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Differential gene expression and neuroimmune responses in the amygdala of rat strains with high and low nervous system excitability

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Motivation and Aim: The aim of this study is to investigate the transcriptome profiles and neuroimmune reactions to stress in the amygdala of rat strains with contrast levels of nervous system excitability. Understanding the molecular and immune mechanisms associated with these differences can provide insights into the genetic and neurobiological factors that may influence excitability, stress responses, and related behavior abnormalities. Previous research has highlighted the importance of genetic predisposition in excitability to severity of neuroinflammation and post-stress changes in physiology. However, comprehensive transcriptomic data specific to the amygdala, particularly in the context of excitability level and neuroimmune interactions, remain unclear. This study seeks to fill this gap by providing gene expression profiles and identifying potential regulatory pathways involved in the differences observed between these rat strains.

Methods and Algorithms: The experiment was conducted on five-month-old male rats from two strains: high threshold (HT) of nervous system excitability (low excitability) and low threshold (LT) of nervous system excitability (high excitability). Each experiment included 24 animals, with 6 in each group in case of rtPCR, ELISA and IHC analysis and 5 in case of RNAseq. The animals were part of the biocollection at the "Pavlov Institute of Physiology" (RAS). Experimental animals expose to long-term emotional and pain stress using the Gecht protocol and control and experimental groups were decapitated 7 and 24 days post-stress. Total RNA was isolated from the right hemisphere's amygdala (extractRNA, Evrogen, Russia), followed by RT-PCR (BioRad C-1000) to assess mRNA levels of IL1b, TNF, IL6, IL10, BDNF, IBA1, GFAP). Protein levels in the left hemisphere were measured using ELISA kits for of IL1b, TNF, IL6, IL10 and IBA1 (SEA563Ra, SEA133Ra, SEC288Ra, Cloud-Clone Corp., China). The left hemisphere was also used for immunohistochemical staining for an astrocytic marker GFAP in amygdala (PAA068Ra02, Cloud-Clone Corp., China).

To analyze the transcriptomes of the amygdala in two rat strains tissue samples were collected and total RNA was extracted from tissues using Trisol reagent and the PureLink RNA micro Kit (Invitrogen). Sequencing was was performed at the Genoanalitika Lab, Moscow, Russia. Libraries for sequencing were prepared using the NEBNext® Ultra™ II RNA Library Prep Kit (NEB). Sequencing was done on a HiSeq1500 (Illumina), generating at least 20 million paired-end 50-nucleotide reads per sample. Read mapping and counting were performed with STAR 2.7.9a. Genome: Rnor_6.0. Annotation: Ensembl v.99. Differential expression: Deseq2 v.1.28.1. Genes

with Padj < 0.05 and |log2FC|>0,38 were designated as differentially expressed genes (DEGs). To identify overrepresented Gene Ontology (GO) terms in a list of DEGs, the DEGs were analyzed for functional enrichment using the DAVID tool.

Results: The RNA-seq analysis revealed significant differences in gene expression between the intact rats of two strains. We identified 152 upregulated and 105 down-regulated DEGs in the amygdala of LT strain compared to the HT strain. Top upregulated genes in LT strain vs HT strain: AABR07041096.1 (lincRNA), Serpinf2, Nit2, Pyroxd2. Pyroxd2 is predicted to enable oxidoreductase activity and is involved in mitochondrial organization. It is located in the mitochondrial matrix. So, upregulation of this gene in the LT rat strain's amygdala suggests increased mitochondrial activity, which could be a response to heightened cellular stress and energy demands. Enhanced mitochondrial function might be necessary to support the increased excitability and metabolic needs of neurons, potentially influencing neuroinflammatory processes and stress-related behaviors. Pdilt – Protein Disulfide Isomerase-like is involved in protein folding and acts as a chaperone. It is located in the endoplasmic reticulum (ER) lumen and is active in germ cell migration and spermatid development. its function in the brain has not been studied, but there is evidence of the expression of this gene in the cortex and pituitary gland of rats [1]. The upregulation of Pdilt in the LT rat strain's amygdala could indicate an increased demand for protein folding and ER stress response. Proper protein folding is crucial for maintaining cellular function, especially under stress conditions. Among the downregulated genes can be noted Ift88, that crucial for intracellular transport processes. Its downregulation could disrupt cellular trafficking and signaling, possibly affecting cellular responses to environmental stressors; Rexo4 is involved in DNA binding, nuclease activity, DNA catabolic process, and DNA repair. Downregulation of Rexo4 may lead to impaired DNA repair mechanisms, potentially increasing genomic instability [2] and vulnerability to neuroinflammation and cellular stress. Alb facilitates fatty acid, modified amino acid, and zinc ion binding. Involved in the regulation of sleep. Reduced Alb levels may affect the transport and availability of essential fatty acids and ions, potentially disrupting neuronal function.

Functional Enrichment: The Gene Ontology (GO) analysis indicates that the DEGs are significantly involved in biological processes (BP) such as oxygen transport, angiogenesis, lipid metabolism and, most importantly, in immunity and innate immunity; cellular components (CC) such as the extracellular matrix and endoplasmic reticulum; and molecular functions (MF) such as oxidoreductase and glycosyltransferase activities.

PCR and ELISA analyses found that in the amygdala of highly excitable rats (LT), stress leads to increased levels of the pro-inflammatory cytokines IL1b and TNF compared to controls. This indicates a pronounced neuroinflammatory response in these rats under stress. Additionally, immunohistochemistry data showed a decrease in the number of GFAP-expressing astrocytes in the amygdala of high excitability rats (LT) under stress. Which is consistent with data on the loss of glial cells in some brain structures in response to stress [3].

Conclusion: The RNA-seq results revealed key differences in gene expression between the LT and HT strains. In LT rats, the upregulation of genes associated with inflammatory and metabolic processes suggests an inherent predisposition to heightened stress responses and neuroinflammation. This aligns with the observed increases in IL1b and TNF levels under stress, indicating that these rats have a molecular profile primed for robust inflammatory responses.

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References

1. Darbellay F., Necsulea A. Comparative transcriptomics analyses across species, organs, and developmental stages reveal functionally constrained lncRNAs. *Mol Biol Evol.* 2020;37(1):240-259. doi 10.1093/molbev/msz212
2. Shcherbinina V., Pavlova M., Daev E., Dyuzhikova N. Rats selected for different nervous excitability: long-term emotional–painful stress affects the dynamics of DNA damage in cells of several brain areas. *Int J Mol Sci*. 2024;25(2):994. doi 10.3390/ijms25020994
3. Fang H., Bing X., Lili W. Loss of glial cells of the hippocampus in a rat model of post-traumatic stress disorder. *Neurochem Res*. 2015;40(5):942-951. doi 10.1007/S11064-015-1549-6