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# Expression of arginine vasopressin and corticoliberin receptor genes in the prefrontal cortex and hippocampus of mice with anxiety-like behavior phenotype

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*Motivation and Aim*: Stress can disrupt normal brain processes and lead to the development of severe mental illnesses such as post-traumatic stress disorder (PTSD) [1]. The Single Prolonged Stress (SPS) protocol is one of the most commonly used rodent behavioral models employed by researchers to study PTSD [2]. This study focuses on how SPS affects changes in the expression of stress hormone receptors vasopressin (AVP) and corticotropin-releasing hormone (CRH) in the prefrontal cortex and hippocampus—brain structures responsible for the formation of fear and the consolidation of memory related to traumatic events [3]. Additionally, the level of neuroinflammation was assessed by counting hippocampal microglial cells responsible for the immune response in the body.
*Methods and Algorithms*: For the experiment, male wild-type mice were selected and divided into a control group and a stress group. The experiment involved 6 animals in the control group and 5 animals in the stress group. The animals were housed in a vivarium at Immanuel Kant Baltic Federal University (IKBFU). Experimental animals in the stress group were subjected to the Single Prolonged Stress (SPS) protocol, which comprised acute and chronic phases, for one month. No additional mental stress was applied to the animals in the control group during this period. At the conclusion of the experiment, behavioral phenotyping was conducted using the "Open Field" and "Elevated Plus Maze" tests. Subsequently, animals from both the control and stress groups underwent decapitation and perfusion. RNA was extracted from the prefrontal cortex and hippocampus of the left hemisphere using ExtractRNA kit (Evrogen, Russia), followed by reverse transcription. Gene expression levels were assessed using real-time PCR (BioRad C-1000). The right hemisphere was utilized for immunohistochemical examination. Serial sections with a thickness of 50 microns were obtained using a cryotome (Cryostat Microtome KD-3000, KEDEE, China). Immunohistochemical staining was performed using the Iba-1 microglial marker (ab178847, Abcam, United Kingdom) with a secondary antibody (ab150073, Abcam, United Kingdom). Iba+ cells in three zones of the hippocampus (CA1, CA2/3, and DG) were manually counted at 20x magnification using a Leica DM4000 B LED fluorescence microscope (LEICA Microsystems, Germany) equipped with LAS V.4.3 software.
*Results*: In the "Open Field" test, mice from the stress group exhibited significantly reduced motor activity and spent more time in the periphery compared to mice from the control group. In the "Elevated Plus Maze" test, males from the stress group spent more time in closed arms than in open arms, unlike males from the control group. Consequently, mice from the stress group displayed decreased exploratory behavior and

elevated levels of anxiety. In the prefrontal cortex of males from the stress group, an increase in the expression levels of arginine-vasopressin receptors Avpr1a and Avpr1b was observed, while the expression of the Avpr2 receptor and the corticotropin-releasing hormone receptor Crhr1 remained unchanged compared to the control group. In the hippocampus of males from the stress group, there was a decrease in the expression levels of Avpr1b and Crhr1 receptors, but no changes in the expression levels of Avpr1a and Avpr2 were observed. The number of Iba+ microglial cells was increased in the stress group compared to the control group in the CA1, CA2/3, and DG regions of the hippocampus, indicating the presence of neuroinflammation in mice from the stress group.

*Conclusion*: Therefore, SPS contributes to a decline in exploratory behavior and an increase in anxiety levels in mice from the stress group. Additionally, SPS induces neuroinflammation in the hippocampus and also has varying effects on the expression profiles of vasopressin and corticotropin-releasing hormone receptors in the prefrontal cortex and hippocampus of mice.

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