1. Project objectives

Reduction of memory based on the drug of abuse-environment association (so called “drug memory”) lies at the foundation of potential pharmacotherapy of substance use disorder, however there is still a lack of empirical evidence explaining the molecular mechanisms and sequence of cellular events occurring during drug abstinence. Suppressing of any behavior or acquired conditional response is a process of new and active learning, and abundant evidence indicates that the glutamategic N-methyl-d-aspartate (NMDA) receptor belongs to the key elements of the above processes. The aim of the project is to verify the hypotheses that the molecular mechanisms associated with NMDA receptors during cocaine abstinence can be critical markers controlling drug seeking and drug relapse.

To this end, we will examine the functional structure and mechanisms ex vivo of the subunits of NMDA receptors and their interactions with scaffolding proteins in different brain areas during cocaine forced abstinence in rats that have earlier self-administered cocaine. If, during the project course, the obtained changes within the NMDA receptors intensify drug craving and relapse, in later studies we will use "in vivo" tools to reverse the existing disturbances. For this purpose, we will apply new peptides penetrating through the cell membranes (that control the association of the NMDA receptor subunit and scaffolding protein) and viral vectors (which change the composition of the NMDA receptor subunits).

In other words, we intend to discover the cellular and molecular mechanisms that occur during cocaine abstinence, explain the phenomenon responsible for drug relapses after periods of abstinence, and use the knowledge gained for the benefit of substance use disorder pharmacotherapy.

2. Studies that will be performed

At the Department of Toxicology of the Faculty of Pharmacy, Jagiellonian University Collegium Medicum, in cooperation with the Laboratory of Drug Addiction Pharmacology, Institute of Pharmacology in Cracow, we intend to examine the various neuroadaptive changes within the NMDA receptor occurring in animals repeatedly treated with cocaine.

In our research plan, the best preclinical behavioral procedures in substance use disorder as well as technologically advanced molecular analyses will be employed. A behavioral model of intravenous cocaine self-administration in rats perfectly reflects the drug addiction cycle, beginning from the occasional drug use, through its abuse and the loss of control over behavioral responses and drug relapse. In this model, the animals are trained to press a lever located in the experimental chamber. Pressing on a lever activates an infusion pump, which causes an intravenous injection of cocaine. Cocaine delivery may be accompanied by positive reinforcements (so called “conditional stimuli) that signal its availability.

Rats, undergone the above cocaine self-administration training, will be forced into several cocaine abstinence conditions (social isolation, enriched environment, extinction training in experimental chambers), followed by drug seeking and relapse. The latter behavioral outcomes will be developed by cocaine priming, conditioned stimuli associated with previous cocaine self-administration or stress factors. With using the “yoked-triad” procedure which depends on pairing a rat actively taking cocaine with an animal that passively receives the drug or with an animal that passively receives saline, appropriate control groups will be generated.

In further preclinical analyses, animals subjected to cocaine self-administration and different abstinence conditions (above), molecular analyses will be performed in the dorsal striatum, prefrontal cortex, hippocampus and basolateral amygdala. These brain structures are involved in the regulation of drug seeking and relapse. Molecular analyses will address NMDA receptor protein interaction with scaffolding proteins (co-immunoprecipitation), the determination of the composition and protein expression of NMDA receptors in the postsynaptic and extrasynaptic membrane fractions (TIF and Western blot) and the cellular mobility of the NMDA receptors and scaffolding proteins (BS3 cross-binding). In order to reverse the molecular changes in NMDA receptor under the influence of forced cocaine abstinence, we plan to use viral vectors (administered intracranially) and new peptides that penetrate cell membranes (administered systemically). The same intervention factors and their specificity will be tested in animal models to assess drug seeking and relapse.

3. Rationale for the research topic

Substance use disorder is a serious disease of the central nervous system, which destroys the health and live of people. It is characterized by the uncontrolled drug taking and drug seeking as well and drug relapse that appear even after a long period of abstinence. High risk coming from this devastating disease prompts researchers to search for effective pharmacotherapies that control drug abuse as well as a lot of studies is focused on investigating the neurobiological basis of the addiction process. Of the psychoactive drugs, cocaine possess a high addictive potential. Cocaine abuse in the last five years has increased alarmingly in Europe and Poland, and cocaine use disorder is still an unsolved medical and social problem in the world.

Cocaine use disorder is characterized by compulsive drug use and loss of control over intake. Impairment evoked by cocaine begins in the mesolimbic dopaminergic system, followed by metabolic deficits within the brain cortical structures that, among others change the assessment of the award (cocaine) value and the effort necessary to get the award. The other important impairments due to cocaine intake are found within the memory and learning processes. The formation of memory can be described as consolidating traces of memory, relying on the generation and stabilization of new connections between nerve cells, whereas the loss of memory is due to the loss of such connection.

In recent years, one of the leading hypotheses postulates the alterations of learning processes as mechanisms leading to drug habits. Modulation of memory association due to habit-driven drug-associated conditional stimulus is considered a potential therapy for addiction. Given the significant involvement of glutamatergic neurotransmission in the control of synaptic plasticity, learning processes and conditioned response, in recent years there has been attention on glutamatergic NMDA receptors and their role in the mechanisms of substance use disorder. NMDA receptors are not static elements of the synaptic membrane; created with four subunits (GluN1, GluN2A, GluN2B, GluN3), they occur in synaptic versus extra-synaptic membranes, they form complexes with other proteins (e.g., scaffolding proteins), are subject of different processes (e.g., membrane trafficking and synaptic plasticity) and - depending on the composition of the subunit and location – perform different physiological functions.

Preclinical behavioral findings on cocaine (and other drugs of abuse) chronic treatment demonstrate the role of NMDA receptors in the drug reward, relapse as well as in the memory consolidation and reconsolidation of drug-cue memories, while some

molecular studies, including those run by our team, provided evidence that demonstrated changes in NMDA receptors and their subunit expression after acute or repeated treatment with cocaine given passive (by the experimenter) and voluntary (with self-administration model). However, the existing data do not allow to create a complete picture of the involvement of NMDA receptors and their subunits in the phenomenon of cocaine use disorder, including drug forced abstinence.

This project is aimed to explain the changes that occur during cocaine abstinence and the discovery of new critical markers involved in the development of substance use disorder. We will also answer the question whether peptides penetrating cell membranes, which control the associated NMDA receptor subunit-scaffolding proteins, based on molecular and pharmacological analyses can be in the future the basis of a modern, rational pharmacotherapy for cocaine use disorder. Because the abused drugs, natural rewards, pathological behaviors produce adaptive changes in the same brain structures, the obtained results may contribute to the discovery of new "general" therapeutic drug strategies and to set a new direction for research. It is worth noting that addiction to psychoactive drugs causes damage and serious problems for medical and social services of an economic nature and therefore the results obtained in this project through the launch of a new research-therapy may help reduce the consequences