Abstract

Effective operation in a dynamically changing environment requires synchronized and undisturbed functioning of numerous neural pathways within the brain. One of the most important neural pathways in the regulation of animal behavior comes from dopaminergic (DA) neurons in the midbrain. The majority of those neurons are concentrated in the ventral midbrain, encompassing the ventral tegmental area (VTA) and the adjacent substantia nigra pars compacta (SNc). Within the VTA structure, the existence of other neuron types was demonstrated. Nevertheless, DA neurons represent the most extensive population. Additionally, this population of neurons is known for its ability to generate action potentials in two distinct modes: non-bursting (regular or irregular). Moreover, DA neurons of VTA participate in various functions, but for this study, their significance in regulating motivation levels is the most important. The activity of DA neurons is controlled by numerous well-described structures that project to them and encode aversions, such as lateral habenula (LHb), rostromedial tegmental nucleus (RMTg), bed nucleus of the stria terminalis (BNST) or lateral hypothalamus (LH). Correspondingly, less is known about the innervation of the VTA originating from the brainstem, especially in the context of the regulation of animal motivation. Based on theoretical premises and the results of preliminary observations, I hypothesized that the nucleus incertus (NI) of the brainstem - involved in generating the stress response - is the source of innervation controlling the activity and related functions of DA neurons in the VTA of the rat brain. Thus, the primary aim of this study was to describe the anatomy, physiology, and potential function of the NI-derived innervation of VTA DA neurons. To achieve this, I used retrograde and anterograde labeling of neuronal pathways combined with immunohistochemical identification of the biochemical nature of the cells forming the studied innervation, extracellular and juxtacellular in vivo recording of DA neuron activity in the VTA, and behavioral tests. During electrophysiological experiments and behavioral tests, I controlled the activity of the studied

neuronal pathway using an optogenetics technique based on selective transfections of NI neurons, which are the source of VTA innervation.

The result of retrograde labeling of neuronal pathways obtained by me, confirmed that NI directly innervates the VTA, with clear lateralization, i.e. significantly stronger ipsilateral innervation. Furthermore, immunohistochemical stainings showed that the majority of NI neurons innervating the VTA are negative for the neuropeptide relaxin-3. In turn, the anterograde labeling of neuronal pathways confirmed the presence and showed a dorsal-ventral gradient in the distribution of NI fibers within the VTA, with their increasing density ventrally. Interestingly, the performed electrophysiological recordings, combined with optogenetic activation of the examined neuronal pathway, showed that a large part of DA neurons located in the VTA react with a short-term reduction in their electrical activity in response to single pulses stimulating the NI-VTA pathway. Moreover, DA neurons decreased their electrical activity during long-term, pulsatile activation of the studied pathway, which was used in the conducted behavioral studies. However, after long-term stimulation ended, the activity level of DA neurons in the VTA returned to baseline. The biochemical nature of VTA neurons whose activity was inhibited by optogenetic activation of innervation from NI, i.e. the presence of tyrosine hydroxylase in cell bodies, was finally confirmed by recording and labeling of neurons in the juxtacellular configuration. Based on the obtained results of anatomical and electrophysiological studies, I planned and carried out observations of changes in animal behavior caused by optogenetic activation of the NI-VTA pathway. The real-time conditioned place preference/aversion test (RT-CPP/A) showed that activation of NI neurons innervating the VTA exerts an aversive effect and/or reduces the animal's motivation. Simultaneously, the animal's behavior in the open field test (OF) and the elevated plus maze test (EPM) did not indicate that optogenetic activation of the NI-VTA pathway caused changes in motor skills, the level of exploration, or fear of animals.

In conclusion, the results of the research conducted during my PhD described the anatomy and physiology of the previously unknown descending pathway from the NI to the VTA that controls the activity of DA neurons. Moreover, the analysis of the results of behavioral tests showed that the studied neural pathway may have an impact on controlling the animals' motivation level. The observations described in my doctoral dissertation allow to consider NI as one of the nuclei of the brainstem, which is responsible for regulating the activity and related functions of DA neurons located in the VTA.