Emotional states such as fear and aversion are among the basic mechanisms underlying animal learning, and any disruption in the regulation of these processes can contribute to the development of cognitive or psychological disorders such as phobias or anxiety disorders. Additionally, they are important adaptive mechanisms, and due to their highly complex nature, numerous brain structures are involved in the processing of aversion. One of the key neuronal groups in this aspect is the lateral habenula (LHb), which constitutes an aversion processing center. This structure is strongly interconnected with many brain centers responsible for, among other things, processing the body's stress response, and plays the role of integrating sensory stimuli with internal states, which enables so-called behavioral flexibility. The LHb is located in the epithalamus and consists mainly of neurons producing glutamic acid. Connections from the LHb reach, among other regions, the brainstem, where another structure important for regulating the stress response and fear memory is located – the nucleus incertus (NI). Both brain centers are therefore involved in similar neural processes and we hypothesize that the LHb-NI interconnection is an extremely important element of the neural circuit controlling the processing of aversive and stressful stimuli. Although the existence of the LHb-NI neuronal pathway has been experimentally confirmed, the studies do not address this topic further. Therefore, the main goal of this project is to investigate the reciprocal connection between the LHb and the NI from an anatomical and functional perspective. In order to fill the knowledge gaps, individual tasks planned in this project will be performed using innovative research techniques.

The effect of LHb stimulation on the electrical activity of NI neurons in deeply anesthetized rats will be investigated using optogenetic stimulation with simultaneous recording of neuronal activity using multielectrode arrays. Analogous recordings will be performed to investigate the effect of NI stimulation on LHb activity. Next, neuronal tract tracing technique will be used to investigate the anatomy and lateralization of the LHb-NI interconnection. The most extensive stage of this project aims to characterize the activity of NI and LHb neurons that form the interconnection between both structures, in response to context associated with aversive stimulus. For this purpose, the NI-LHb or LHb-NI pathway will be visualized by injection of viral vectors carrying genes for fluorescent proteins. Animals will undergo a classical fear conditioning procedure. On the test day, selected groups will be exposed to the aversive stimulus or only to the context associated with it. Such setup will allow for separation of responses to the aversive context from the responses to aversive stimulus itself. The activity of individual structures will be assessed using immunohistochemical staining, which allows visualization of the c-Fos protein, a marker of increased neuronal activity. Additionally, colocalization of the c-Fos protein with an artificially injected fluorescent protein will be examined, which will allow for determining the role of neurons forming the studied connection in mediating the response to an aversive context.

In summary, the LHb-NI neuronal circuit seems to be crucial in generating coordinated responses to aversive and stressful stimuli. The obtained results will contribute to a deeper understanding of one of the key mechanisms underlying a brain function crucial for animal survival. Thanks to the use of advanced experimental techniques, the data will be highly reliable and will provide a solid basis for future research plans. It will also be the foundation for the search of new and improving the existing methods of treating disorders related to aversion processing and anxiety.