Summary

The studies described in this thesis explore the role of 5-HT$_{1B}$ receptors in serotonin (5-HT) function in rats and in genetically modified mice lacking 5-HT$_{1B}$ receptors.

In chapter 1 relevant literature is reviewed. The serotonergic system is complex as 5-HT exerts its function through 14 different receptor subtypes. Serotonergic neurons are located in the raphe nuclei and project to brain structures throughout the mammalian forebrain, including the hippocampus, frontal cortex and striatum. In the mammalian brain, 5-HT$_{1B}$ receptors are expressed either as an inhibitory 5-HT autoreceptor or as a 5-HT$_{1B}$ heteroreceptor. 5-HT$_{1B}$ autoreceptors are localised on serotonergic nerve terminals controlling 5-HT release. 5-HT$_{1B}$ heteroreceptors are present on non-serotonergic neurons, suggesting that this receptor subtype modulates other neurotransmitters and is involved in a variety of functions.

The serotonergic system is an important target in the treatment of psychiatric disorders. Selective serotonin reuptake inhibitors (SSRIs) are widely used in the treatment of depression and anxiety disorders, but a clinical problem is the delayed therapeutic effect. This delayed onset of action suggests that adaptive changes may occur. Previous preclinical studies have indicated a role of both cell body 5-HT$_{1A}$ and terminal 5-HT$_{1B}$ autoreceptors in the effects of SSRIs. Moreover, dysfunction of 5-HT$_{1B}$ receptors has been associated with aggression, impulsivity, alcoholism and drug abuse. More insight in the functional role of 5-HT$_{1B}$ receptors contributes to our understanding of this receptor in psychiatric disorders. A limitation in research on 5-HT$_{1B}$ receptors has been the relative restricted selectivity of 5-HT$_{1B}$ receptor antagonists. Therefore, the generation of 5-HT$_{1B}$ KO mouse has provided an interesting model to study 5-HT$_{1B}$ receptors.

In the first part of this thesis, the role of 5-HT$_{1B}$ receptors in the effects of SSRIs on 5-HT levels was studied by comparing these effects in wildtype mice with mice lacking the 5-HT$_{1B}$ receptor by means of in vivo microdialysis. Activation of 5-HT$_{1B}$ autoreceptors reduces the release of 5-HT. Thus in the absence of 5-HT$_{1B}$ autoreceptors, increased extracellular 5-HT levels and an increased response to SSRIs might be expected. Previous studies have indicated regional effects of SSRIs and therefore the effects of SSRI on 5-HT output were examined in different brain structures. Upon systemic administration of a SSRI, both cell body and terminal 5-HT autoreceptors are activated. To examine the contribution of terminal autoreceptors on 5-HT levels, and to circumvent the effects of raphe 5-HT autoreceptors, a SSRI was locally administered into the brain structures of interest by reversed microdialysis.

Chapter 2 describes a study on the role of 5-HT$_{1B}$ autoreceptors in the effects of SSRIs in the hippocampus. Both systemic and local administration of a SSRI
resulted in an enhanced 5-HT response in 5-HT\textsubscript{1B} knockout mice relative to wildtypes, indicating that terminal 5-HT\textsubscript{1B} autoreceptors in the hippocampus limit the acute effects of SSRIs.

No difference was observed between the two genotypes in response to a lower concentration of the locally applied SSRI. This finding indicates that 5-HT reuptake sites might be increased in the hippocampus of 5-HT\textsubscript{1B} KO mice to compensate for the loss of 5-HT\textsubscript{1B} autoreceptors.

In chapter 3 the effects on 5-HT output of locally administered fluvoxamine into the medial PFC are described. A locally applied SSRI resulted in an augmented response in 5-HT\textsubscript{1B} KO mice relative to wildtype mice. Blockade of 5-HT\textsubscript{1B} receptors with a selective 5-HT\textsubscript{1B} receptor antagonist augmented the effect of a SSRI on 5-HT output in wildtype mice, further supporting that 5-HT\textsubscript{1B} autoreceptors limit the effects of (local) SSRIs in this brain structure.

Chapter 4 describes a study on the role of 5-HT\textsubscript{1B} receptors in the interaction between 5-HT and dopamine in the dorsal striatum. The two genotypes showed an identical dose-response to local administration of fluvoxamine, indicating that 5-HT\textsubscript{1B} receptors do not play a prominent role in the regulation of striatal 5-HT release. In contrast, stimulation of 5-HT\textsubscript{1B} receptors reduced 5-HT levels in wildtype mice, indicating that 5-HT\textsubscript{1B} autoreceptors are functionally present in the striatum. A role of striatal 5-HT\textsubscript{1B} receptors in dopamine outflow could not be confirmed in 5-HT\textsubscript{1B} receptor knockout mice. The putative selective 5-HT\textsubscript{1B} receptor agonist CP93129 increased dopamine outflow in 5-HT\textsubscript{1B} receptor knockout mice to the same extend as in wildtype mice. Therefore, this effect of CP93129 cannot be mediated by striatal 5-HT\textsubscript{1B} heteroreceptors. Possibly the effect of CP93129 on dopamine involves 5-HT\textsubscript{2}, 5-HT\textsubscript{3} or 5-HT\textsubscript{4} receptors. The effect of CP93129 on dopamine outflow was from a neuronal origin (tetrodotoxin dependent), but was not attenuated by 5-HT\textsubscript{2} receptor blockade. The 5-HT releaser fenfluramine and the SSRI fluvoxamine increased 5-HT and dopamine levels, but no difference was observed between the genotypes, further supporting that 5-HT\textsubscript{1B} receptors localised in the striatum do not modulate dopamine outflow in this brain structure.

In the second part of this thesis, studies are presented on the effects of NAS-181, a new selective 5-HT\textsubscript{1B} receptor antagonist, in rat frontal cortex. In chapter 5, the effect on 5-HT levels of NAS-181 was compared with two other 5-HT\textsubscript{1B} receptor antagonists. NAS-181 and GR127935, but not SB224289, attenuated the suppressant effect of a 5-HT\textsubscript{1B} receptor agonist on 5-HT levels. The three antagonists reduced 5-HT levels when given alone, but these effects were absent in the presence of a SSRI, suggesting some partial agonistic properties of these compounds. NAS-181 was found to be a selective 5-HT\textsubscript{1B} receptor antagonist.

In chapter 6, the hypothesis was tested whether the effect of a 5-HT\textsubscript{1B} receptor antagonist depends on extracellular 5-HT levels. Previous studies have shown
that administration of a 5-HT\textsubscript{1B} receptor antagonist alone has no effect on 5-HT levels. Different strategies were used to enhance extracellular 5-HT levels. NAS-181 augmented the effect of a locally applied SSRI. Whereas no additional effect of NAS-181 was observed when 5-HT levels were increased by depolarization induced 5-HT release (with potassium chloride) or by preventing degradation of 5-HT (with a mono-oxidase inhibitor). Interestingly, in the presence of a SSRI, the depolarization induced release of 5-HT was augmented by NAS-181. The data provide some evidence that the effect of a 5-HT\textsubscript{1B} receptor antagonist depends on extracellular 5-HT levels, but strongly suggest that additional 5-HT reuptake inhibition is required to detect any effect of 5-HT\textsubscript{1B} receptor antagonists on 5-HT levels by means of in vivo microdialysis.

The main results are discussed in chapter 7. The main advantage of a knockout model is its selectivity. In addition, the 5-HT\textsubscript{1B} receptor antagonist NAS-181 can be a valuable pharmacological tool to study 5-HT\textsubscript{1B} receptors in rodents. The studies in mice and rats described in this thesis clearly show that 5-HT\textsubscript{1B} autoreceptors limit the acute effects of local administration of a SSRI in the hippocampus and frontal cortex, but not in the striatum. These findings support the idea that blockade of both 5-HT reuptake sites and 5-HT\textsubscript{1B} autoreceptors might be a potential interesting augmentation strategy for treatment with SSRIs. The mice data indicate some evidence for compensatory changes in 5-HT\textsubscript{1B} KO mice, supporting the importance of 5-HT\textsubscript{1B} receptors in 5-HT neurotransmission. In contrast to the well-established role of 5-HT\textsubscript{1B} autoreceptors, relative little is known about 5-HT\textsubscript{1B} heteroreceptors. The previous reported role of 5-HT\textsubscript{1B} receptors in striatal dopamine release could not be confirmed in the 5-HT\textsubscript{1B} KO mice.