

Stress is an inherent attribute of life in modern society. The phenomenon of stress, itself, it is not a threat to the functioning of the biological organism. Moreover, it was an important adaptive mechanism that played a key role in the evolution of vertebrates. However, when the ongoing stress is too intense or lasts too long, it can have serious consequences for mental and somatic health. The primary goal of stress research is to understand the underlying neuronal mechanism of this phenomena. Among the brain structures involved in generating the stress response, the nucleus incertus (NI) seems to play a pivotal role. NI is located in the brainstem just below the fourth ventricle of the brain. NI consists mainly of neurons that produce gamma amino butyric acid (GABA), however there are also neurons that produce other equally important substances. This structure innervates many important areas throughout the brain. Interestingly, the architecture of neurons located in NI enables them to receive information about stress sent from other areas of the brain. The results of research on this structure revealed several functions in which NI is involved. Firstly, it is involved in the formation of memory traces. Disturbances in NI functioning, results in blocking the formation of memories, especially those related to stressful situations. Another role in which NI is involved is the so-called social re-cognition. The conducted research showed that damage to neurons in NI disturbs the course and dynamics of social interactions between two individuals of the same species. From the perspective of this project, the most important function of NI is its participation in generating the body's response to stressful situations. The anatomical location of NI in the brain and the architecture of its neurons put this structure in the right position to process information about the stress phenomenon. The results of research, especially behavioural and biochemical studies, provided some premises linking the activity of NI with the phenomenon of stress. Nevertheless, there is still no direct observation of how and which neurons within NI respond to aversive stimuli. Moreover, there is also a lack of information on to which structures in the brain NI neurons, potentially responsive to the aversive stimulus, transmit this signal. Finally, there is also a lack of observation of the biochemical consequences of aversive stimulation for NI neurons. The latest technologies will be used to fill this gap in our knowledge. The responses of NI neurons to the aversive stimulus will be recorded using electrophysiological techniques. Information on potential connections of NI neurons responding to aversive stimuli will be obtained by using the technique to visualize neural connections and to manipulate cell activity. Retrogradely transported viral vectors, will be used to introduce the gene into neurons innervated by NI. Due to this, it will be possible to induce the expression of light sensitive ion channels - enabling the selective activation of these - neurons with light - and the expression of fluorescent proteins visualizing the axons of these neurons. To determine the biochemical consequences of stress on the biochemistry of NI neurons, a training phase of fear conditioning will be performed. Immediately afterwards, modern technology will be used to visualize the mRNA of molecules specific to neurons in NI. In conclusion, the experiments planned in this project will allow to describe in detail the mechanism by which NI neurons participate in generating the organism response to stress. This mechanism will be investigated on the physiological, anatomical, functional, and biochemical field. The results obtained in this way will expand our current knowledge about the NI and how the phenomenon of stress is represented in our brains. Since onset and development of many severe and impairing mental disorders such as depression, schizophrenia and PTSD is affected by stress, understanding its mechanisms is key for improving medical therapies and to help patients.