# TIME-COURSE ANALYSIS OF HORMONAL AND NEUROPLASTIC CHANGES IN RATS EXPOSED TO PRENATAL STRESS

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## INTRODUCTION

Perinatal life is a period of high plasticity and vulnerability to stress. Indeed, a growing body of evidence suggests that early life adversities are implicated in early programming of adult chronic diseases, including mood disorders. Accordingly, exposure to stress during gestation in rats has a strong impact on development and can cause long-term abnormalities in adult behavior that resemble those found in human subjects affected by major depression**.** Two systems have emerged as major vulnerability elements and can be considered markers for depression-related dysfunctions: the hypothalamus-pituitary-adrenal (HPA) axis, closely related to stress-responsiveness, and neuronal plasticity, an array of mechanisms that may contribute to structural modifications and to powerlessness to adapt or respond to environmental changes.

## MATERIALS AND METHODS

**ANIMALS AND STRESS PROCEDURE**

We exposed pregnant Sprague-Dawley rats to repetitive immobilization three times a day for 45 minutes in transparent Plexiglas cylinders under a bright light (6.500 lx) from day 14 of pregnancy until delivery. Stress sessions were conducted during the light phase but at differing periods of the day in order to reduce possible habituation to repeated restraint stress. We carried out all the subsequent analysis in the offspring, both in males and females, at different time points: immediately after birth (PND1), during infant life (PND7), at weaning (PND21), during adolescence (PND40) and in the adult life (PND62), in order to create a time-profile of the changes under investigation.

**ANALYSIS OF RNA**

The brain areas of interest (hippocampus and prefrontal cortex) were rapidly dissected and used for the isolation of total RNA. The analysis of BDNF mRNA levels was performed by Real-Time Quantitative PCR.

**PLASMA CORTICOSTERONE ASSAY**

Samples of blood from each rat were collected in heparinized tubes. Plasma was separated by centrifugation (5000 rpm for 10 min) and the levels of corticosterone were determined using a radioimmunoassay-based (RIA) kit (Pantec, Cat. No. 07-120103). These levels were calculated using a standard curve generated from standards containing 0-1000 ng/mL of corticosterone.

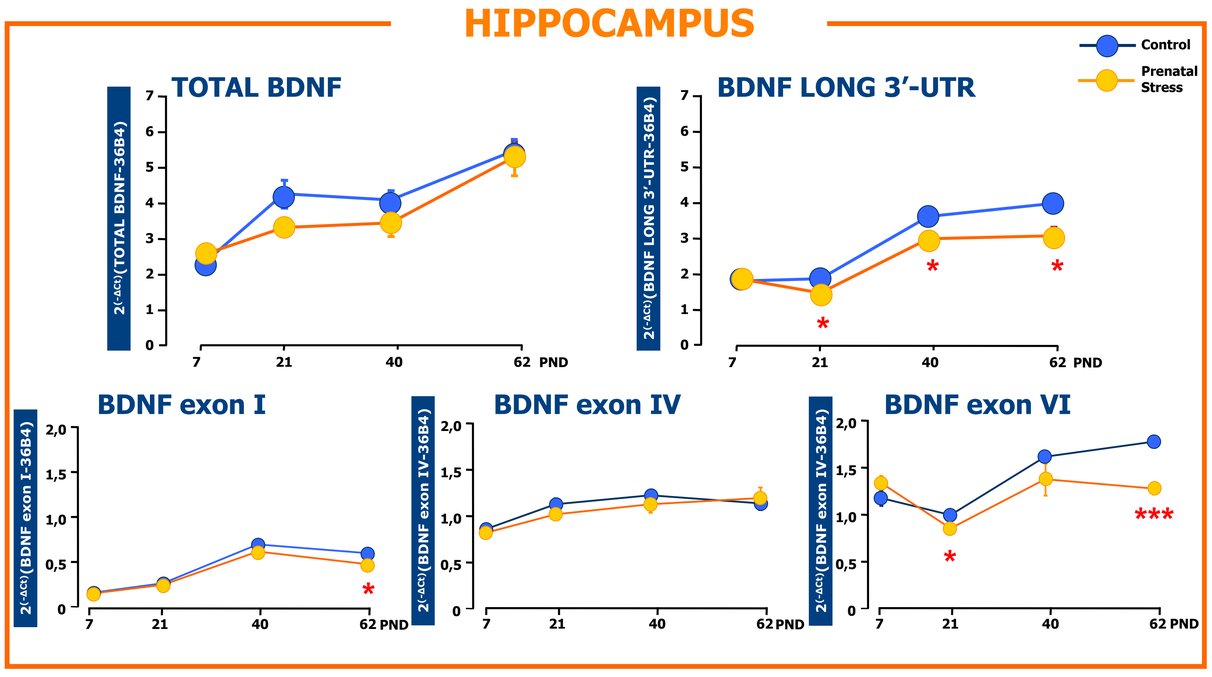
**STATISTICAL ANALYSIS OF DATA**

Data from gene expression and corticosterone analysis were analyzed with Student's *t*-test. Pearson product moment correlations (r) between levels of glucocorticoid receptor mRNA and corticosterone plasma levels were performed to evaluate the correlation in single animals. Significance for all tests was assumed at least at $p<0.05$. Data are presented as means ± SEM.

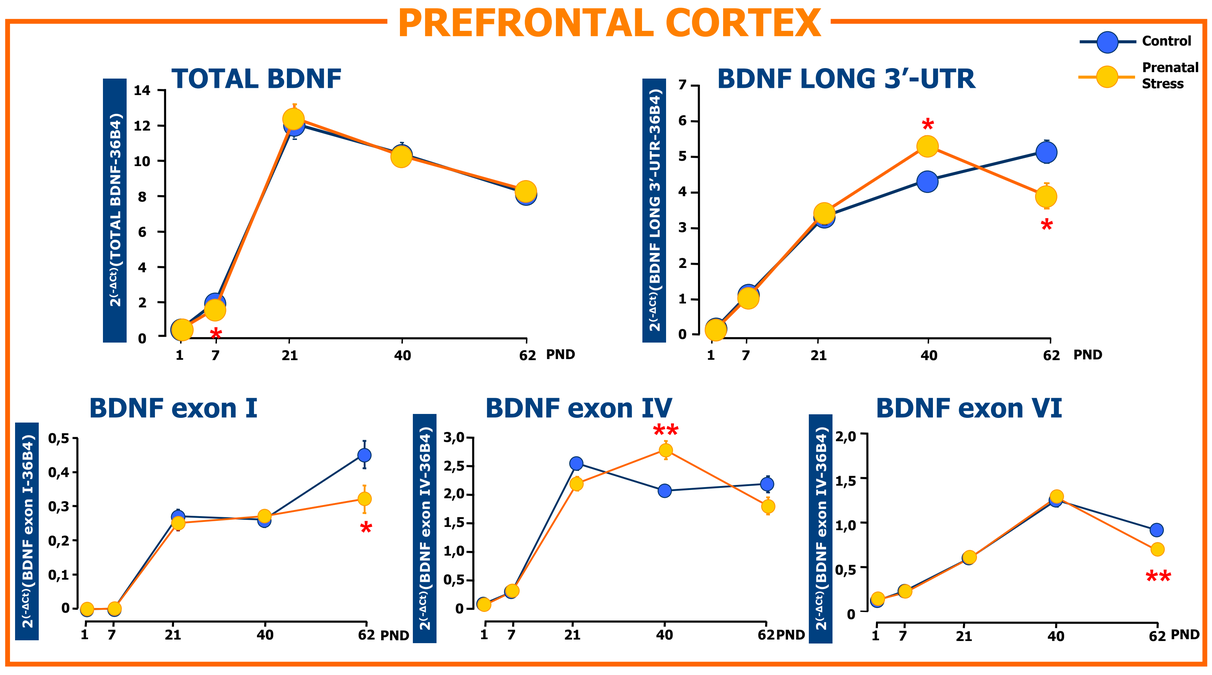
## ANALYSIS OF BDNF EXPRESSION

### MALES

#### HIPPOCAMPUS

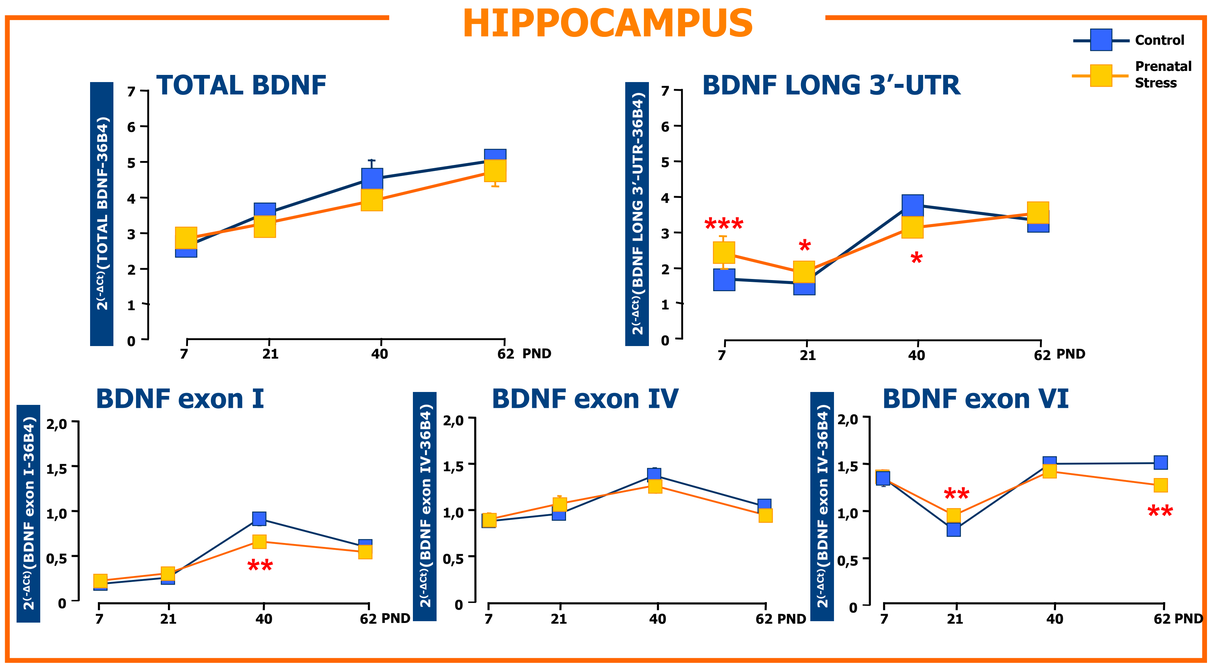



#### PREFRONTAL CORTEX

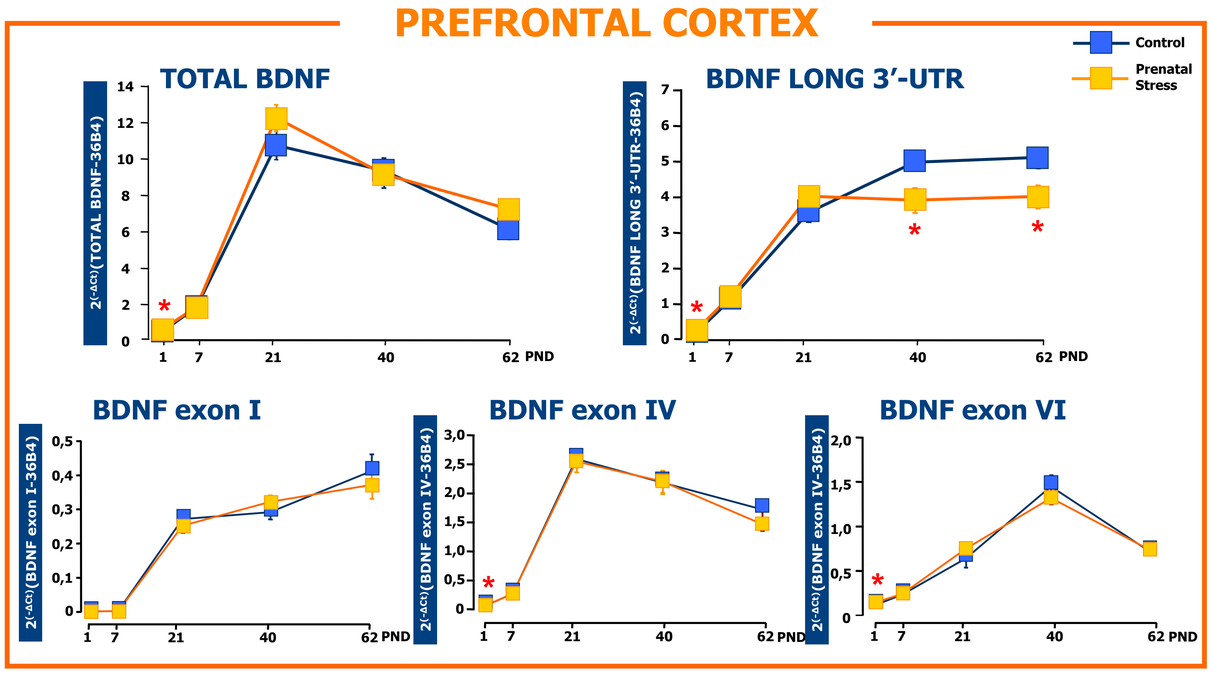



### FEMALES

#### HIPPOCAMPUS




#### PREFRONTAL CORTEX



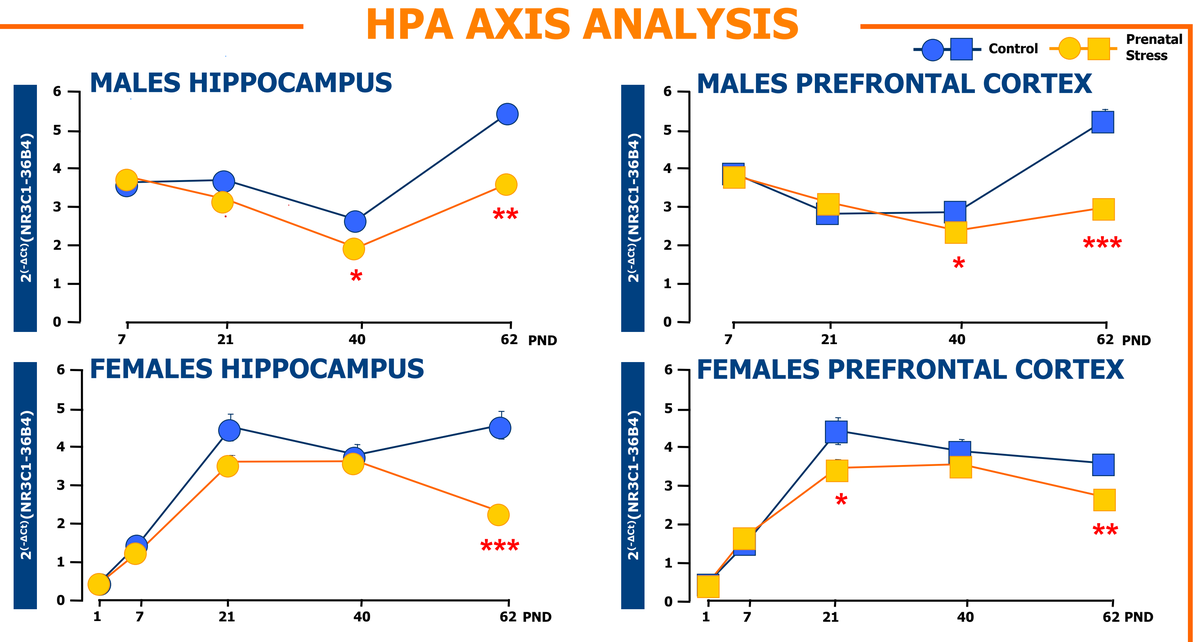

We found that rats exposed to prenatal stress have reduced expression of the neurotrophin BDNF, an effect that becomes fully manifest between adolescence and adulthood, but that is not present at earlier stages of development.

The changes found in adult rats that were exposed to stress during gestation are due to the differential regulation of specific BDNF transcripts in the two regions analyzed. Within the males' hippocampus the effect seems to be gender-specific, as we can see a reduced expression of BDNF long 3'-UTR transcripts starting from PND 21 that is mirrored by a reduced expression of BDNF exon VI. In the females, the time course of BDNF long 3'-UTR is different, although, similar to the males, there is a significant down regulation of BDNF exon VI at adulthood.

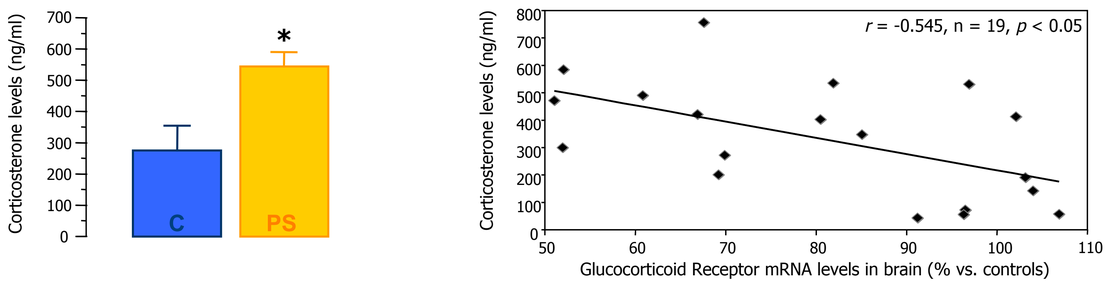
In the prefrontal cortex, both males and females show reduced mRNA levels of BDNF long 3'-UTR at adulthood, which is probably due to changes of BDNF exon IV and BDNF exon VI.

** p<0.05 vs. control; ** p<0.01 vs. control; *** p<0.001 vs. control*

## HPA AXIS ANALYSIS



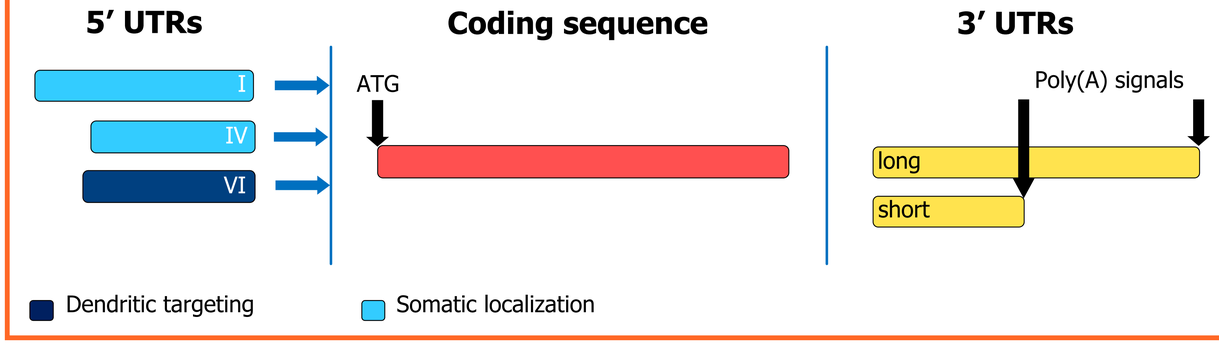

The expression of glucocorticoid receptors was significantly decreased in adult animals prenatally exposed to stress, and this effect is present in the hippocampus as well as in the prefrontal cortex of male and female rats.




In the left panel the levels of circulating corticosterone are shown, while in the right panel we display the correlation analysis between glucocorticoid receptor mRNA levels in the brain and the levels of circulating corticosterone.

** p<0.05 vs. control; ** p<0.01 vs. control; *** p<0.001 vs. control*

## BDNF GENE STRUCTURE




## SUMMARY

- Stress during the last week of gestation influences the expression of BDNF. The differential changes observed in the hippocampus and the prefrontal cortex of both males and females may be due to changes of different BDNF transcripts.
- Prenatal stress is also responsible for some changes borne by the HPA axis. In particular, this adverse event suffered by pregnant rats is responsible for the reduction of glucocorticoid receptor in the brain of the pups at different time points, and this correlates with an increase in circulating corticosterone levels.
- Our data provide further support to the notion that *in-utero* exposure to stress leads to permanent functional changes in the offspring.
- Particularly with BDNF, we demonstrate that some of these alterations are not present before adolescence or young adulthood, and may therefore result from developmental derangement of the physiological maturation of the neurotrophin. This suggests that early intervention may prove effective in preventing the manifestation of the full blown phenotype that may be associated with deterioration of neuronal plasticity in key brain structures .

## ACKNOWLEDGMENTS

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**No potential conflict of interest**