Opioid use disorder (OUD) is among the most important crises of contemporary societies. Significant increase in the use of novel, synthetic opioid analgesics (e.g. oxycodone) has exacerbated this crisis both in Poland and worldwide. Opioid use leads to neuroadaptations maintained through abstinence which have a causal role in the development of OUD symptoms, including withdrawal syndrome and drug craving, both leading to the recurrent opioid use that occurs despite adverse physical, psychological, or social consequences. In OUD, expression of these major symptoms occur during abstinence when exposition to the cues associated with opioid use (conditional stimuli, CS) and withdrawal syndrome leads to opioid craving. Such craving is enforced by appetitive motivation to experience rewarding effects of an opioid and by negative motivation (negative reinforcement mechanisms) to alleviate dysphoric states experienced during opioid withdrawal. Prevention of relapse to opioid taking and ability to decrease negative symptoms of withdrawal are primary goals of OUD recovery. Opioid craving remains resistant to most forms of clinical treatment, driving the search for alternatives such as harm reduction strategies, including maintenance therapy, during which abused opioid is substituted with weaker/slower acting opioid (e.g. methadone or buprenorphine). Remarkably, 60 years after the introduction of substitution/maintenance therapy, little work has been devoted to identifying the brain circuitry or neurotransmitter's pathways through which this treatment operate. In addition, substitution therapy drugs has abuse potential themselves and lead to several side effects, limiting safety of the treatment.

Recently, of opioid receptors structure as well as their protein models have been demonstrated. In addition, recent studies indicates that functional effects of opioid receptor activation might depend on G proteins or β-arrestin activation. These findings lead to the development of novel functionally selective ligands of opioid receptors – biased agonists, which selectively activates only one of those opioid receptor-coupled proteins and thus potentially leading to different behavioral effects. One of such biased agonist is PZM21 – and μ opiod receptor ligand activating G proteins but not β-arrestin. In rodents, PZM21 has no rewarding effects suggesting little abuse potential and thus better alternative for currently used opioids in the substitution therapy.

Project goal: our goal is to identify the neurobiological mechanisms that underlie opioid craving and withdrawal as well as identification of potential new pharmacological treatments of the OUD symptoms. The first aim of the project is a mapping of neural activity and identification of circuity underlying opioid withdrawal and craving using cutting-edge anatomical, optogenetic and neurophysiological technologies. Furthermore, the recent discovery of novel biased opioid receptor agonists opens new possibilities to explore their effects on behavioral and neural functions of craving and withdrawal and may open new pathways to refined pharmacological therapies of OUD. The second aim of the project is examining the therapeutic potential of selected novel biased agonists of the μ and κ opioid receptors in the attenuation of oxycodone craving and seeking as well as opioid withdrawal.

Description of research: we will use an innovative combination of *in vivo* optogenetics targeting activated cells, fluorescent tracers, advanced immunohistological and molecular imaging techniques, fiber photometry, single-cell recordings. We will use behavioral pharmacology in wild type and transgenic rats to demonstrate neuronal circuitry underlying oxycodone (using intravenous oxycodone self-administration) seeking and protracted withdrawal. Receptor- and brain region-specific interrogation of the opioid craving and withdrawal is likely the most useful avenue towards the identification of selective compounds or receptor mechanisms that modulate distinct circuitries involved in OUD symptoms.

Reasons for attempting a particular research topic. Critical steps in advancing the research field are to reveal if opioid craving and withdrawal activate specific neuronal populations in the brain. We anticipate that by identifying and manipulating genetically- and activity- marked neuronal subpopulations, we will accomplish progress towards circuit specific control of OUD symptoms. More importantly, identification of brain regions via which substitution therapy has its effects might provide a necessary route to control opioid seeking behavior and evaluate new therapeutic approach for the treatment of OUD. Together, the anticipated results would encourage pharmacological treatment of opioid craving and withdrawal.

Substantial results expected. Major goals of this project, potentially leading to the development of more effective OUD therapies in the future, is to identify neurophysiological underpinnings of opioid-seeking and withdrawal as well as brain mechanisms and pharmacological treatments capable of attenuation of OUD symptoms. Together, the anticipated results would encourage pharmacological treatment of opioid craving and withdrawal.