Improving pattern separation and cognition

Summary

In this dissertation we studied cognitive processes with emphasis on pattern separation and spatial memory. Pattern separation is the process of transforming highly similar sensory inputs into distinct, dissimilar representations. It takes place in the hippocampus and is thought to be used in memory formation and retrieval. Impaired pattern separation performance has been recognised to be a predictor for the development of cognitive impairments in humans such as dementia and is present in patients with schizophrenia and post-traumatic stress disorder (PTSD). Moreover, the role of the serotonin 1A receptor (5-$HT1_AR$) in pattern separation processes was studied. The functioning of different 5-$HT1_AR$ subpopulations was assessed on a behavioral and biochemical level in order to evaluate different possibilities to utilize this receptor subtype (and subpopulations) for treatment possibilities in different psychiatric and neurological disorders. This is described in more detail in **Chapter 1**, which gives a general introduction on cognitive enhancement, pattern separation & spatial memory, and the the 5-$HT1_AR$. Furthermore, this chapter contains the aim of this dissertation; "The aim of this dissertation was to gain more insight in the neuronal signaling pathways involved in behavioral pattern separation processing and spatial memory, through investigation of the potential of different pharmacological agents to either impair or enhance these processes."

Chapter 2 elaborates more on the pattern separation proces and describes different paradigms to assess memory processes in rodents. The object recognition task (ORT) is a widely used paradigm to measure object memory processes in rodents. Recently, the memory process known as pattern separation has received increasing attention, as impaired pattern separation can be one of the cognitive symptoms of multiple neurological and psychiatric disorders. In the search for an easily implemented task for rodents that can be used to measure pattern separation, we developed a task derived from the ORT and the object location task (OLT), which we called the object pattern separation (OPS) task. This task aims to measure spatial pattern separation per se, which utilizes memory processes centered in the DG and CA3 region of the hippocampus. Adult male C57BL/6 mice and adult male Wistar rats were used to validate different object locations which can be used to measure spatial pattern separation. Furthermore, different inter-trial time intervals were tested with the most optimal object location, to further evaluate pattern separation-related memory in mice. We found that specific object locations show gradual effects, which is indicative of pattern separation, and that the OPS task allows the detection of spatial pattern separation bi-directionally at intermediate spatial separations. Thus, object locations and time intervals can be specifically adjusted as needed, in order to investigate an expected improvement or impairment. We conclude

that the current spatial OPS task can be best described as a specific version of the ORT, which can be used to investigate pattern separation processes.

In **Chapter 3** the focus is on cognition enhancement and the role of 5HT1$_A$Rs on the pattern separation process and spatial memory in rodents. Several studies were performed to increase our understanding of the role this receptor, its different subpopulations and their different ligands, plays in enhancing pattern separation processes and spatial memory.

The 5-HT$_{1A}$R can exert differential effects on cognition-related neurotransmission depending on the location (or subpopulation) of the receptors. A subpopulation of 5-HT$_{1A}$ autoreceptors are located in the raphe nuclei, such as the dorsal raphe nucleus (DRN), and exert inhibitory effects on serotonergic transmission, whereas 5-HT$_{1A}$ heteroreceptors are mainly located in limbic regions, such as the hippocampus and cortical regions, and can exert indirect stimulatory effects. The study outlined in this Chapter aimed to identify how different 5-HT$_{1A}$R subpopulation activity mediates cognition, specifically spatial pattern separation performance, following acute and chronic stimulation.

Male Wistar rats were treated with either F13714, a biased agonist which preferentially activates 5-HT$_{1A}$ autoreceptors, or F15599, a biased agonist that preferentially activates 5-HT$_{1A}$ heteroreceptors, both acutely and chronically for 14 days. Body temperature measurements were taken daily. Object pattern separation (OPS) performance was measured directly after acute treatment and at day 15 of chronic treatment. Animals were sacrificed after behavioral testing to measure 5-HT$_{1A}$R density and cognition-related markers in the hippocampus and DRN.
Acute treatment with F13714 impaired OPS performance, whereas chronic treatment increased performance to vehicle levels. Body temperature was measured as a functional correlate of 5-HT$_{1A}$ receptor stimulation. It dropped from day 4 onwards and in parallel the number of 5-HT$_{1A}$ receptors decreased in the DRN. F15599 enhanced OPS performance both acutely and chronically and caused an acute drop in body temperature, which rose again during chronic treatment. Furthermore, BDNF levels and doublecortin positive newborn neurons increased in the dorsal hippocampus after chronic F15599 treatment.
In conclusion, these studies showed the divergent effects of two 5-HT1$_A$R biased agonists. Subpopulation specific targetting of 5-HT1$_A$Rs might prove to be a valuable tool for pattern separation impairments. The studies outlined in this Chapter indicate that chronic treatment with F13714 may result in desensitization of 5-HT$_{1A}$ autoreceptors,which causes a reversal of the initial impairment measured in OPS performance. Chronic post-synaptic stimulation of 5-HT$_{1A}$ heteroreceptors with F15599 may have therapeutic potential to treat pattern separation impairments.

Assessment of the potential of 5-HT_{1A} heteroreceptor stimulation on a rodent cognitive-deficit model would be the next logical step. **Chapter 4** introduces a preclinical pharmacological model for cognitive impairments associated with schizophrenia (CIAS). Cognitive deficits are a key feature of schizophrenia that can severely impact daily functioning. Stimulation of 5-$HT1_A$ receptors to improve cognition has led to differential effects, dependent on their location. Conventional non-biased 5-$HT1_A$ receptor agonists can elicit positive effects which are believed to be regulated through post-synaptic 5-$HT1_A$ heteroreceptors, but these can be counteracted by simultaneous activation of the 5-$HT1_A$ autoreceptors. Therefore, selective targeting of post-synaptic 5-$HT1_A$ heteroreceptors with biased agonists such as F15599 could be more effective to alleviate cognitive deficits in schizophrenia.

Here, the NMDA glutamate receptor antagonist ketamine (30mg/kg, IP) was used to induce schizophrenia-like cognitive deficits in rats. Three weeks after sub-chronic (5-days) ketamine or vehicle treatment, cognitive performance was measured in the OPS task, which relies on hippocampal processes. An attentional set-shifting task was used to assess medial prefrontal cortex-regulated behavioral flexibility. Acute treatment with F15599 (0.04mg/kg IP) was compared to vehicle treatment to evaluate the effects of selective 5-$HT1_A$ heteroreceptor activation on cognitive performance in both control rats and the schizophrenia-like rats.

In both tasks the ketamine-treated rats showed an impaired performance compared to control animals. Acute treatment with F15599 reversed the pattern separation deficit up to the performance level of control animals. Administration of F15599 prior to learning a new response-strategy in the set-shifting task, improved performance in the schizophrenia-like rats. These data show that selective activation of 5-$HT1_A$ heteroreceptors can be a successful treatment to relieve schizophrenia-related deficits that originate from both hippocampal and/or prefrontal cortex centered processing.

Chapter 5 describes another mechanism to enhance spatial memory processes in preclinical pharmacological rodent models for cognitive impairment. Upregulation of cyclic guanosine monophosphate (cGMP) through the inhibition of specific phosphodiesterases (PDEs) has previously been shown to improve memory performance. The current study aimed to target cGMP upregulation differently, using the soluble guanylate cyclase (sGC) stimulator riociguat, and investigate the acute effects on memory in both healthy mice and a biperiden induced memory deficit mouse model. Biochemical measurements were performed on hippocampal tissue to further elucidate the role of the nitric oxide (NO) – sGC – cGMP signaling pathway in memory function. Acute administration with a low dose of riociguat was able to enhance working-, short- and long-term spatial memory as measured with the object location task or Y-maze continuous alternation task. Pharmacokinetic measurements within brain tissue of acutely treated mice showed very poor or no brain penetration

of riociguat. Western blots revealed an increase in activation of vasodilator stimulated phosphoprotein (VASP) at the behaviorally active dose of riociguat. No other effects were found on memory-related hippocampal plasticity measures including activation of CREB, AMPA receptor trafficking and PSD95. These findings support the assumption that the memory enhancing effects are due to a non-central effect. In this respect, further research is needed to investigate the possible contribution of hemodynamic or metabolic effects which are known to be regulated by sGC-cGMP signaling.

After validating the OPS paradigm, it was decided to share a highly detailed protocol of this novel task with the scientific community. This protocol was published and is outlined in **Chapter 6**. In this protocol we describe how to implement a simple and robust OPS task in mice and rats that we have previously established and validated. This two-trial memory task utilizes specific object locations so differences in performance can be calibrated with the extent of object movement. Changes in performance are indicative of spatial pattern separation. In contrast to other pattern separation tasks, the OPS task allows detection of spatial pattern separation performance bi-directionally. Furthermore, the OPS task is cheaper and easier to use and interpret than other tasks which use more than two objects or which are touch-screen based. The entire protocol, from vivarium acclimatization to training of the animals, takes approximately 30 days. After successful training, the animals can be tested repeatedly and three OPS experiments (n = 20-24 per experimental day) can be performed per week. A standard level of expertise undertaking behavioral studies in rodents is sufficient to successfully integrate this paradigm into an existing rodent test battery.

To summarize, this dissertation showed that spatial pattern separation can be successfully measured using the OPS task in both healthy rodents and a rat model for CIAS. The task lends itself for easy application and adaptation to study both the enhancing and impairing effects of different drug treatments. The experiments in this dissertation provide evidence that 5-HT1_ARs mediate pattern separation performance and that selective targeting of post-synaptic heteroreceptors could potentially be a successful strategy to enhance pattern separation performance and schizophrenia-related cognitive impairments. We provided evidence that chronic stimulation of 5-HT1_A ¬heteroreceptors with F15599 increases hippocampal plasticity which possibly mediates the beneficial effects on pattern separation. Acute treatment with F15599 can alleviate ketamine-induced schizophrenia-like cognitive impairments, possibly through enhanced cortico-hippocampal signaling resulting in restoral of glutamatergic and GABAergic levels. However, it must be noted that further experiments are needed to elucidate the exact role of 5-HT1_ARs in cognitive functioning. The studies truly highlight the complexity of 5-HT1_AR signaling which appears to be highly dependent on the localization of the receptors within different brain structures.