Parkinson's Disease (PD) is the second most common neurodegenerative disorder, known for its impact on movement. The death of dopaminergic neurons is a main cause of movement disturbances, which appears to be due to the presence of aggregates of a misfolded protein called α-synuclein, leading to the formation of aggregates called Lewy Bodies. Apart from movement dysfunctions, the pre-motor and non-motor dysfunctions such as sleep disturbances, anxiety, depression or cognitive impairments are the features of PD, which significantly reduce the patients' quality of life. These symptoms do not respond to classical PD therapies – which replace neurotransmitter dopamine, thus they have been tentatively linked to other than dopaminergic neuronal pathways, such as noradrenergic and serotonergic systems, which are also disturbed in PD. Importantly, pre- and non-motor symptoms might be the first warning signs that could help diagnose PD earlier, allowing for more effective neuroprotective treatment at early stages of the disease. Many studies have shown that dysfunctions in the noradrenergic and serotonergic systems in the Locus Coeruleus (LC) and Raphe Nuclei (RN) occur at the same time or even before the loss of dopaminergic neurons in the Substantia Nigra (SN).

The pattern of neuronal degeneration in LC and RN starts in the brainstem nuclei and is linked with accumulation of α-synuclein aggregates in this area. Patients with initial pathology in the brainstem are vulnerable to develop pre-motor and non-motor symptoms, which correlate with the spread of pathological α-synuclein in the brain, including LC and RN. There are two hypothesis about how PD develops: 1) brain-first, 2) gut-first theories. The gut-first theory, proposed by Braak and colleagues suggests that the pathology starts peripherally in the organism and moves into the brain from the dorsal motor nucleus of the vagus nerve (DMV) located in the brainstem, spreading through the brain in a specific sequence. This progression aligns with the clinical course of PD, starting with pre-motor and non-motor symptoms and advancing to severe motor and non-motor symptoms.

Recently, scientists have been able to replicate in laboratory α-synuclein aggregation using α-synuclein preformed fibrils in both: *in vitro* (in cells), *in vivo* (in animals) models. However, most models focus on dopaminergic neurons *in vitro* or *in vivo*, and do not fully replicate the brainstem pathology seen in patients. No previous studies showed early non-motor symptoms in α-synucleinopathy models involving the direct role of the LC and RN.

Our project aims to fill this gap by studying the progression of α-synuclein pathology in the brainstem's noradrenergic and serotonergic neurons using unique *in vitro* mice primary serotonergic and noradrenergic neuronal cultures and a novel *in vivo* mouse model. We will investigate how misfolded α-synuclein aggregates affect these neurons at the cellular level and in live animals after introducing α-synuclein preformed fibrils to the DMV or LC and RN in the brain. Also, we are planning to verify the biochemical and behavioral aspects of proposed *in vitro* and *in vivo* models. It is worth noting that such characterizations and evaluations have never been done yet, and there are still limited cellular or no existing animal models that fully mimic all PD symptoms. Reliable *in vitro* or *in vivo* models are crucial for studying α-synuclein pathology progression. Understanding the connection between α-synuclein, LC, RN, and early PD diagnosis is essential for developing future therapies and diagnostics.

This project will provide evidence for progressive spreading of α-synuclein pathology from the brainstem and its possible links to pre- and non-motor symptoms of PD. Additionally, it will establish and characterize novel, more relevant to early human PD models of α-synucleinopathy in noradrenergic and serotonergic neurons for further research explorations and therapeutic developments.