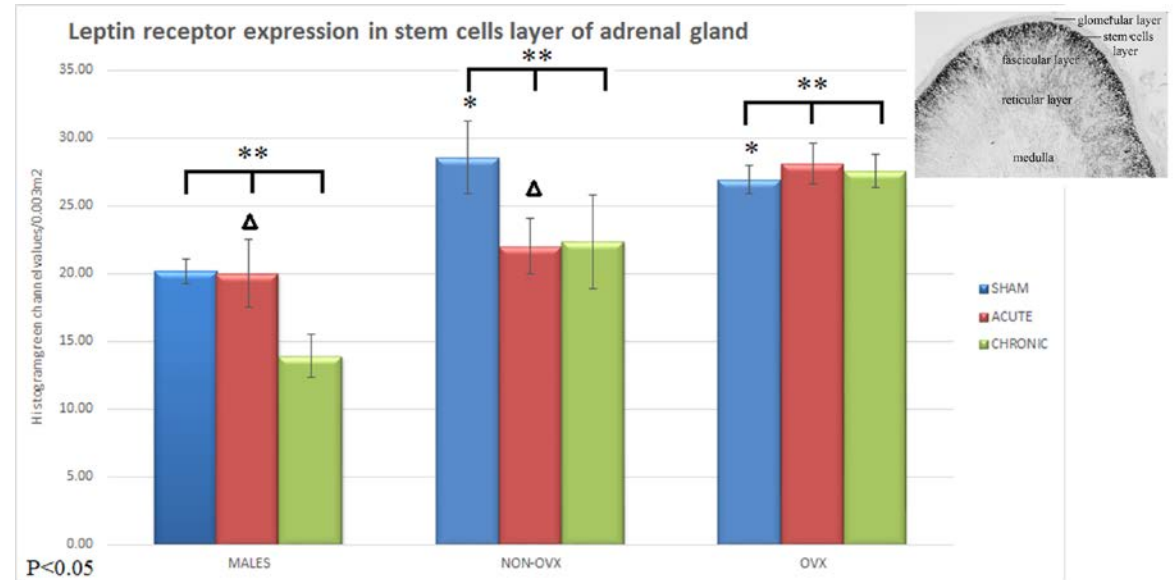


- Adrenal glands of 7-months-old rats were immunostained with leptin receptor (Ob-R) primary antibody and secondary FITC-conjugated fluorescent antibody
- DAPI was used to stain nuclei
- Images were photographed by confocal microscope and quantified by ImageJ software

Immunostaining of stem cells zone with Ob-R

Tukey test (▲ for males compared to NON-OVX, $F=6.56$; * for NON-OVX compared to OVX, $F=6.2$).

**Kruskal-Wallis test: males ($\chi^2=12.31$; $p=0.002$), NON-OVX ($\chi^2=12.11$; $p=0.002$) and OVX ($\chi^2=15.15$; $p=0.0005$).



Conclusion

- Leptin might be involved in general stress regulation at the level of adrenal gland
- Since leptin regulates glucose uptake and inhibits stress-induced apoptosis this finding might indicate its important role in stem cells survival and long-term effect on stress response by adrenal gland
- Future experiments: long term survival of adrenal gland stem cells labeled with Ob-R/BrdU

Thank you for your attention!

Marija Heffer

Srećko Gajović

Irena Labak

Vedrana Ivić

Senka Blažetić



*Why yes, I'm a bit stressed.
Why do you ask?*