It is estimated that each year, 38.2% of the European Union (EU) population (or 164.8 million people) suffer from a mental disorder: 7.8%suffer from depression, which is also the most burdensome disorder of all diseases in the EU as well as the most burdensome and costly (costs in EU- 105 billion euro) brain disease. The classical antidepressant drugs (ADs) which affect monoaminergic systems were introduced into the clinic over 70 years ago, and are characterized by a slow onset of action, the poor efficacy and a high resistance rate. Therefore, there is a large need for more effective ADs with faster onsets of action, higher remission rates, anti-suicide properties, and fewer side effects.

There are several powerful substances that show these properties, one is ketamine, the other is scopolamine. Both produce rapid and long-lasting antidepressant effects and both can be drugs of abuse, showing hallucinogenic properties, among others.

Scopolamine, (another name Hyoscine) is a naturally derived alkaloid of species from the Solanaceae family, (e.g., Datura stramonium, Scopolia carniolica, and Hyoscyamus niger). Scopolamine is a nonselective antagonist of muscarinic acetylcholine (M1-M5-ACh) receptors and is widely used as a butyl bromide salt in the treatment of abdominal pain, irritable bowel syndrome and bladder spasms (in its form it does not penetrate into the brain), whereas a hydrobromide derivative (which enters the brain) is used as a motion sickness reliever and as a preoperative medication

The antidepressant effects of scopolamine were described for the first time by Furey and Drevets in 2006. The mechanism responsible for the antidepressant effect of scopolamine remains unclear. According to Witkin (2014), M1 and M2 muscarinic receptor antagonism seem to play the main role in this effect. Scopolamine induces considerable adverse effects including memory impairment, drowsiness as well as visual disturbances, which hamper its use as a psychiatric drug. Our preliminary studies (Podkowa et a., 2016, 2018) have shown that to overcome these limitations one can combine scopolamine with metabotropic glutamate (mGlu) receptor ligands. Administration of low doses of scopolamine together with low doses of AMN082, a positive allosteric modulator (PAM) of mGlu7 receptors or with low doses of an antagonist of group 2/3 mGlu receptors LY341495 allows for an enhancement of antidepressant effects of scopolamine and reduction of adverse effects.

Here we will continue that line of research investigating which subtype of muscarinic receptors is responsible for antidepressant effects of scopolamine as well as which subtypes of group II/III mGlu receptors are involved in interactions with scopolamine. Hopefully, we will identify antagonists of muscarinic M1-M2-M3-M4 or M5 receptors with an antidepressant efficacy, that will interact with mGlu receptors in such a way, that a marked reduction of adverse effects will be observed together with the enhancement of antidepressant effects.

The following hypotheses will be tested:

1. The selective antagonists or NAMs of muscarinic (M1-M5) receptors or ligands of mGlu2/3 or mGlu7 receptors exert antidepressant activity in different animal tests/models.
2. After combined administration of various antagonists muscarinic receptors and mGlu 2/3/7/ ligands, including orthosteric and allosteric ligands, positive allosteric modulators [PAMs], or [NAMs], the antidepressant activity is enhanced and the adverse effect profile is reduced.
3. The combined administration of M and mGlu ligands is accompanied by changes in the release of glutamate, GABA, and acetylcholine or monoamines in the relevant brain structures. The interactions will be investigated on the behavioral level (see above) as well as by the *in vivo* microdialysis and by electrophysiology. The functional effects of various receptor ligands on prefrontal cortex (PFC) circuitry will be studied using the whole-cell patch clamp technique
4. It has been demonstrated that the antidepressant effects of scopolamine require mTORC1 signaling and are associated with increased glutamate transmission and synaptogenesis. The investigation of the antidepressant signature of combined administration of M and mGlu receptor ligands will be carried out.
5. Receptor oligomerization might provide an additional level of signaling diversity and complexity provided by cross-talk between two different receptors. We will investigate if muscarinic receptor subtypes and mGlu2/3 or mGlu7 receptors-remain in close vicinity- and can-form heterocomplexes.

The current project has been developed by a group that has done pioneering work in the area of investigation of the antidepressant effects of mGlu receptor agents including mGlu5 receptor antagonists mGlu2/3 receptor antagonists and mGlu7 receptor agonists.

The project will lead to a better understanding of the mechanism of action of antidepressant drugs and in the future may help to develop better treatment of depression.