English summary

The aim of the studies described in this thesis was to investigate different cellular aspects of the outgrowth and connectivity of the brain 5-HT system. The 5-HT system has the remarkable feature that although there are only few 5-HT neurons, localized in the midbrain raphe nuclei, virtually every brain area receives dense 5-HT innervations. These innervations contain several 5-HT varicosities, where 5-HT is released both synaptically and as volume transmission. Due to several 5-HT receptors which are present in the brain, 5-HT has many functions and influences a wide variety of processes. Moreover, the 5-HT system is implicated in several psychopathological processes such as anxiety and depression. Despite the high social and economical importance of these conditions, there is a lot of uncertainty about the involved mechanisms. Therefore, a further cellular characterization of the outgrowth and connectivity of the 5-HT system is of high interest. We used several experimental approaches to study different cellular aspects of the outgrowth and connectivity of the 5-HT system.

In **chapter 2** we used organotypic slice cultures to study 5-HT outgrowth and connectivity and the effect of pharmacological manipulations in vitro. This revealed that the 5-HT neurite density was not affected by blocking the SERT or application of a 5-HT_{1A} agonist, but the density was reduced by application of a 5-HT_2 agonist. This was presumably due to inhibited outgrowth of the 5-HT system.
In **chapter 3**, we investigated the trafficking of SERT. We expressed SERT tagged with the fluorescent protein mCherry in hippocampal neurons. We showed that mCherry-SERT is transported in vesicles which displayed a dynamic trafficking.
In **chapter 4** we used the same approach as in chapter 3, but now we studied the localization and trafficking of Tph2, the rate limiting enzyme in 5-HT synthesis. We tagged Tph2 with EGFP and studied the localization of Tph2-EGFP in hippocampal neurons. In axons Tph2-EGFP displayed a punctate distribution and co-localized with Synaptophysin-mCherry, a marker for synaptic terminals, suggesting that Tph2-EGFP localized to synaptic terminals.
In **chapter 5** we studied the effect of a SNP in the presynaptic gene Piccolo on the trafficking and localization of SERT. Knockdown of Piccolo in neurons did not affect mCherry-SERT trafficking. The SNP in Piccolo possibly affects the localization of SERT, although future research is needed to further resolve this issue.
In **chapter 6** we crossed SERT-Cre mice with floxed Munc18-1 mice to specifically abolish Munc18-1 in SERT expressing neurons. This resulted in early

postnatal lethality and a rapid degeneration of the 5-HT system. We used heterozygous mice to study the effect of removal of one Munc18-1 allele in SERT expressing neurons on behaviour using a phenotyping experiment. Basal behaviour, conditioning and anxiety related behaviour were not affected.

In **chapter 7** we describe our approach to create mice which have Cre expression specifically in 5-HT neurons. Moreover, we show that we have generated transgenic lines with a floxed stop cassette which can be used for spatial regulation of transgene expression upon Cre mediated recombination. In the future, these mice can be used to specifically label 5-HT neurons, or to specifically remove a gene in 5-HT neurons.