Altered expression of genes *Npas4* and *Nr1d1* in adult female mice with history of early-life stress

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Introduction

Stressful events early in life alter neuronal plasticity of the brain regions that regulate social behavior. Molecular mechanisms of this alterations remain unclear. Previous work have shown that brief and prolonged separation of pups from their mothers leads to enhanced social behavior in adult female mice [1].

Immediate early genes are strongly involved in synaptic plasticity [2], their products play a role in several distinct processes required for long-term synaptic changes and memory formation [3].

The specific aim of the present study was to characterize the expression of immediate early genes in the prefrontal cortex and dorsal hippocampus of adult female mice as a marker of modified neuroplasticity elicited both by stress early in life and by previous social interaction.

To exclude effect estrous cycle of female mice on gene expression level, correlational analysis between gene expression and serum estradiol was performed. In addition, correlational analysis between gene expression an behavioral parameters was performed.

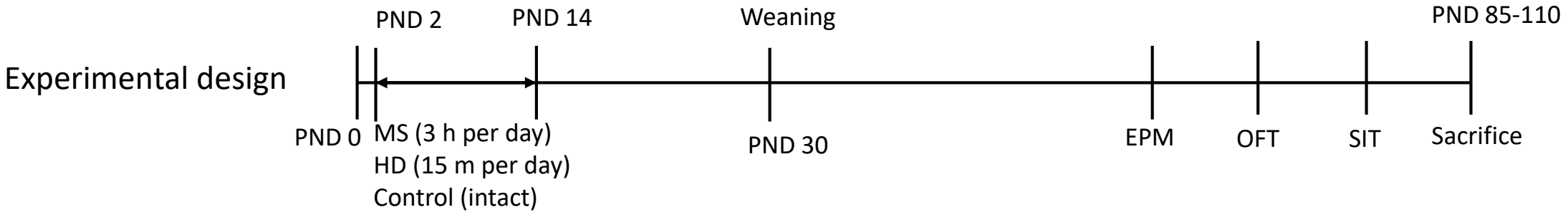
Materials and methods

We used brief (15 min per day) and prolonged (3 h per day) maternal separation prosedure for 14 days. As adults, mice were subjected to elevated plus maze, open field, and the social interaction test (one test per day).

After last day of test, mice were decapitated. Brains were removed, prefrontal cortex was dissected and snap frozen in liquid nitrogen, the rest of the brain was embedded in the Tissue-Tek O.C.T. Compound. Frozen brains were cut into coronal sections in a cryostat Microm HM 550. Two 150 µm sections were dissected and dorsal hippocampus was isolated from slices.

Gene expression level analysis was performed by real-time PCR, evaluation the expression of followed genes: *Egr1*, *Npas4*, *Arc*, *Homer1a*, *Homer1b/c*, *Nr3c1*, *Nr3c2*, and *Nr1d1*.

Serum 17β-estradiol was evaluated by using enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocols.

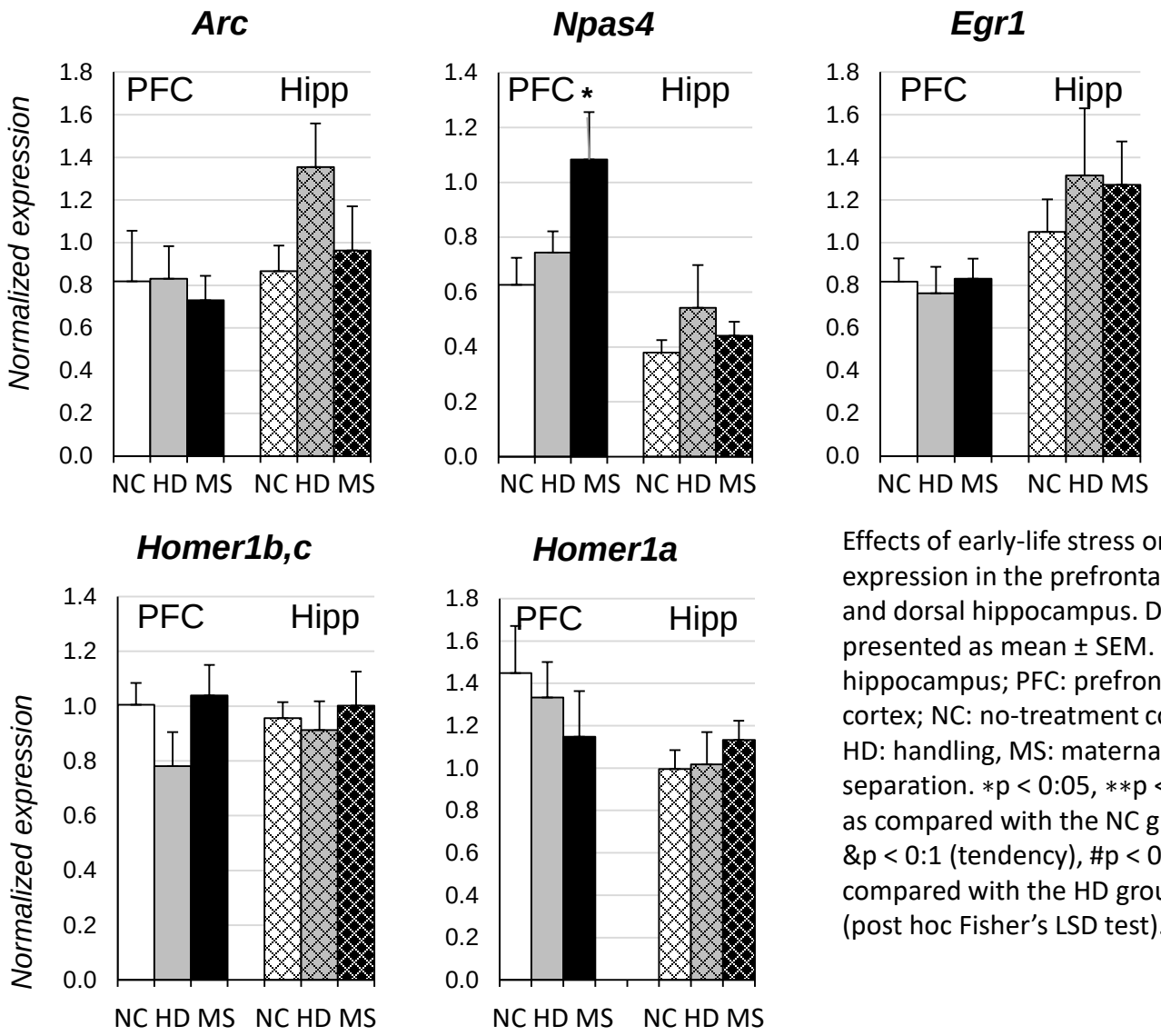


Results

We found that prolonged maternal separation increases expression level of gene *Npas4* in prefrontal cortex and *Nr1d1* in prefrontal cortex and dorsal hippocampus (Fig.1), expression level of other genes remain unchanged.

Npas4 is a transcription factor, which controls expression of other genes like *Arc*, *Egr1* and *c-Fos*. Usually enhanced *Npas4* expression accompanied with upregulation of genes including *Arc*, *Egr1 и Bdnf*. In this study, expression of these genes did not exhibit significant change. Despite of lack of significant changes, correlation analysis revealed strong correlation between expression levels of genes *Npas4*, *Arc*, *Egr1* and *Bdnf* in prefrontal cortex. Alteration of expression of these genes has not found in dorsal hippocampus, as well as correlation in expression level. These observed changes in expression level suggest that the observed alterations are specific to the prefrontal cortex.

Correlational analysis between expression levels of the studied genes and behavioral parameters revealed positive correlation between genes in prefrontal cortex (*Npas4*, *Egr1*, *Nr3c1* and *Nr3c2*) and one in hippocampus (*Homer1a*) with level of social behavior.



Effects of early-life stress on gene expression in the prefrontal cortex and dorsal hippocampus. Data are presented as mean ± SEM. Hipp: hippocampus; PFC: prefrontal cortex; NC: no-treatment control; HD: handling, MS: maternal separation. ∗p < 0:05, ∗∗p < 0:01 as compared with the NC group; &p < 0:1 (tendency), #p < 0:05 as compared with the HD group (post hoc Fisher's LSD test).

Nr1d1 is a gene encoding the nuclear receptor, also known as REVERBa, which control gene transcription. Its most studied function is regulation of circadian rhythm. Enhances expression of this gene can be related with social behavior through clock genes activity in prefrontal cortex and hippocampus.

Glucocorticoids may influence on expression of clock genes due to presence of glucocorticoid response elements in their promoters, thus synchronization of peripheral and central circadian oscillators occurs. In this experiment, there is no alterations in *Nr3c1* and *Nr3c2* expression in prefrontal cortex and hippocampus. After stress, there was no changes in *Nr3c1* and *Nr3c2* expression in males, but prolonged maternal separation increased *Nr3c2*/*Nr3c1* mRNA ratio in hippocampus and hypothalamus.

For most genes, we did not detect a significant correlation between estradiol concentration and gene expression; this finding allows us to compare gene expression levels without considering the stage of the cycle.

ACKNOWLEDGMENT

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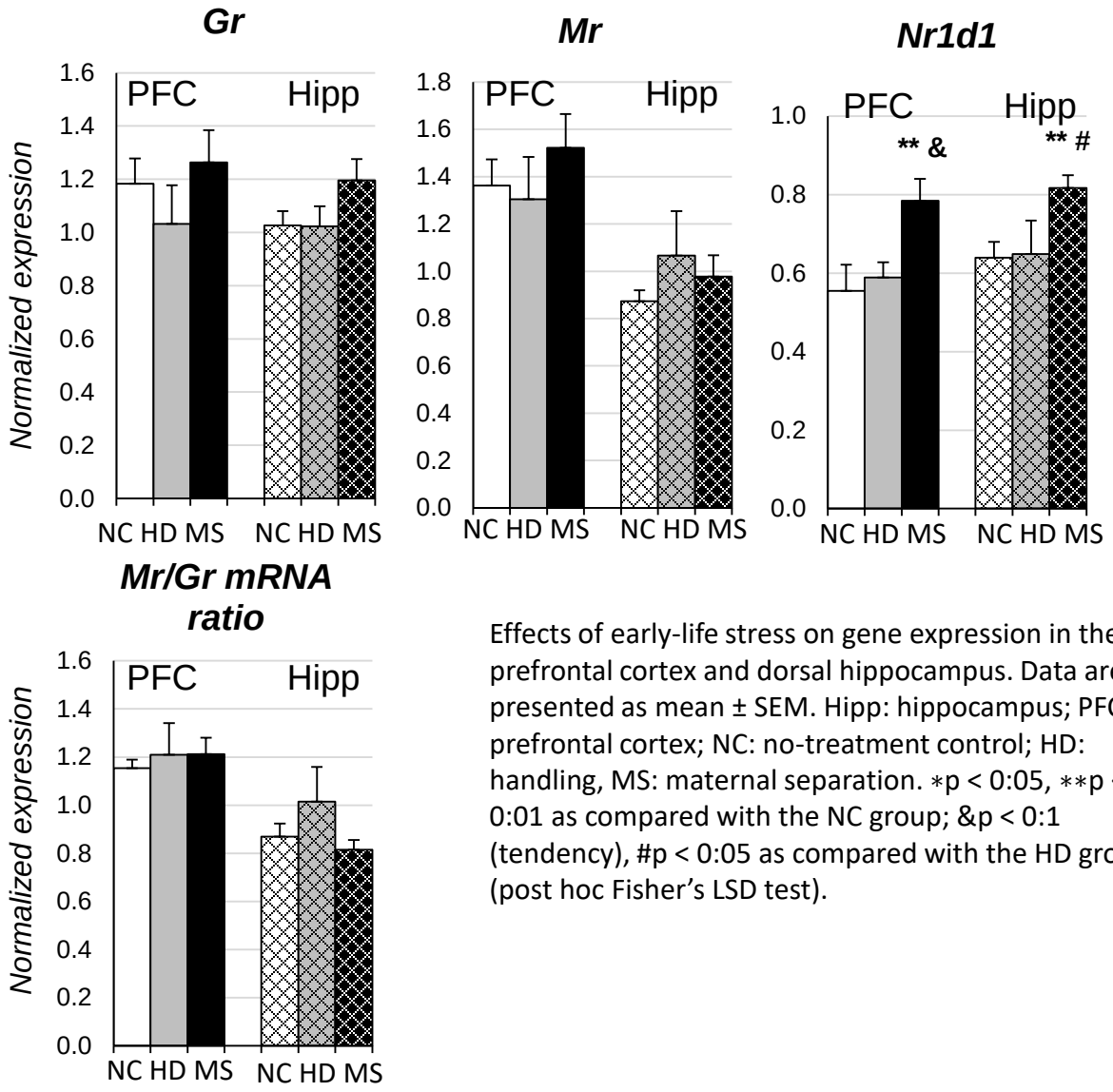

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REFERENCES

1. Bondar NP, Lepeshko AA, Reshetnikov V V. Effects of Early-Life Stress on Social and Anxiety-Like Behaviors in Adult Mice: Sex-Specific Effects. Behav Neurol. 2018;2018: 32–34.
2. Okuno H. Regulation and function of immediate-early genes in the brain: Beyond neuronal activity markers. Neurosci Res. 2011;69: 175–186.
3. Minatohara K, Akiyoshi M, Okuno H. Role of immediate-early genes in synaptic plasticity and neuronal ensembles underlying the memory trace. Front Mol Neurosci. 2016;8: 1–11.