In the first year of the project, the neuronal circuit responsible for maternal and adult social behaviours as well as their hormonal background was addressed. In the first study, the role of preoptic GABAergic inhibitory neurons was addressed in parenting, anxiety and depression. Pup exposure and forced swimming resulted in similar *c-Fos* activation pattern in neurons expressing vesicular GABA transporter in the preoptic area with generally stronger labeling and different distributional pattern in females than in males. Chemogenetic stimulation of preoptic GABAergic cells resulted in elevated maternal motivation and caring behavior in females and mothers but aggression towards pups in males. Behavioral effects were the opposite following inhibition of preoptic GABAergic neurons suggesting their physiological relevance. In addition, increased anxiety- and depression-like behaviors were found following chemogenetic stimulation of the same neurons in females while previous pup exposure increased only anxiety-like behavior suggesting that not the pups, but overstimulation of the cells can lead to depression-like behavior. A sexually dimorphic projection pattern of preoptic GABAergic neurons was also identified, which could mediate sex-dependent parenting and associated emotional behaviors. In conclusion, we identified GABAergic neurons in the preoptic area to increase pup-directed behaviors in females and possibly a separate group of GABAergic preoptic neurons eliciting pup-directed aggression in males. We also demonstrated that GABAergic preoptic neurons responsible for parenting behavior could lead to depression-like behavior if overstimulated.

According to the above experiments, the preoptic area plays a crucial role in the control of maternal behaviors. Still, our knowledge on the preoptic gene expressional changes in mothers is limited. Therefore, we addressed this question by transcriptome sequencing in rats. Transcriptome sequencing was first applied in the preoptic region of rat dams in comparison to a control group of mothers whose pups were taken away immediately after parturition and did not exhibit caring behavior 10 days later. Differentially expressed genes were found and validated by quantitative RT-PCR, among them NACHT and WD repeat domain containing 1 (Nwd1) is known to control androgen receptor (AR) protein levels. The distribution of Nwd1 mRNA and AR was similar in the preoptic area. Therefore, we focused on this steroid hormone receptor and found its reduced protein level in rat dams. To establish the function of AR in maternal behavior, its antagonist was administered intracerebroventricularly into mother rats and increased pup-directed behavior of the animals. AR levels are suppressed in the preoptic area of mothers possibly mediated by altered Nwd1 expression in order to allow sustained high level care for the pups. Thus, our study first implicated the AR in the control of maternal behaviors.

Next, we addressed the pathways providing inputs from the thalamus to the preoptic area to control social behaviors. We previously demonstrated that tuberoinfundibular peptide of 39 residues (TIP39), is induced in mother rats in the posterior intralaminar thalamic nucleus (PIL). Here, we described evidence that TIP39 and the thalamo-hypothalamic pathway containing TIP39 is involved in the regulation of maternal as well as direct contact adult social behavior.

TIP39 expression was increased in the PIL of female rats kept together with other females while social isolation reduced TIP39 levels, a finding consistent with the reduction of TIP39 in mother rats following removal of her litter. PIL TIP39 neurons demonstrated c-Fos activation in response to conspecifics but only if direct contact was allowed between the animals. Chemogenetic activation of PIL neurons increased the number of social touches between rats. The physiological importance of PIL TIP39 neurons was demonstrated by the reduction of maternal motivation when the cells were chemogenetically inhibited. Furthermore, an antagonist of the PTH2 receptor also reduced maternal motivation. To reveal which projections of TIP39 PIL neurons are involved in social behavior, cell specific neuronal tracing was performed, which revealed distinct projection pattern of calbindin (TIP39) and GABAergic (VGAT+) PIL neurons. The most intense projection of calbindin (TIP39) neurons was to the medial preoptic area (MPOA). The selective chemogenetic stimulation of the PIL-MPOA pathway was performed using double viral injections (a retrogradely virus expressing Cre injected into the MPOA coupled with a Cre dependent virus into the PIL) and also by using local CNO administration directly into the MPOA. The selective stimulation of the PIL-MPOA projection increased direct interactions during social behavior. In addition, direct contact during social interaction caused increase in neuronal activity in the MPOA. The results suggest that posterior thalamic PIL neurons convey socially relevant information to a variety of different forebrain centers, among which the MPOA is involved in the processing of social touch. Thus, we identified an important novel component of the social brain network, which may increase the motivation for affiliative direct contact interactions.

In the second year of the project, the effect of manipulation of neuronal activity in the PIL on social behaviors was addressed. In the first study, chemogenetic stimulation and inhibition of neurons that were previously activated by a conspecific was performed. Activity-dependent tagging of PIL neurons was performed in rats experiencing physical social contacts. Cre recombinase was introduced to the cells using a Tet-On system, where the presence of the antibiotic provided a time window for conspecific-induced c-Fos expression, which in turn activates Cre recombinase in activated neurons. Following activation or inhibition of neurons induced by CNO, the animals increased and decreases social grooming behavior, respectively. Next, the role of posterior intralaminar thalamic nucleus (PIL) neurons projecting to the preoptic area was described. Following the injection of rAAV-EF1a-mCherry-IRES-Cre virus into the preoptic area, Cre recombinase appeared in the PIL. By administering a second Cre-dependent virus to the PIL, the role of the PIL neurons projecting to preoptic area was determined. In this experiment, stimulation of the PIL to preoptic area projecting cells, social grooming behavior was increased. In further studies, CNO was injected locally into the preoptic area to selectively stimulate or inhibit the terminals originating in the PIL, an approach, which confirmed the role of this projection in the control of social grooming. In summary, experimental manipulation of activated neurons and PIL neurons projecting to the preoptic area had a significant impact on the social behavior of the animals. Thereby, we discovered a novel neuronal pathway from the PIL to the medial preoptic area (MPOA) involved in control of social grooming. We found that neurons in the PIL and MPOA were naturally activated by physical contact between female rats and also by chemogenetic

stimulation of PIL neurons. We proposed that the discovered neuronal pathway facilitates physical contacts with conspecifics.

Neurons projecting from the PIL to the MPOA express the neuropeptide parathyroid hormone 2 (PTH2, the other name is Tuberoinfundibular peptide of 39 residues-TIP39) and central infusion of its receptor antagonist diminished social grooming. We also described available knowledge on this peptide It acts via its endogenous class B G-protein coupled receptor, the parathyroid hormone 2 receptor (PTH2R). The peptide is expressed in the brain by a small number of neurons with a highly restricted distribution, which in turn project to a large number of brain regions that contain PTH2R. The role of this peptide neuromodulator system was established in the control of nociception, fear and fear incubation, anxiety and depression-like behaviours, and maternal and social behaviours. It also influences thermoregulation and potentially auditory responses. TIP39 probably exerts direct effect on the neuronal networks controlling these behaviours based on the localization of PTH2R and local TIP39 actions. In addition, TIP39 also affects the secretion of several hypothalamic hormones providing the basis for indirect behavioural actions.

We showed similarity in the anatomical organization of the PIL and the distribution of PTH2 receptor in the MPOA between the rat and human brain.

In the third year of the project, gene expressional alterations in the medial preoptic area were investigated in response to social isolation. Correlating gene expression levels and their molecular functions with behavioral analysis is still challenging due to the complexity of behavioral regulation and accompanying physiological changes. The medial prefrontal cortex is involved in the formation of social behavior. We aimed to determine RNA level changes between groups of male rats kept socially or solitarily for 3 weeks. The social behaviour of rats was measured using 3 chamber test and a direct social interaction test. Their anxiety-like behaviour was measured by the elevated plus maze test and the open field test. Forced swimming test was used to assess the depression-like behaviour of the animals. More than 30 genes differed between the groups according to criteria of $\log2FC > \pm1$ and adjusted p-value < 0.05. We measured 5 genes with RT-PCR, which were all validated. These validated genes differentially expressed between the groups were Ndst4, Rgs9, HTr2c, Pdyn and Lrrc10b. The level of these genes decreased as a result of social isolation. Based on the known functions of the genes, HTr2C and Rgs9 are of particular interest as they may play an important role in depression and social behaviour.

In the fourth year of the project, we summarized of results on social behavioural control. The medial preoptic area (MPOA) has long been associated with maternal and male sexual behaviors. Recent advances in neuroscience uncovered the cellular networks involved, while also implicating the MPOA in other social behaviors such as affiliative touch and aggression. These social interactions depend on sensory input from conspecifics, primarily through olfactory and somatosensory modalities in rodents. Notably, these inputs bypass the cerebral cortex and directly influence the MPOA. Hormonal signals also act directly on MPOA neurons. The MPOA, in turn,

regulates social responses through projections involved in reward and motor output, positioning it as a key brain center for instinctive social behavior. While critical components of MPOA circuits have been identified, this synthesis of new findings lays the groundwork for future research to explore the mechanisms through which the MPOA governs social interactions.

Our results were regularly presented at scientific conferences, such as the FENS meeting, Social Behavioural Meeting in Erice, 2023, the International Neuroendocrine Society Meeting, the International Brain Research Organization Meeting and the Regulatory Peptide meeting. Participating university students presented their data at scientific student conferences, and doctoral students on PhD days. We also presented our results to the lay public during Brain Awareness Week and Researchers' Night events.