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Distinctive hypothalamic gene expression profiles in hypertensive ISIAH and normotensive WAG rats exposed to a single short-term restraint stress

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Motivation and Aim: An increase in blood pressure is one of the adaptive manifestations of the stress response, which can represent a significant health risk factor in modern human lifestyles. There is no doubt that there is a relationship between psychoemotional stress and hypertension. However, the study of the genetic basis of the increased blood pressure response to psychoemotional stress remains incompletely studied to date. The study was aimed to identify the strain-specific neural networks providing differences in hypothalamic response to stress in hypertensive ISIAH and normotensive WAG rats.
Methods and Algorithms: The experiment was carried out on three-month-old male hypertensive ISIAH/Icgn and normotensive WAG/GSto-Icgn rats. Transcriptome sequencing (RNA-Seq) of the hypothalamus was performed on 4 groups of rats: (1) ISIAH_control; (2) WAG_control; (3) ISIAH_stress; (4) WAG_stress. There were 7 rats in each group. In all rats, basal systolic blood pressure was measured using the tail-cuff method under light ether anesthesia to prevent emotional stress during the measurement. Seven days after measuring basal blood pressure, two groups of rats (ISIAH and WAG) were subjected to restraint stress by placing the rat in a tight wire mesh cage for 2 hours. After completion of the stress procedure, blood pressure was measured by a tail cuff method without anesthesia, and rats were immediately decapitated. The hypothalamus was isolated and homogenized in the ExtractRNA reagent (Evrogen, Russia). Plasma corticosterone concentrations were measured using the immunoassay (Corticosterone ELISA Kit, Abcam). Pair-end sequencing of cDNA libraries was performed on the DNBSEQ platform (DNB-SEQ Technology) with a read length of 150 base pairs and sequencing depth of more than 30 million uniquely mapped reads. All samples were analyzed as biological replicates. The study protocol was approved by the Bioethical Council of the federal research center Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia), protocol No. 115 of December 20, 2021. FastQC software (version 0.11.5) was used to assess the quality of sequencing data. Sequencing data were mapped to the rat reference genome mRatBN7.2/rn7 (rn7 assembly Wellcome Sanger Institute Nov, 2020) using the STAR software package, version 2.7.10a. Differential gene expression (DEG) assessment was performed with DESeq2 v1.30.1 using surrogate variable analysis (SVA) to account for unwanted variations in the data caused by possible random biases during sample preparation. The significance threshold for determining DEGs was chosen taking into account correction for multiple comparisons and corresponded to adjusted p-value < 5 %.

Results: Groups of DEGs that describe the general response to stress, independent of the genotype of rats, as well as strain-specific responses of the hypothalamus to stress, were identified in ISIAH_stress/ISIAH_control and WAG_stress/WAG_control comparisons. Functional annotation of DEGs made it possible to identify the biological processes that change most significantly in each rat strain. It is assumed that different degrees of activation of oxidative phosphorylation and manifestations of oxidative stress may be significant events in the formation of strain-specific features of the hypothalamic response to stress. It is suggested that alternative changes in the expression of the *Npas4* gene (neuronal PAS domain protein 4), which is downregulated in the hypothalamus of control WAG rats and induced in the hypothalamus of hypertensive ISIAH rats, is a key phenomenon for understanding inter-strain differences in the hypothalamic stress response in these rat strains. The strain-specific response to stress in the hypothalamus of ISIAH rats is characterized by downregulation of most DEGs. At the same time, induction of transcription of the *Fos* and *Jun* genes may play a decisive role in the activation of neuronal functions in these rats. Taking into account our previous data that the induction of *Fos* gene transcription occurs in concert with an increase in blood pressure in ISIAH rats [1], we can assume the existence of an association between the functioning features of the hypothalamic gene networks we identified and a sharp increase in blood pressure in ISIAH rats exposed to stress.
Conclusion: Distinctive differential expression of a large number of genes is observed in hypothalamic profiles of hypertensive ISIAH and normotensive WAG rats exposed to single short-term restraint stress. The data obtained may be potentially useful in the selection of molecular targets for the development of pharmacological approaches to the correction of stress-induced pathologies associated with neuronal excitability, taking into account the hypertensive status of patients.
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References

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