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The impact of stress on pro-inflammatory cytokines and glial markers expression in rat strains with different excitability levels

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Motivation and Aim: Stress is a critical factor in the development of post-stress disorders, yet its underlying mechanisms remain unclear. Research indicates that stress is associated with neuroinflammation, which plays a significant role in anxiety and depression. This study focuses on the pro-inflammatory cytokines Interleukin 1 beta (IL-1β) and tumor necrosis factor (TNF), produced during pathological conditions and stress responses [1]. GFAP and Iba1 are glial markers of astrocytes and microglia respectively. This research aims to assess changes in pro-inflammatory cytokines and glial markers in the brain of rats with different genetically determined levels of nervous system excitability at different periods after stress exposure.

Methods and Algorithms: The experiment was carried out on male five-month-old rats of two strains: with a high threshold (HT) of nervous system excitability (low excitable) and a low threshold (LT) of nervous system excitability (highly excitable). Each experiment involved 24 animals, 6 in each group. The animals belong to the biocollection of the “Pavlov Institute of Physiology" (RAS) [2]. Experimental animals were subjected to psychogenic long-term emotional and pain stress in accordance with the Gecht protocol: every day for 15 days the animals were exposed to 6 unsupported (10 seconds each) and 6 reinforced current (2.5 mA, 2 ms) light signals with a probability of 50 %. Animals of the control and experimental groups were decapitated 7 and 24 days after the end of stress. For immunohistochemical studies, additional perfusion was performed. Total RNA was isolated from the amygdala, hippocampus and prefrontal cortex of the right hemisphere (extractRNA, Evrogen, Russia), reverse transcription was performed, and the mRNA level was assessed using RT-PCR (BioRad C-1000). The corresponding structures of the left hemisphere were used to assess protein levels (SEA563Ra, SEA133Ra, SEC288Ra kits, Cloud-Clone Corp., China) by ELISA. For immunohistochemical study, the left hemisphere was taken, the animal brain was cut on a cryotome (Cryostat Microtome KD-3000, KEDEE, China) in 50 μm thick slices and subjected to immunohistochemical staining for astrocytic marker (PAA068Ra02, Cloud-Clone Corp., China) with secondary antibody (A-21207, Thermo Fisher Scientific, USA). Data were analyzed under a Leica DM4000 B LED fluorescence microscope (LEICA Microsystems, Germany) with LAS V.4.3 software. Cells were counted at a magnification of 20×. Three regions of interest each were analyzed in the prefrontal cortex (IL, PrL, Cg1) at 2.70, 2.20, 1.70 from bregma and in the hippocampus (CA1,

CA2/3 and DG) at –2.30, –3.30, –4.80 from bregma [3]. In the amygdala, cells were counted without distinguishing individual regions at levels –2.12 to –2.56 of bregma. Cells were counted in 3 fields of view in each region of interest. Astrocytic cell counts were calculated on 8 slices from each animal using the ImageJ analysis program.

Results: Motor activity decreased in both strains after 24 days, and exploratory activity in the Elevated Plus Maze test also decreased; however, in the LT strain, these changes were evident in a wider range of measured parameters. TNF expression increases in the amygdala in LT strain rats on day 24 after stress at both the mRNA and protein levels. In the hippocampus, TNF at the mRNA level is significantly higher in the stressed group of the HT strain 24 days after stress compared with the control. The level of IL-1β protein significantly increased only in the amygdala of stressed LT rats 24 days after stress, while mRNA expression remained at the control level. Iba1 expression increases in the amygdala in low excitable HT rats 7 days after stress at the mRNA level, while no changes are observed at the protein level. The number of GFAP+ cells decreases in the amygdala, CA1, CA3 and DG of hippocampus and in prefrontal cortex in LT rats 7 days after stress. In HT rats, at the same time point after stress, the number of cells decreases only in the CA1 area of hippocampus.

Conclusion: Thus, in rats with high and low hereditary excitability of the nervous system, different levels of expression of pro-inflammatory and glial markers and their specific changes under the influence of stress were revealed. In LT rats, these changes were evident in a wider range of measured parameters, indicating a heightened sensitivity to stress which is in line with their more pronounced behavioral response to stress. However, in HT rats, specific changes such as increased TNF mRNA in the hippocampus and altered Iba1 expression in the amygdala suggest that they are also significantly affected by stress. These unique patterns observed in HT rats indicate a need for more detailed studies on the long-term consequences of stress in this strain. Further research could elucidate the delayed effects of stress and the potential for developing chronic conditions, providing insights into tailored interventions for individuals with different genetic predispositions.

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