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Risk factors for the development of post-stress neuroinflammation: studies on rat strains with contrast excitability of the nervous system

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Motivation and Aim: The prevalence of disorders such as depression and anxiety is increasing worldwide and leads to disability of up to 18 % of people [1]. At the same time, the mechanisms of pathogenesis of these diseases remain unclear. Stress is considered one of the factors that, in combination with the biological vulnerability of the organism, can lead to the manifestation of psychopathologies. It is known that chronic stress leads to the development of neuroinflammation, which is now considered as a possible cause of cellular plasticity disturbances, which leads to dysfunctions in the nervous tissue.

The aim of this work was to evaluate the dynamics of post-stress peripheral and neuro-inflammation in rat strains with contrast excitability of the nervous system.

Methods and Algorithms: Experiments were carried out on adult male rats of two strains with high (LT – low threshold) and low (HT – low threshold) levels of the peripheral and central nervous system excitability. The severity and dynamics of inflammatory response was evaluated by assessing the number of microglial cells in the brain (prefrontal cortex, hippocampus and amygdala), the shift of the leukocyte formula and the expression of proinflammatory cytokines in the blood and brain.

Experimental animals were subjected to psychogenic prolonged emotional and pain stress according to the protocol of K. Hecht: every day for 15 days the animals were exposed to 6 unsupported and 6 current-reinforced (2.5 mA, 2 ms) light signals. According to the scheme, combinations of conditional and unconditional stimuli were not repeated, but alternated with a probability of 0.5, which did not allow the animals to develop a conditioned reflex. Stressed animals and the corresponding control groups were decapitated at four time points: 1) 24 hours after exposure to stress; 2) 7 days after exposure to stress; 3) 24 days after exposure to stress and 4) 60 days after.

rtPCR was used to evaluate changes in the expression levels of IL1 β and TNFα genes. The number of microglial cells was evaluated by immunohistochemical staining (Iba1+). The cells calculation was carried out using a fluorescent microscope Axio imager A2 (Carl Zeiss, Germany). The neutrophils /lymphocytes ratio (N:L ratio) was evaluated using a blood smear stained by Giemsa, and the phenotype of blood cells was determined in accordance with the recommendations for hematological evaluation in rats.

Distribution of data was evaluated by Shapiro-Wilk test using the GraphPad Prism software (v. 6, GraphPad Software Inc., La Jolla, CA). Non-parametric tests (Mann–

Whitney U tests, Fisher's exact test, Wilcoxon) were also performed with the GraphPad Prism software.

Results: In response to stress, a significant change in N:L ratio compared with the control animals was observed in stressed HT rats 24 hours after stress exposure. No significant differences were found between the LT control and stressed groups. We also found significant interstrain differences in the cytokine levels in the animal's blood already before exposure of these animals to any stress. A higher level of IL1 β mRNA was found in the blood of highly excitable LT strain rats compared to low-excitable HT strain animals. However, we did not find any effect of stress on the expression of the studied cytokines in the blood of highly excitable LT animals at any time points. But we observed a significant increase in the expression of IL1 β in the blood of HT strain compared to the control after 24 hours. Perhaps in this case we observe a normal pro-inflammatory reaction in the early stages of stress [2], which disappears by day 7.

Highly excitable intact LT rats showed significantly fewer Iba1+ cells in the hippocampus compared to low excitable HT rats. In HT stressed rats we found a significant increase in microglial cells number only in the CA1 region 7 days after stress (compared to the HT control). In LT rats the number of microglial cells in all studied areas of the hippocampus was significantly increased 7 days after long-term stress and 24 days after stress exposure, the number of microglial cells did not differ from the control.

Analysis of the mRNA level of inflammatory cytokines in the brains of animals with varying levels of excitability showed regional heterogeneity of poststress changes. At 24 days after the stress stimulus, the level of IL1 β mRNA increased in the stressed animals of both the LT and HT strains compared to the control groups of these strains. In the PFC, 24 hours after stress exposure there was a significant decrease in the level of IL1 β mRNA in the experimental group of low-excitable HT rats compared to control animals. At 24 days after the stress, there was a significant increase in the level of IL1 β mRNA in highly excitable animals (LT) compared to the control. In the amygdala of highly excitable animals, stress led to increased IL1 β expression 24 days after the stress. A significant decrease in TNFα mRNA expression levels compared to the control observed 24 hours and 7 days after stress in animals of the HT strain (low-excitable).

Conclusion: We found that animals with high and low excitability have specific features in the severity and dynamics of post-stress peripheral and neuroinflammation. Thus, genetically determined excitability of the nervous system is a promising marker of individual vulnerability to stress, since it affects the severity and dynamics of post-stress neuroinflammation.

References

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