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Aims and outline

  Box 1. Volume neurotransmission
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The fundamental importance of the central serotonergic system is reflected in the extensive innervation of brain areas by serotonin (5-HT) nerve fibers and the diversity of the serotonin receptor family. To date, seven 5-HT receptor families with 14 different receptor subtypes are identified. Detailed research on each of the different subtypes is necessary to understand the complexity of the serotonergic system and the functional role of the different receptor subtypes. Dysfunction of the central serotonergic system has been implicated in the pathophysiology of several psychiatric disorders. The serotonergic system is an important target in the treatment of psychiatric disorders like schizophrenia, depression and anxiety. Selective 5-HT reuptake inhibitors (SSRIs) are drugs that enhance 5-HT levels by preventing the reuptake of 5-HT. SSRIs were introduced in the 1980s and since then widely used in the treatment of depression and some anxiety disorders. A major clinical problem is, however, that it requires several weeks before a therapeutic effect is achieved. This delayed onset of action suggests that adaptive changes occur. Pre-clinical studies indicated a role of 5-HT autoreceptors in the delayed of action of SSRIs. 5-HT$_{1B}$ receptors are present in the brain both as autoreceptors, controlling the release of 5-HT at serotonergic nerve terminals, and as heteroreceptors modulating other neurotransmitters, suggesting that this receptor is involved in a variety of functions. Therefore, 5-HT$_{1B}$ receptors may be a potential target to augment treatment with 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 subtype in 5-HT neurotransmission and in its role in psychiatric disorders.

Central serotonergic system

Serotonergic innervation of the forebrain
In the 1950s, the existence of 5-HT in the mammalian brain was first reported (Twarog and Page, 1953). Serotonin is a bioamine and is synthesized from tryptophan, an amino acid originating from the diet. Cell bodies containing 5-HT are located in nine discrete cell clusters, located near the midline of the brainstem (Dahlstrom and Fuxe, 1964). The most caudal raphe nuclei project mainly to the gray matter of the spinal cord, while the 5-HT neurons innervating the forebrain mainly originate in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN). The 5-HT cells in the raphe nuclei innervate virtually all forebrain areas (Steinbusch, 1981) as shown in Figure 1. The DRN is the most extensively studied and is the larger nucleus with about 11,500 serotonergic cells, and the
General introduction

MRN contains about 1100 serotonergic cells. The serotonergic system appears similar in primates and non-primates in the distribution of 5-HT cell bodies and their projection network (Jacobs and Azmitia, 1992). In primates, however, the raphe nuclei are more laterally localised and the DRN is more highly organized. Both neuroanatomical and functional mapping studies have indicated that the projections from the DRN and MRN have distinct patterns of distribution, with some forebrain areas receiving mixed innervation of these nuclei and others receiving relatively selective DRN or MRN inputs. For example, the frontal cortex and striatum appear to be predominantly innervated by the DRN, while the dorsal part of the hippocampus is rather exclusively innervated by the MRN and the ventral hippocampus receives mixed input from both DRN and MRN (see Jacobs and Azmitia, 1992, McQuade and Sharp, 1997). Moreover, serotonergic connections between the raphe nuclei exist. The main source of 5-HT fibers reaching the DRN arises either from the DRN itself or from the MRN (Jacobs and Azmitia, 1992). Inputs to the DRN arise from many sources, from the locus coeruleus (noradrenergic), the substantia nigra (dopaminergic), periaqueductal gray (neuropeptides), the hypothalamus, and the lateral habenula (see Jacobs and Azmitia, 1992). The DRN also receives input from the medial prefrontal cortex (mPFC), other cortical areas and hypothalamic nuclei (Hajos et al., 1999, Peyron et al., 1998).

**5-HT receptors**

5-HT exerts its function through activation of different receptor subtypes. To date, seven different 5-HT receptor families (5-HT\(_{1-7}\)) with 14 subtypes are identified. The 5-HT receptors are classified as 5-HT\(_{1A,1B,1D,1E,1F}\), 5-HT\(_{2A,2B,2C}\), 5-HT\(_{3}\), 5-HT\(_{4}\), 5-HT\(_{5A,5B}\), 5-HT\(_{6}\) and 5-HT\(_{7}\) (Hoyer et al., 1994, Barnes and Sharp, 1999). The 5-HT receptor subtypes display a distinct pattern of distribution within the CNS. 5-HT receptors are all G-protein coupled receptors, except 5-HT\(_{3}\) receptors, which are ion channel coupled. In this chapter 5-HT\(_{1}\) receptors will be discussed, details on other 5-HT receptor subtypes and their function can be found in recent review (Barnes and Sharp, 1999).

**Regulation of 5-HT neurotransmission**

**5-HT\(_{1A}\) autoreceptors**

5-HT is released at nerve terminals through exocytosis, triggered by action potential induced depolarisation. Presynaptically located 5-HT transporters terminate the action of 5-HT (see box 1). After reuptake, extravesicular 5-HT is deaminated by mono-oxidase (MAO), and subsequently oxidated by aldehyde dehydrogenase to its metabolite 5-HIAA. Electrophysiological studies have demonstrated that the neuronal activity of raphe cells is inhibited by 5-HT sensitive receptors on the cell bodies and dendrites of serotonergic cells in the raphe nuclei (Sprouse and Aghajanian, 1987). Activation of these receptors results in inhibition of neuronal activity, and subsequent in diminished 5-HT release at the nerve terminals. It is well known that somatodendritic autoreceptors are of the 5-HT\(_{1A}\) receptor subtype. Selective drugs for 5-HT\(_{1A}\) receptors, like 8-OH-DPAT, have been found to inhibit raphe cell firing (Sprouse and Aghajanian, 1987, Arborelius et al., 1995). In vivo microdialysis studies have clearly shown that activation of 5-HT\(_{1A}\) receptors decreases output of 5-HT in several brain areas, including the dorsal and median raphe nuclei, frontal cortex, hippocampus and striatum and amygdala (Hjorth and Sharp, 1991, Bosker et al., 1996, Bosker et al., 1997a). Moreover, raphe 5-HT neurons may be controlled through postsynaptic 5-HT\(_{1A}\) receptors located in the mPFC (Hajos et al., 1999, Casanovas et al., 1999) and in the amygdala (Bosker et al., 1997b). This long feedback loop may be restricted to specific serotonergic projection areas, since activation of 5-HT\(_{1A}\) receptors in the hippocampus, a brain structure rich in 5-HT\(_{1A}\) receptors, has no effect on 5-HT release in this brain area (Kreiss and Lucki, 1994).
What is the source of 5-HT in the raphe nuclei? Ultrastructural studies reported that axon terminals are observed in the raphe nuclei, but only few terminals make synaptic contacts (Jacobs and Azmitia, 1992). Extracellular 5-HT levels are twice as high in the raphe nuclei as in 5-HT projection areas (Bel and Artigas, 1992). Therefore it is thought that the source of 5-HT in the raphe is mainly from somatodendritic origin, and differs from the 5-HT release at nerve terminals in the projection areas (see Pineyro and Blier, 1999). Nevertheless, stimulation of these receptors by locally applied 5-HT₁₅ receptor agonists reduces 5-HT output in the raphe nuclei, indicating that extracellular 5-HT in the DRN and MRN is modulated by somatodendritic 5-HT₁₅ autoreceptors (Adell et al., 1993, Bosker et al., 1994, Bosker et al., 1996).

5-HT₁₅ autoreceptors
On serotonergic nerve terminals, the release of 5-HT is controlled by inhibitory 5-HT₁₅ autoreceptors. In vitro release studies indicated the existence of a terminal autoreceptor of the 5-HT₁₅ receptor subtype (Engel et al., 1986, Maura et al., 1986, Hoyer and Middlemiss, 1989). The negative feedback on 5-HT output by 5-HT₁₅ autoreceptors was supported by in vivo microdialysis studies using local administration of the 5-HT₁₅ receptor agonist CP93129, a compound that does not cross the blood-brain barrier, into different brain structures, including the hippocampus and the frontal cortex (Hjorth and Tao, 1991, Bosker et al., 1995, Adell et al., 2001). Interestingly, an in vivo electrophysiology study in mice demonstrated that stimulation of 5-HT₁₅ receptors following intravenous applied CP94253, a centrally active compound, increased firing rate of dorsal raphe 5-HT neurons (Evrard et al., 1999). This was unexpected, as previous studies performed in rat found no evidence for such an excitatory control of DRN neuron firing activity through 5-HT₁₅ receptors (Sprouse et al., 1997). Nevertheless, a microdialysis study in rats indicated that stimulation of 5-HT₁₅ receptors by CP93129 reduced 5-HT release in the DRN and even more markedly in the MNR (Adell et al., 2001). These findings are supported by a voltammetry study showing that CP93129 reduced 5-HT release both in the DRN and MRN (Hopwood and Stamford, 2001). The intravenous administration of CP94253 to anaesthetised rats did not affect DRN 5-HT neurons, but showed a biphasic effect on the firing rate of MNR causing an increase after a high dose and decrease after lower doses of CP94253 (Adell et al., 2001). The localisation of 5-HT₁₅ receptors in the raphe is not completely clear, but is presumably on 5-HT nerve terminals. Autoradiographic studies demonstrated binding of 5-HT₁₅ receptors in the raphe nuclei (Boschert et al., 1994), while 5-HT₁₅-like immunoreactivity could not be
Box 1. Volume neurotransmission

Ultrastructural analysis of the serotonergic system demonstrated that 5-HT neurotransmission is predominantly non-synaptic (Bunin and Wightman, 1999). Neurotransmission through synaptic junctions, or wiring transmission, is the classic concept of a neurotransmitter, which is released by exocytosis into the synaptic cleft, activates postsynaptic receptors, and subsequent the neurotransmitter is removed from the synaptic cleft by reuptake sites. Volume neurotransmission, also referred to as paracrine or non-synaptic neurotransmission, can occur when receptors are located extrasynaptically and if released neurotransmitter reaches the extrasynaptic space at sufficiently high concentrations to activate its receptor (Zoli et al., 1999). It has been reported that 5-HT systems have a great spillover of 5-HT out of the synaptic junction (Bunin and Wightman, 1998). Consistent with the notion of volume neurotransmission, 5-HT transporters have been demonstrated along 5-HT cell axons and perisynaptic area, suggesting a broad range of 5-HT uptake sites beyond synaptic junctions in rat brain (Pickel and Chan, 1999, Tao-Cheng and Zhou, 1999). Furthermore, 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptors predominate at extrasynaptic and nonsynaptic sites (Boulenguez et al., 1996, Sari et al., 1999, Riad et al., 2000). In the DRN, some 5-HT varicosities are junctional, but most are non-junctional (Chazal and Ralston, 1987). In most 5-HT projections areas, for example in the frontal cortex, hippocampus and neostriatum, varicosities are also predominantly non-junctional, whereas, for example in the substantia nigra (SN) pars reticulata almost all varicosities are of the junctional type (Moukhles et al., 1997). The relative frequency of junctional and non-junctional synaptic contacts in different 5-HT projection areas indicates region specific actions of 5-HT. Moreover, the non-junctional network of most 5-HT projection areas is in line with the general view that 5-HT acts as a global modulatory system.

detect in the raphe (Sari et al., 1999). Taken together, these findings suggest that 5-HT\textsubscript{1B} receptors localised in the MRN may affect 5-HT release. In addition to 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} autoreceptors, there is substantial evidence that 5-HT\textsubscript{1D} receptors localised in the DRN may act as autoreceptors controlling the release of 5-HT (Pineyro et al., 1995a;Pineyro et al., 1996, Starkey and Skingle, 1994, Davidson and Stamford, 2000, Moret and Briley, 1997). Why are multiple 5-HT\textsubscript{1} receptor subtypes needed for the autoregulation of 5-HT? It has been suggested that 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors, each different located, may have different roles in controlling 5-HT release (Stamford et al., 2000).
5-HT₁B receptors

Species differences
Literature on 5-HT₁B receptors is sometimes confusing due to the use of old nomenclature. Originally, 5-HT₁B receptors were found in species like rat, hamster, mouse, and opossum, while 5-HT₁Dβ receptors were found in human, guinea pig, cow and dog. Cloning of the mouse 5-HT₁B receptor (Voigt et al., 1991, Adham et al., 1992, Maroteaux et al., 1992) revealed a high homology of 96% in the transmembrane domain with the 5-HT₁Dβ receptor (Jin et al., 1992). The receptors are considered as species homologues and are now classified as r5-HT₁B and h5-HT₁B receptors (Hartig et al., 1996). Despite the high homology, rodent 5-HT₁B receptors bind certain β-adrenoceptor antagonists, such as propranolol and pindolol, with a much higher affinity than human 5-HT₁B receptors (Adham et al., 1994). This pharmacological difference is due to only one amino acid difference in the seventh transmembrane domain of the receptor (Oksenberg et al., 1992). For intracellular signal transduction, 5-HT₁B receptors (like all 5-HT₁ receptors) are negatively coupled to adenylate cyclase through Gᵢₒ proteins (Adham et al., 1992).

Distribution
5-HT₁B receptors are expressed throughout the brain, the highest binding densities are found in the basal ganglia, substantia nigra, globus pallidus, and moderate binding densities in the striatum, cortical areas, hippocampal areas, thalamic nuclei and deep cerebellar nuclei (Maroteaux et al., 1992, Bruinvels et al., 1993, Sari et al., 1999). In both the DRN and MRN, mRNA for 5-HT₁B receptors was detected, and 5-HT₁B receptor mRNA levels were markedly reduced following lesion of the serotonergic system, indicating that 5-HT₁B receptors are synthesised in serotonergic neurons within the raphe nuclei (Doucet et al., 1995). But not in all projection areas where binding sites for 5-HT₁B receptors are demonstrated, mRNA for this receptor could be detected. This mismatch between binding sites and mRNA of 5-HT₁B receptors, combined with lesion studies, indicated that 5-HT₁B receptors are present as presynaptic receptors, both on serotonergic and non-serotonergic nerve terminals (Boschert et al., 1994). For example, in the substantia nigra high binding densities are found, but no mRNA for 5-HT₁B receptors, while in the striatum, the projection area of the SN, both 5-HT₁B receptors binding sites and mRNA was demonstrated. Taken together, this mismatch indicated that 5-HT₁B receptors are present on projection neurons from to striatum to the substantia nigra.
**Pharmacology**

Research on 5-HT$_{1B}$ receptors has been complicated because most available ligands are not selective (see Hoyer et al., 1994). The most potent agonists include CP93129, CP94253, RU24969, 5-CT and a potent antagonist is methiothepin, but all these compounds also have (some) affinity for other 5-HT receptor subtypes, particularly for 5-HT$_{1A}$ receptors (Barnes and Sharp, 1999). For example, CP93129 is a potent agonist with a 200 times greater selectivity for 5-HT$_{1B}$ than for 5-HT$_{1A}$ receptors (Macor et al., 1990). A more selective 5-HT$_{1B}$ receptor antagonist is GR127935, although this compound does not discriminate between 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Skingle et al., 1995). SB224289, is also a selective 5-HT$_{1B}$ receptor antagonist (Gaster et al., 1998). Both GR127935 and SB224289 are selective antagonists for both rodent and human 5-HT$_{1B}$ receptors, but these compounds have been found to display partial agonistic properties at h5-HT$_{1B}$ and 5-HT$_{1D}$ receptors in vitro, although there is no in vivo evidence for such properties (Pauwels, 1997, Millan et al., 1999). As outlined above, there is a pharmacological difference between human and rodent 5-HT$_{1B}$ receptors. Recently, a new selective rodent 5-HT$_{1B}$ receptor antagonist, NAS-181, has come available that has been shown to enhance 5-HT synthesis and metabolism (Berg et al., 1998, Stenfors et al., 2000).

Interestingly, 5-HT moduline, an endogenous peptide has been discovered that was found to interact with 5-HT$_{1B}$ receptors in a non-competitive inhibitory way (Massot et al., 1996, Rousselle et al., 1998). In mice lacking 5-HT$_{1B}$ receptors binding of 5-HT moduline is absent, indicating that the 5-HT$_{1B}$ receptor is the specific target for this peptide (Cloez-Tayarani et al., 1997). 5-HT moduline appears capable of altering the reactivity of 5-HT$_{1B}$ receptors making it less sensitive to an agonist. Behavioural studies suggest that 5-HT moduline may be involved in conditions related to anxiety and stress (Grimaldi et al., 1999, Chennaoui et al., 2000).

5-HT$_{1B}$ heteroreceptors

Studies in hippocampal synaptosomes have indicated a modulatory role of 5-HT$_{1B}$ receptors in acetylcholine release (Maura et al., 1986, Bolanos-Jimenez et al., 1995, Sarhan and Fillion, 1999). Furthermore, a microdialysis study has demonstrated an increase of acetylcholine levels following 5-HT$_{1B}$ receptor stimulation with CP93129 in the frontal cortex (Consolo et al., 1996). In the dorsal subiculum, glutamate release has been reported to be inhibited through 5-HT$_{1B}$ receptors localised in the CA1 area of the hippocampus (Boeijinga and Boddeke, 1993, Ait Amara et al., 2001). Microdialysis studies have shown that stimulation of 5-HT$_{1B}$ receptors with locally applied CP93129 results in increased dopamine (DA)
levels in the striatum (Galloway et al., 1993, Benloucif et al., 1993), in the prefrontal cortex (Iyer and Bradberry, 1996), and in the nucleus accumbens (Yan and Yan, 2001). Effects mediated by 5-HT_{1B} receptors are general inhibitory in nature, thus the increases of DA observed following 5-HT_{1B} receptor stimulation are presumably indirectly mediated effects. Electrophysiological studies have reported that activation of 5-HT_{1B} receptors inhibits the release of GABA from terminals that innervate DA neurons in the substantia nigra and the ventral tegmental area, resulting in disinhibition of DA neurons (Johnson et al., 1992, Cameron and Williams, 1994, Morikawa et al., 2000).

**Behavioural pharmacology**

Most studies on the behavioural effects of 5-HT_{1B} receptor stimulation, compared the effects of 5-HT_{1} receptor agonists like TFMPP and mCPP, or mixed 5-HT_{1A/1B} receptor agonists like RU24969 and eltoprazine with selective 5-HT_{1A} receptor agonists like 8-OH-DPAT, or in combination with 5-HT_{1B} receptor antagonists, like cyanopindolol and propranolol. Stimulation of 5-HT_{1B} receptors has been found to be involved in a variety of behaviours. Aggressive behaviour in rodents is reduced by so-called serenics, compounds with high affinity for 5-HT_{1B} receptors such as eltoprazine, a 5-HT_{1A/1B} receptor agonist (Olivier and Mos, 1992). Furthermore, 5-HT_{1B} receptors have been implicated in locomotor activity (Geyer, 1996), thermoregulation, sexual behaviour, feeding behaviour, and anxiety (see for a review Chopin et al., 1994).

**Psychiatric disorders and 5-HT_{1B} receptors**

**5-HT and stress**

It is well known that stress affects the serotonergic system (Chaouloff et al., 1999). For example, the stress-related neuropeptide corticotropin-releasing factor (CRF) modulates the neuronal firing activity of the DRN (Lowry et al., 2000, Kirby et al., 2000) and several in vivo microdialysis studies have shown that stress affects extracellular 5-HT in the hippocampus (Adell et al., 1997, Kirby et al., 1995, Linthorst et al., 2000). The functional responsiveness of 5-HT_{1B} receptors, as measured on 5-HT and acetylcholine release in hippocampal synaptosomes, has been shown to be reduced after acute restraint stress, suggesting that 5-HT_{1B} receptors desensitize after stress (Bolanos-Jimenez et al., 1995). Learned helplessness is a model in which some (but not all) animals that are exposed to inescapable foot shock stress, display learned helpless behaviour when tested 24 hours later in a foot shock avoidance paradigm. The learned helplessness
paradigm models some aspects of stress-related disorders including depression and post-traumatic stress disorder. Increases in 5-HT\textsubscript{1B} receptor mRNA levels in the DRN have been observed in helpless rats, suggesting increased synthesis of 5-HT\textsubscript{1B} autoreceptors (Neumaier et al., 1997). Furthermore, in the cortex of helpless rats, increased 5-HT\textsubscript{1B} receptor binding sites were observed, while cortical levels of extracellular 5-HT were decreased, supporting the hypothesis that a deficit in 5-HT release is associated with learned helpless behaviour (see Moret and Briley, 2000).

**5-HT autoreceptors and the action of SSRIs**

SSRIs are widely used in the treatment of depression; however, it takes several weeks of chronic administration before a therapeutic effect is achieved. This delayed therapeutic onset of SSRIs and other antidepressants can be explained by adaptive changes in 5-HT autoreceptors (see for an extensive review Pineyro and Blier, 1999). At the beginning of treatment with a SSRI, extracellular 5-HT levels are increased. Subsequently, the increased 5-HT levels in the raphe nuclei results in inhibition of 5-HT neuronal firing (Sprouse and Aghajanian, 1987), 5-HT synthesis (Barton and Hutson, 1999) and release (Adell and Artigas, 1991, Hjorth and Auerbach, 1994). After sustained administration of a SSRI, the 5-HT\textsubscript{1A} autoreceptor desensitizes and firing activity is restored in the presence of the SSRI (Blier et al., 1998, Hjorth et al., 2000). A limiting role of 5-HT autoreceptors in the acute effects of SSRIs is supported by several microdialysis studies (see box 2) that have used 5-HT\textsubscript{1A} receptor antagonists (Invernizzi et al., 1997, Hjorth, 1993, Trillat et al., 1998) and 5-HT\textsubscript{1B} receptor antagonists (Rollema et al., 1996, Cremers et al., 2000, Gobert et al., 2000). Moreover, administration of SSRIs combined with both 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor blockade results in a synergistic increase of extracellular 5-HT (Gobert et al., 1997, Dawson and Nguyen, 2000, Sharp et al., 1997). Furthermore, following chronic SSRI treatment changes in 5-HT\textsubscript{1B} receptors have been demonstrated, suggesting a role of 5-HT\textsubscript{1B} receptors in the mechanism of action of SSRIs (O’Connor and Kruk, 1994, el Mansari et al., 1995, Newman et al., 2000). That adaptive changes of 5-HT autoreceptors are implicated in the delayed therapeutic effect of SSRIs in depression is supported by the acceleration of the antidepressant response of SSRIs when combined administration with the 5-HT\textsubscript{1A} autoreceptor antagonist pindolol in patients (see box 3).
Box 2. In vivo brain microdialysis

Microdialysis studies have provided important information on the function of the 5-HT system, and particularly contributed to the understanding of the in vivo effects of antidepressants on extracellular 5-HT (and other neurotransmitters) in different brain structures. The in vivo brain microdialysis technique was developed in the 1980s, initially to study DA neurotransmission (Zetterstrom et al., 1983) and soon followed by studies on extracellular 5-HT (Kalen et al., 1988). In this technique, a small probe with a permeable membrane is inserted in a brain structure of interest while artificial cerebrospinal fluid is perfused through the probe. Neurochemicals that passively diffuse across the membrane, are collected in the perfusion fluid, and are subsequently measured by means of high-pressure liquid chromatography with electrochemical detection (HPLC-ECD). Studies with tetrodotoxin added or Ca$^{2+}$ omitted from the perfusion fluid indicated that extracellular 5-HT is of neuronal origin. Moreover, autoreceptor induced changes in 5-HT output support that the sampled neurotransmitter is directly related to neurotransmission. Therefore, it is assumed that extracellular 5-HT, sampled by means of in vivo microdialysis reflects functional release of 5-HT (Westerink and Timmerman, 1999). The levels of 5-HT in dialysates are in general low (femtomole range), therefore usually samples of 10 to 20 minutes are collected to yield 5-HT levels above detection limit. The amount of 5-HT in dialysis samples is dependent on the surface (active tip) of the dialysis membrane and on the flow rate of the perfusion fluid. In many studies, a SSRI is added to the perfusion fluid to increase 5-HT levels. To study 5-HT$_{1B}$ autoreceptors, adding a SSRI to the perfusion fluid is not desirable, because this increases extracellular 5-HT and subsequently activates 5-HT$_{1B}$ autoreceptors. Microdialysis studies have been performed mostly in rat and guinea pig, but with the generation of mutant mice, the application of the microdialysis technique in mice became of interest. The relative small brain of mice restricts the method to larger brain structures like the frontal cortex, hippocampus or striatum.

Obsessive compulsive disorder

SSRIs are not only used as antidepressants, but are also effective in the treatment of obsessive compulsive disorder (OCD), an anxiety disorder (Hollander, 1998, Blier and de Montigny, 1999). In depression it requires several weeks before a therapeutic effect of SSRI is obtained, while in OCD patients treatment with SSRIs requires higher dosages with an even later therapeutic
onset to obtain an anti-obsessional effect. It was hypothesized that this later therapeutic onset in OCD (about 8 weeks) is necessary to desensitize terminal 5-HT receptors in the orbital frontal cortex (el Mansari et al., 1995). The orbital frontal cortex is thought to be involved in OCD, based on changes in brain activity (glucose metabolism) of OCD patients demonstrated by means of in vivo imaging studies, and moreover, these changes could be restored by chronic treatment with SSRIs (Saxena et al., 1999). Pharmacological studies in humans are limited due to restricted availability of drugs, but anti-migraine drugs such as sumatriptan and zolmitriptan, both 5-HT_{1B/1D} receptor agonists, can be used and have been shown to increase plasma growth hormone (Whale et al., 1999). A role of 5-HT_{1B/1D} receptors in OCD, was suggested by a study indicating that OCD symptoms were worsened in patients following a challenge with sumatriptan (Dolberg et al., 1996), but this could not be confirmed by others using sumatriptan or zolmitriptan (Ho Pian et al., 1998, Boshuisen and den Boer, 2000). Interestingly, children with oppositional defiant disorder (ODD), a disorder associated with aggressive and impulsive behaviour, showed an increased response to sumatriptan on growth hormone (Snoek et al., 2002).

Variations in the 5-HT_{1B} receptor gene
The serotonergic system provides interesting candidate genes, including the 5-HT_{1B} receptor gene, for studies on genetic polymorphism in psychiatric disorders (see for a review Veenstra-VanderWeele et al., 2000). Polymorphisms, or variations, in the 5-HT_{1B} receptor gene may be important in the development of pathophysiology and may be important in determining the response to drugs of abuse. At least three polymorphisms (G861C, T-261G and T371G) in the human 5-HT_{1B} gene are known and in a preliminary family based study the presence of a linkage disequilibrium between a 5-HT_{1B} receptor gene polymorphism (G861C) and OCD was found (Mundo et al., 2000). In a Finnish population, an association between antisocial alcoholism and a 5-HT_{1B} receptor polymorphism (G861C) was suggested (Lappalainen et al., 1998), although such an association of 5-HT_{1B} receptors to antisocial substance abuse was not found by others (Kranzler et al., 2002). Also a postmortem study could not identify a relationship between suicide, major depression, alcoholism or pathological aggression with 5-HT_{1B} receptor binding or the G861C genotype (Huang et al., 1999). Nevertheless, studies on genetic polymorphism need examination of large populations, thus future studies may reveal or exclude involvement of 5-HT_{1B} receptor gene variations in different psychiatric disorders.
Box 3. Augmentation strategies for SSRI-treatment

There is some clinical evidence indicating that co-administration of pindolol, a β-adrenoceptor and 5-HT\textsubscript{1A} receptor antagonist, has an accelerating effect on the treatment with SSRLs (Artigas et al., 2001), although larger controlled studies could not support this finding (McAskill et al., 1998). It is debated whether the effects of pindolol on SSRIs are mediated through blockade of 5-HT\textsubscript{1A} autoreceptors, as a PET study revealed that not more than 40% of 5-HT\textsubscript{1A} autoreceptors are occupied at the pindolol dosages used (Rabiner et al., 2001). Moreover, a preclinical study in guinea pigs suggested that the observed effects on 5-HT output by pindolol might be due through β-adrenoceptor blockade (Cremers et al., 2001). Furthermore, pindolol has been shown to display partial agonistic properties at 5-HT\textsubscript{1A} receptors (Sprouse et al., 2000, Arborelius et al., 2000). Although the findings with pindolol seem conflicting, it indicates the clinical potential of blockade of 5-HT autoreceptors with more selective ligands at the start of treatment with a SSRI may have a beneficial effect in patients with major depression.

5-HT\textsubscript{1B} receptor knockout mice

Due to a lack a selective receptor antagonists, the generation of constitutive 5-HT\textsubscript{1B} receptor knockout mice yielded a new model to study the functional role of 5-HT\textsubscript{1B} receptors. By homologous recombination in embryonic stem cells, homozygous mutant mice lacking both copies of the gene encoding the 5-HT\textsubscript{1B} receptor were generated (see for details Saudou et al., 1994).

**Behavioural phenotype**

Locomotor activity in 5-HT\textsubscript{1B} receptor knockout mice is normal, but a role of the 5-HT\textsubscript{1B} receptors in locomotor activity is supported by the finding that the locomotor response to RU24969, a 5-HT\textsubscript{1A/1B} receptor agonist, is absent in the knockouts (Saudou et al., 1994, Ramboz et al., 1996). In a resident intruder paradigm, 5-HT\textsubscript{1B} receptor knockout mice are more aggressive relative to wildtype mice, which is in line with previous findings that activation of 5-HT\textsubscript{1B} receptors reduces aggressive behaviour in rodents (Saudou et al., 1994, Bouwknecht et al., 2001a). 5-HT\textsubscript{1B} knockout mice display increased exploratory behaviour (Malleret et al., 1999) and response to novelty (Zhuang et al., 1999). Increased impulsivity in 5-HT\textsubscript{1B} receptor knockout mice has been suggested (Brunner and Hen, 1997) and is
supported by findings that 5-HT$_{1B}$ receptor knockouts show more impulsive behaviour in an operant paradigm of decision making and response inhibition (Pattij et al., 2002). The altered exploratory behaviour and the observed increased aggressive and impulsive behaviour, suggest that 5-HT$_{1B}$ receptor knockout mice are more reactive to mild disturbances in their environment. This suggestion is further supported by the finding that physiological reactions to mild disturbances are increased in these knockouts (Bouwknecht et al., 2000a, 2001a). The 5-HT$_{1B}$ receptor knockout mouse has been proposed as a model for substance abuse (Scearce-Levie et al., 1999). 5-HT$_{1B}$ receptor knockout mice show an increased vulnerability to cocaine and an increased propensity to self-administer this drug (Rocha et al., 1998). Increased alcohol consumption is reported in 5-HT$_{1B}$ receptor knockout mice (Crabbe et al., 1996), although this finding could not be replicated by others (Phillips et al., 1999, Risinger et al., 1999, Bouwknecht et al., 2000b, Gorwood et al., 2002).

Serotonergic system of 5-HT$_{1B}$ KO mice

In the absence of inhibitory 5-HT$_{1B}$ autoreceptors, increases in 5-HT levels may be expected. Serotonergic neurons in the DRN of 5-HT$_{1B}$ receptor knockout mice displayed normal firing properties and a normal response to 5-HT$_{1A}$ autoreceptor activation (Evrard et al., 1999). Electrically evoked release of 5-HT was increased in the raphe and hippocampus brain slice of 5-HT$_{1B}$ receptor knockout mice (Pineyro et al., 1995b), but a microdialysis study showed that depolarisation evoked 5-HT release in the hippocampus and cortex was similar in wildtype and knockouts and furthermore, basal 5-HT levels were normal in these brain structures (Trillat et al., 1997). In brain tissue of 5-HT$_{1B}$ receptor knockouts decreased 5-HT levels were found in some brain areas, such as the spinal cord and nucleus accumbens, but not in most other forebrain regions examined, suggesting normal biosynthesis of 5-HT in the main serotonergic projection areas including the cortex and hippocampus (Ase et al., 2000). These normal properties of the 5-HT system may be the result of compensatory changes in 5-HT$_{1B}$ receptor knockout mice. Changes in 5-HT transporters have been observed in some brain structures, together with increased 5-HT innervation in the amygdalo-hippocampal nucleus and the ventral hippocampus of the knockouts, indicating region specific adaptive changes in the serotonergic system to compensate for the loss of 5-HT$_{1B}$ receptors (Ase et al., 2001).

After acute administration of the SSRIs paroxetine and fluoxetine, augmented 5-HT levels were reported in the hippocampus, but not in the striatum of 5-HT$_{1B}$ receptor knockout mice (Malagie et al., 2001, Knobelman et al., 2001a), and in the frontal cortex an augmented response was only found after a lower dose of
paroxetine (Malagie et al., 2001). Possibly, upon systemic administration of SSRIs, the contribution of terminal 5-HT_{1B} receptors on 5-HT output in the striatum and frontal cortex was obliterated by the simultaneous activation of 5-HT autoreceptors in the raphe.

**Aims and outline**

The aim of this thesis is to explore the role of 5-HT_{1B} receptors in 5-HT function, by comparing the neurochemical effects of different serotonergic drugs on extracellular 5-HT and DA levels in wildtype and 5-HT_{1B} receptor knockout mice, and in rats using a selective 5-HT_{1B} receptor antagonist.

In part I of this thesis, in vivo microdialysis studies in wildtype and 5-HT_{1B} receptor knockout mice are described. To examine the functional role of the 5-HT_{1B} autoreceptors in the regulation of 5-HT release, the effects of 5-HT_{1B} receptors stimulation and administration of SSRIs in different brain structures were compared in the two genotypes. The SSRI fluvoxamine was locally administered into serotonergic projection areas to circumvent activation of 5-HT autoreceptor in the raphe nuclei that affect 5-HT output at nerve terminals. The effects of SSRIs, systemic paroxetine and local fluvoxamine, on hippocampal 5-HT levels are described in chapter 2. The role of 5-HT_{1B} receptors in 5-HT output in the mPFC was assessed in chapter 3 by local administration of fluvoxamine and by co-administration of the selective 5-HT_{1B} receptor antagonist NAS-181. In chapter 4, the interaction between 5-HT and DA in the striatum was examined as previous studies in rat indicated that stimulation of 5-HT_{1B} receptors increases DA outflow. To assess, both the role of 5-HT_{1B} autoreceptors on 5-HT output and the role of 5-HT_{1B} heteroreceptors on DA output, 5-HT_{1B} receptors were stimulated directly by a 5-HT_{1B} receptor agonist and indirectly by enhancing 5-HT levels through local administration of a SSRI and a 5-HT releaser.

In part II, the effect of NAS-181, a new selective rodent 5-HT_{1B} receptor antagonist, on extracellular 5-HT was evaluated using microdialysis in rat frontal cortex. In chapter 5, the potency of NAS-181 to antagonize the decrease in 5-HT induced by a 5-HT_{1B} receptor agonist was compared with two other 5-HT_{1B} receptor antagonist, both in the presence and absence of a SSRI. In the study presented in chapter 6, tested the hypothesis whether the effect of a 5-HT_{1B} receptor antagonist depends on extracellular 5-HT levels by using different strategies to increase cortical 5-HT levels combined with administration of NAS-181. In chapter 7, the main findings are discussed and concluded with future directions.