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Distribution of Serotonin Receptors and Transporters in the Human Brain: Implications for Psychosis

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Though this be madness, yet there is method in it.

William Shakespeare

ABSTRACT

The serotonin (5-HT) system is thought to be involved in different psychiatric disorders, including depression and anxiety disorders, and is a major target for the pharmacological treatment of these conditions. The involvement of the 5-HT system in psychosis has been suggested, as 5-HT receptor agonism is a common mechanism of different classes of hallucinogenic drugs and several antipsychotics are 5-HT receptor antagonists.

In this study, the distribution of 5-HT transporters and G-protein-coupled 5-HT receptors in the human brain was characterized using whole hemisphere autoradiographic techniques. In addition, a pilot investigation was performed to compare the densities of 5-HT binding sites in brain tissue from patients who suffered from schizophrenia-like psychosis and control subjects.

We found higher levels of 5-HT transporters in several regions of the greater limbic lobe (subcallosal area, anterior cingulate gyrus, posterior uncus, insular and entorhinal cortices, and the temporal pole) as compared to the isocortex. Higher levels of 5-HT_{1A} receptors were also found in limbic cortices (subcallosal area, temporal pole, hippocampus, and entorhinal cortex) compared to isocortical structures. Dense binding to 5-HT_{1B} receptors was found in the ventral striato-pallidal system of the human brain. 5-HT_{1B} receptor mRNA expression was detected in the striatum with the highest levels in ventral striatal regions. We found no evidence for mRNA expression in the substantia nigra and pallidum, where the highest levels of receptor binding sites were found, in support of the localization of 5-HT_{1B} receptors in axon terminals in these regions. Conversely, high levels of mRNA expression was identified in thalamic nuclei, where binding to 5-HT_{1B} receptors was very low or absent, suggesting the localization of these receptors in thalamic projections. 5-HT_{1B} receptor binding sites and mRNA expression were detected in the isocortex with a region- and layer-specific distribution pattern. 5-HT_{1D} receptors seemed to be confined to the substantia nigra and pallidum, where densities were markedly lower as compared to 5-HT_{1B} receptor densities. The use of a high sensitivity radioligand allowed the detection of 5-HT₄ receptors in low-density regions, including thalamus and raphe nuclei. Our results demonstrate that 5-HT₆ receptors are concentrated in nigrostriatal regions, whereas 5-HT₇ receptors are densely localized in the anterior thalamus, hippocampal formation and the anterior cingulate gyrus, regions involved in the modulation of learning and memory and affective behavior, respectively.

There was a trend towards lower levels of 5-HT transporters in the striatum and the temporal cortex, as well as lower levels of 5-HT_{2A} receptors in cortical regions and 5-HT₇ receptors in the lateral frontal cortex and pulvinar thalamus of psychotic patients compared to controls. Data also indicated lower levels of 5-HT_{1A} and 5-HT_{1B} receptors in the hippocampal formation, and in the ventral pallidum and orbitofrontal cortex, respectively. Future large-scale studies are required to verify these findings.

It can be concluded that the different 5-HT receptors have unique distribution patterns in the human brain, reflecting their different physiological effects. The general localization in regions belonging to limbic cortico-striato-pallido-thalamic circuits is consistent with the documented role for 5-HT in the modulation of mood and emotion, as well as with the suggested involvement of this system in the pathophysiology of psychiatric disorders.

LIST OF PUBLICATIONS

- I. Varnäs K, Hall H, Bonaventure P, Sedvall G. Autoradiographic mapping of 5-HT_{1B} and 5-HT_{1D} receptors in the post mortem human brain using [³H]GR 125743. *Brain Research* 2001; **915**:47-57.
- II. Varnäs K, Hurd YL, Hall H. Regional expression of 5-HT_{1B} receptor mRNA in the human brain. *Synapse* 2005; **56**:21-28.
- III. Varnäs K, Halldin C, Pike VW, Hall H. Distribution of 5-HT₄ receptors in the post-mortem human brain - an autoradiographic study using [¹²⁵I]SB 207710. *European Neuropsychopharmacology* 2003; **13**:228-234.
- IV. Varnäs K, Thomas DR, Tupala E, Tiihonen J, Hall H. Distribution of 5-HT₇ receptors in the human brain: a preliminary autoradiographic study using [³H]SB-269970. *Neuroscience Letters* 2004; **367**:313-316.
- V. Varnäs K, Halldin C, Hall H. Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain. *Human Brain Mapping* 2004; **22**:246-260.
- VI. Varnäs K, Thomas DR, Tupala E, Tiihonen J, Räsänen P, Halldin C, Hirst WD, Hall H. Brain 5-HT₄, 5-HT₆ and 5-HT₇ receptor densities in schizophrenia-like psychosis: A whole hemisphere autoradiographic study. *Manuscript*.

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LIST OF ABBREVIATIONS

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine, serotonin
8-OH-DPAT	8-Hydroxy-2-(di-n-propylamino)tetralin
AC	Adenylyl cyclase
CP 93129	3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo(3,2-b)pyrid-5-one
cpm	Counts per minute
CSF	Cerebrospinal fluid
dpm	Disintegration per minute
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
GABA	Gamma aminobutyric acid
G-protein	Guanine nucleotide binding protein
GR 125743	N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide
GTI	Serotonin-O-carboxymethyl-glycyl-tyrosinamide
LSD	Lysergic acid diethylamide
M100907	(R)-(+)-4-[1-hydroxy-1-(2,3-dimethoxyphenyl)methyl]-N-2-(4-fluorophenylethyl)-piperidine
mRNA	Messenger ribonucleic acid
NAS-181	2-[[3-(morpholinylmethyl)-2H-chromen-8-yl]oxy]methyl]morpholine
NMDA	N-Methyl-D-aspartate
NOS	Not otherwise specified
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PLC	Phospholipase C
PET	Positron emission tomography
RNase	Ribonuclease
SB-207710	(1- <i>n</i> -butyl-4-piperidinyl) methyl-8-amino-7-iodo-1,4-benzodioxane-5-carboxylate
SB-258585	4-Iodo-N-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzene-sulphonamide
SB-269970	3-[2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl]phenol
SPET	Single photon emission tomography
SSC	Standard saline citrate
SSRIs	Selective serotonin reuptake inhibitors
WAY-100635	N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-N-(2-pyridinyl)-cyclohexanecarboxamide trihydrochloride

Abbreviations of brain regions are in accordance to Mai et al. ¹⁶³ and are explained in the figures where they appear.

BACKGROUND

Serotonin (5-hydroxytryptamine, 5-HT) was identified and isolated over 50 years ago when it was characterized as a vasoconstrictive substance in serum ²³¹. Subsequent studies also demonstrated the presence of 5-HT in brain tissue ²⁶⁹, where it has a major role as a neurotransmitter.

The brain 5-HT system has been implicated in a variety of physiological functions, including sleep ²⁷⁰, mood and social interaction ²⁹⁰, cognition ^{43, 180}, feeding behavior ¹⁴⁹, thermoregulation ⁶¹ and pain control ²³⁶. Disturbed 5-HT function has been suggested for several psychiatric disorders, such as depression ⁶⁶, anxiety disorders ^{104, 186, 293} and schizophrenia ¹⁷⁹. In addition disturbed function of the 5-HT system is associated with suicidal behavior ^{204, 296, 297}, aggression dyscontrol ^{153, 154} and impulsivity ⁷³.

The 5-HT system is one of the main targets for the pharmacological treatment of several psychiatric disorders. In particular, the selective serotonin reuptake inhibitors (SSRIs) are used in the treatment of depression and a variety of other conditions, including obsessive compulsive disorder and panic disorder ²⁰¹. Knowledge of the detailed distribution of different 5-HT receptor subtypes in the human brain could be relevant for the understanding of the role of 5-HT in the pathophysiology of psychiatric disorders and for the development of psychoactive drugs targeting the 5-HT system.

There has been considerable interest for the possible involvement of the 5-HT system in psychotic disorders, including schizophrenia. This hypothesis was initially based on findings that the hallucinogenic indoleamine LSD showed 5-HT antagonistic effects ^{98, 285}. The more recent interest in the 5-HT system in relation to psychosis is mainly due to the high affinity of atypical antipsychotics for 5-HT receptors ^{176, 237}.

The main focus of this thesis is the anatomical localization of 5-HT receptors in the human brain and the involvement of the 5-HT system in psychosis. Therefore, the background section will give a brief description of brain 5-HT pathways, the distribution and functional implications of 5-HT receptor subtypes, and summarize some of the evidence linking 5-HT to psychosis. This study has been focused on seven of the main G-protein coupled 5-HT receptor subtypes, for which radioligands are available and which have been implicated in psychiatric disorders.

BRAIN SEROTONIN PATHWAYS

The anatomical localization of 5-HT pathways in the central nervous system was initially delineated in the rat brain by Dahlström and Fuxe⁶², who demonstrated that 5-HT cell bodies are concentrated to the raphe nuclei of the brainstem. Later investigations have found a similar distribution of 5-HT neurons in the human brain^{12, 14, 15, 124}. Nuclei of ascending serotonergic projections are mainly confined to the dorsal and median raphe nuclei in the mesencephalon and rostral pons (Fig. 1). These nuclei project to forebrain structures through two major pathways: a lateral projection through the internal capsule, innervating lateral cortical regions, and a medial projection through the medial forebrain bundle to the hypothalamus, basal forebrain and amygdala. The medial pathway also innervate medial cortical regions and hippocampus through fibers in the cingulum bundle^{12, 124}, see Fig. 1. In addition to the ascending 5-HT pathways, a caudal group of neurons in the lower pons and medulla sends descending serotonergic projections to the brainstem and spinal cord^{12, 124}. Although biochemical data of brain 5-HT concentrations shows large variability between different investigations, it may be concluded from these studies that highest levels of 5-HT are present in the raphe nuclei, substantia nigra, striatum and hypothalamus of the human brain. Lower levels have been detected in cerebellum and cerebral cortex, where 5-HT seems to be concentrated to sensory and limbic cortical regions¹².

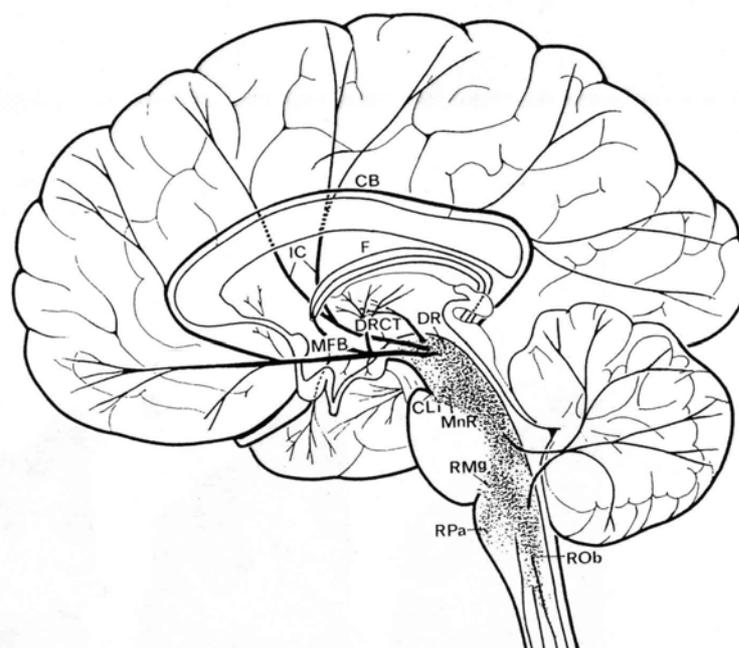


Figure 1. Anatomical localization of the serotonergic neuronal pathways in human brain. Mediosagittal view showing the distribution of serotonin-containing nerve cells within the raphe nuclei and major ascending projections. CB, cingulum bundle; CLi, caudal linear nucleus; DR, dorsal raphe nucleus; DRCT, dorsal raphe cortical tract; F, fornix; IC, internal capsule; MFB, medial forebrain bundle; MnR, median raphe nucleus; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus¹.

¹ Reprinted with permission from the Handbook of Experimental Pharmacology, Anatomy of central serotonergic projection systems, H.G. Baumgarten and Z. Grozdanovic, vol. 129, pp. 41-89, Fig. 1, 1997. © Springer-Verlag Berlin Heidelberg 1997.

5-HT RECEPTORS

In the late 1950s, Gaddum and Picarelli ⁹⁹ demonstrated the presence of two different classes of 5-HT receptors in the guinea-pig ileum. These receptors were referred to as the type D and type M, as their functions were blocked by dibenzylamine and morphine, respectively. The development of the radioligand binding technique in the 1970s made the classification underlying the currently used nomenclature possible. Two distinct 5-HT receptors (5-HT₁ and 5-HT₂) were identified using the radioligands [³H]5-HT, [³H]spiroperidol and [³H]LSD ²¹⁶. Bradley et al. ³⁴ proposed a classification scheme for 5-HT receptor subtypes based on pharmacological criteria. They defined three classes of 5-HT receptors denoted 5-HT₁-like, 5-HT₂ and 5-HT₃, of which the latter two corresponded to D and M types, respectively. Moreover, radioligand binding studies allowed the subdivision of 5-HT₁ receptors into 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subtypes ^{125, 212, 215}. However, the 5-HT_{1C} receptor subtype was later defined as a member of the 5-HT₂ receptor family based on pharmacological and transductional characteristics and termed 5-HT_{2C} ¹³⁰. A fourth 5-HT receptor subtype, with low sensitivity to 5-HT₁, 5-HT₂, and 5-HT₃ receptor ligands was later identified and was denoted 5-HT₄ ⁸¹. These earlier subdivisions were based on functional criteria. Later on, molecular biology studies enabled the cloning of these and several additional subtypes including 5-HT_{1E}, 5-HT_{1F}, 5-HT_{5A}, 5-HT_{5B}, 5-HT₆ and 5-HT₇ ¹²⁷.

To date, seven serotonin receptor families have been identified which are further subdivided into at least 14 distinct receptor subtypes based on pharmacological and structural characteristics, and transductional mechanisms ¹²⁷ (Fig. 2, Table 1). Except for the 5-HT₃ receptor, which is a ligand-gated ion channel, all known 5-HT receptors are G-protein coupled. Members of the 5-HT₁ receptor family are negatively coupled to adenylyl cyclase, 5-HT₂ receptors couple to the hydrolysis of inositol phosphates and 5-HT₄, 5-HT₆ and 5-HT₇ receptors are positively coupled to adenylyl cyclase ¹²⁷.

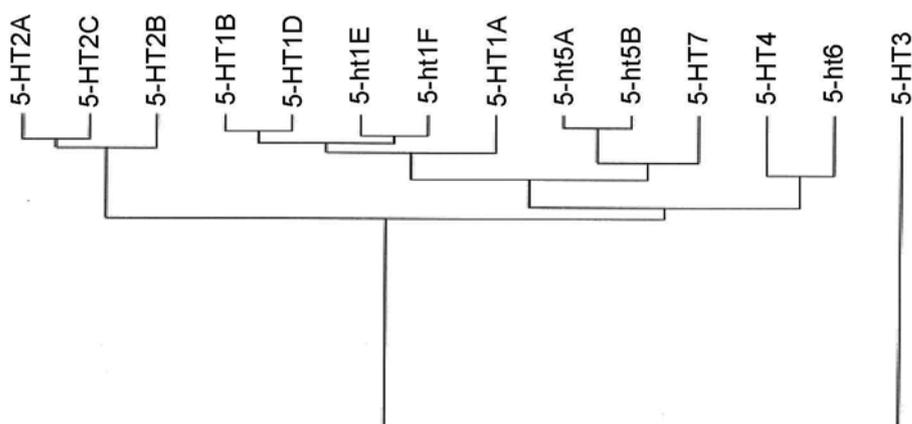


Figure 2. Phylogenetic tree showing relationship between human 5-HT receptor protein sequences (except 5-HT₅ receptors, which are murine in origin). The length of each branch correlates to the evolutionary distance between receptor subtypes ².

² Reproduced from: Neuropharmacology, Vol. 38, N.M. Barnes and T. Sharp, A review of central 5-HT receptors and their function, pp. 1083-1152, © 1999, with permission from Elsevier.

Table 1. Characteristics of 5-HT receptor subtypes (modified from references 127 and 151)

Receptor subtype	Coupling	Agonists	Antagonists	Possible clinical indications (CNS)	Brain regions of highest density	Ref.
5-HT _{1A}	AC (-)	8-OH-DPAT Buspirone	WAY-100635	Anxiety (agonists) ^a , depression (antagonists)	Cortex, hippocampus raphe nuclei	146
5-HT _{1B}	AC (-)	Anpirtoline Sumatriptan	GR 125743 SB 224289 NAS-181	Migraine (agonists) ^a , depression and anxiety (antagonists)	Basal ganglia, cortical regions	191, 243
5-HT _{1D}	AC (-)	PNU-109291 PNU-142633 Sumatriptan	GR 125743	Migraine (agonists) ^a	Basal ganglia	19
5-HT _{1E}	AC (-)	-	-	Unknown	Cortex, striatum, hypothalamus	19, 39
5-HT _{1F}	AC (-)	LY334370 LY344864	-	Migraine (agonists)	Hippocampus, cortex	19, 39
5-HT _{2A}	PLC (+)	DOI DOB DOM	M100907 Eplivanserin Fanaserin	Psychosis (antagonists), depression and anxiety (antagonists)	Isocortical regions	152
5-HT _{2B}	PLC (+)	BW-723C86 Ro-600175	SB-204741 LY-287375 RS127445 S-35526		Cerebellum, lateral septum, dorsal hypothalamus, amygdala	152
5-HT _{2C}	PLC (+)	BVT-933 IL 639 WAY-161503 OrG-12962 Ro-600332	SB-247853 Org-38457 RS 102221	Obesity (agonists), depression and anxiety disorders (antagonists)	Choroid plexus, basal ganglia, hippocampus, ventromedial hypo- thalamus	152
5-HT ₃	Ion channel (Na ⁺ /K ⁺ / Ca ²⁺)	2-Me-5-HT m-chloro- phenyl- biguanide	MDL 72222 Ondansetron Tropisetron Granisetron Zacopride	Emesis (antagonists) ^a	Area postrema, n. tractus solitarius, trigeminal n., n. nervus vagus, > amygdala, hippocampus	23, 58
5-HT ₄	AC (+)	Prucalopride LS-650155 RS 67333 RS 67506	GR 113808 SB-204070 SB-207266 LY353433	Cognitive dysfunction (agonists) Anxiety (antagonists)	Basal ganglia, hippocampus	24
5-HT _{5A}	AC (- ?)	-	-	Unknown	Cortex, hippocampus, cerebellum	193, 209
5-HT ₆	AC (+)	EDMT	Ro 04-6790 Ro 63-0563 SB-271046 SB-258585	Cognitive dysfunction (antagonists)	Striatum > cortical regions	287
5-HT ₇	AC (+)	-	SB-258719 SB-269970 DR-4446	Depression (antagonists)	Thalamus, hypothalamus, hippocampus	267

AC, adenylyl cyclase; PLC, phospholipase C

^a In clinical use as registered drugs

5-HT_{1A} receptors

The brain distribution pattern of 5-HT_{1A} receptors has been extensively characterized. Highest levels are found in the hippocampal formation, superficial layers of the cortex and the raphe nuclei of the human brain^{111, 214}. In general, the regional brain mRNA expression is similar to the distribution pattern of the 5-HT_{1A} receptor protein^{44, 187, 219}, indicative of a somatodendritic localization of this receptor subtype. Presynaptic 5-HT_{1A} receptors are located in serotonergic neuronal cell bodies and dendrites of the raphe nuclei²⁵⁴. This receptor population constitutes autoreceptors controlling the firing rate of 5-HT neurons²⁵⁵. In forebrain regions, 5-HT_{1A} receptors are localized postsynaptically on target neurons. At the cellular level, these receptors are located in cortical pyramidal neurons, in pyramidal neurons of the hippocampus and in granule cells of the dentate gyrus⁴⁴.

The 5-HT_{1A} receptor has been implicated in the pathophysiology of several neuropsychiatric disorders including anxiety, major depressive disorder and schizophrenia. The involvement of this receptor subtype in anxiety is supported by behavioral experiments with animals lacking 5-HT_{1A} receptors^{119, 207, 228}. Furthermore, the 5-HT_{1A} receptor partial agonist buspirone is clinically used in the treatment of generalized anxiety disorder²⁵⁶. PET studies have reported lower levels of 5-HT_{1A} receptors in depressed patients compared to controls^{78, 240} and it has been hypothesized that treatment with tricyclic antidepressants may increase the sensitivity of postsynaptic 5-HT_{1A} receptors²². In addition, the 5-HT₁ receptor antagonist pindolol has been used in combination with SSRIs to accelerate the onset of the antidepressant effect through blockade of somatodendritic 5-HT_{1A} autoreceptors⁶. There is evidence for higher levels of 5-HT_{1A} receptors in *postmortem* brain tissue from patients with schizophrenia compared to controls (for review, see ref. 16), and several atypical antipsychotics display partial agonist activity at 5-HT_{1A} receptors^{8, 47, 136, 196}. The 5-HT_{1A} receptor has also been considered as a target for the treatment of cognitive disorders, including Alzheimer's disease, as antagonists for this receptor subtype may attenuate the memory dysfunction induced by muscarinic receptor antagonists in rodents¹⁸⁸.

5-HT_{1B} receptors

The 5-HT_{1B} and 5-HT_{1D} receptors were previously considered to be species specific variants, in rodents and non-rodents, respectively¹²⁶. Molecular biological studies later showed that genes coding for each one of the two subtypes are expressed in the brain of rodents^{115, 283} and primates, including humans²⁷⁶. 5-HT_{1B} receptors have been shown to be involved in the modulation of the synaptic release of serotonin⁹⁰ and other transmitters^{173, 241}, indicative of their role as terminal auto- and heteroreceptors, respectively.

Due to the limited selectivity of the available pharmacological tools for human 5-HT_{1B} and 5-HT_{1D} receptor subtypes, these are not easily distinguished¹²⁷. Therefore, the distribution patterns of 5-HT_{1B} receptors in the human brain have been characterized using non-selective radioligands. Autoradiographic studies using non-selective radioligands to visualize 5-HT_{1B} receptors in tissue sections covering selected brain regions have found high levels of binding sites in the basal ganglia of the human brain^{25, 40}.

The mRNA expression pattern of 5-HT_{1B} receptors has been extensively described in the rodent^{31, 39, 76} and guinea-pig brain²⁶. While receptor binding sites are present in high levels in the substantia nigra and globus pallidus, mRNA expression is not found in these regions^{26, 31}. Based on the mismatch between the localization of 5-HT_{1B} receptors and their mRNA expression in the rodent brain, it has been suggested that 5-HT_{1B} receptors are located in axon terminals in regions of the basal ganglia³¹. In addition immunohistochemical studies at the light and electron microscopic level have demonstrated that 5-HT_{1B} receptors are concentrated to terminals²⁴². The information available on the 5-HT_{1B} receptor mRNA expression in the human brain is limited, as the studies have been restricted to a few selected regions including striatum, dorsal raphe nucleus and hippocampus^{21, 135, 156}.

The 5-HT_{1B} receptor is implicated in the pathophysiology and treatment of migraine, as 5-HT_{1B/1D} receptor agonists show antimigraine efficacy²⁶⁴. On the other hand, the role of 5-HT_{1B} receptors in psychiatric disorders has not been clearly established. It has been suggested that 5-HT_{1B} receptors may be hypersensitive in depression and anxiety disorders and that compounds targeting 5-HT_{1B} receptors may have a possible role in the treatment of these disorders¹⁹¹. For instance, the densities of 5-HT_{1B/1D} receptors in the cortex⁸⁷ and the 5-HT_{1B} receptor mRNA expression in the dorsal raphe nucleus¹⁹⁴ have been found to be elevated in rats displaying a maladaptive behavior in response to uncontrollable stress in the learned helplessness depression model. In addition, recent studies have demonstrated anxiolytic and antidepressant-like effects of 5-HT_{1B} receptor selective compounds in animal behavioral models^{128, 260}.

Results from pre-clinical studies suggest that stimulation of 5-HT_{1B} receptors may disrupt prepulse inhibition⁸⁰ and modulate dopamine release in the striatum^{32, 241}. This indicates that these receptors may be involved in the mechanisms underlying diseases in which prepulse inhibition is disturbed, such as schizophrenia³⁵. It has recently been demonstrated that a number of antipsychotic drugs show inverse agonist activity for the human 5-HT_{1B} receptor¹⁰, although the clinical implication for this interaction is not clear.

Evidence from rodent studies support a potential role of 5-HT_{1B} receptor antagonists in the treatment of cognitive dysfunction. Thus, improved spatial memory performance has been observed in 5-HT_{1B} receptor knock-out mice¹⁶⁴ and 5-HT_{1B} receptor antagonists improve memory performance in rats^{181, 295}.

5-HT_{1D} receptors

The characterization of the human 5-HT_{1D} receptor has been hampered by the lack of selective ligands (see section above). Therefore, the distribution of this receptor subtype in the human brain has not been clarified. On the other hand the distribution pattern of the 5-HT_{1D} receptor in the rat brain has been mapped using the non-selective 5-HT_{1B/1D} receptor radioligand [¹²⁵I]GTI in the presence of the compound CP 93129, which shows selectivity for rat 5-HT_{1B} versus 5-HT_{1D} receptors³⁸. Low receptor densities were found, with concentration to regions of the basal ganglia of the rat brain³⁸. *In situ* hybridization studies have demonstrated low levels of expression of 5-HT_{1D} receptor mRNA expression in the striatum with no expression in the substantia nigra and

pallidum of the rat ³⁹ and guinea-pig brain ²⁶. Therefore, it is likely that 5-HT_{1D} receptors are located in nerve terminals, similar to the 5-HT_{1B} receptor ^{26,39}.

For the characterization of the localization of 5-HT_{1D} receptors in the human brain, ketanserin has been used to define the binding of 5-HT_{1B/1D} receptor radioligands to 5-HT_{1D} receptors ^{25, 50}. However, this compound shows limited selectivity for 5-HT_{1D} versus 5-HT_{1B} receptors ²⁹². Studies using this methodology have provided indications for the presence of 5-HT_{1D} receptors in the substantia nigra, central gray ^{25, 50}, globus pallidus and spinal cord nuclei ⁵⁰. The available information on the 5-HT_{1D} receptor mRNA expression in the human brain is limited to a few studies indicating expression of mRNA coding for this receptor in the dorsal raphe nucleus ²¹ and the CA3 of the hippocampus ²⁰⁸.

Physiological functions mediated by this receptor subtype are poorly understood as most 5-HT_{1D} receptor-active compounds also interact with the 5-HT_{1B} receptor subtype ¹⁹. Consequently, functional information concerning this receptor subtype in psychiatric disorders is limited. It has been suggested that compounds in the triptan group may mediate some of their effects by interaction with the 5-HT_{1D} receptor ²⁶⁴, and selective 5-HT_{1D} receptor agonists have recently been developed as potential novel antimigraine agents ¹⁷⁵.

5-HT_{1E} and 5-HT_{1F} receptors

mRNA encoding the 5-HT_{1E} receptor has been detected in the striatum, medial hypothalamus and entorhinal cortex of the human brain ³⁹. The localization of the 5-HT_{1E} receptor binding sites on the other hand has not been characterized, due to the lack of selective radioligands.

Expression of 5-HT_{1F} receptor mRNA has been detected in the guinea-pig cerebral cortex, hippocampal formation and amygdala ³⁹. 5-HT_{1F} receptor binding sites as defined by the 5-CT-insensitive component of the specific [³H]sumatriptan binding have been mapped to the human frontal cortex, brainstem nuclei and spinal cord ⁵⁰.

The physiological functions mediated by the 5-HT_{1E} receptor subtype are poorly defined due to the absence of selective pharmacological tools ¹⁴⁶. On the other hand, 5-HT_{1F} receptor selective agonists have been developed as putative novel antimigraine drugs ²²⁷.

5-HT_{2A} receptors

The 5-HT_{2A} receptors are predominantly found in the isocortex, with lower intensities in subcortical structures ^{113, 213}. Within the striatum, 5-HT_{2A} receptors are concentrated to the striosomes, with markedly lower levels in the matrix compartment ¹⁵⁷. At the cellular level, cortical 5-HT_{2A} receptors are primarily localized in pyramidal neurons, but have also been detected in interneurons ^{44, 133, 280}. Moreover, evidence for presence of 5-HT_{2A} receptors has been found in dopaminergic neurons of the ventral tegmental area ^{72, 132, 198} and substantia nigra ¹³², indicating a role in the regulation the activity of dopamine pathways.

There are several lines of evidence supporting a role of 5-HT_{2A} receptors in psychosis. First, interaction with 5-HT_{2A} receptors is a common mechanism of psychotomimetic indoleamines and phenethylamines³. Second, *postmortem* studies have reported lower densities of 5-HT_{2A} receptors in cortical regions of schizophrenic patients compared to controls^{20, 45, 68, 107, 147, 189, 225}. Third, there is evidence for the association of a 5-HT_{2A} receptor polymorphism (102T/C) with schizophrenia¹⁵⁵. Fourth, 5-HT_{2A} receptor agonism disrupts prepulse inhibition²⁵⁰ and the 5-HT_{2A} receptor antagonist M100907 have demonstrated antipsychotic efficacy in animal models²⁵⁹. Finally, several antipsychotics show high affinity for 5-HT_{2A} receptors¹⁷⁶. It has recently been recognized that 5-HT_{2A} receptor-selective compounds may modulate working memory²⁷⁹, which is commonly disturbed in patients with schizophrenia⁸⁹.

5-HT_{2A} receptors have also been implicated in the treatment of depression and anxiety disorders. Several antidepressants are 5-HT_{2A} receptor antagonists and the 5-HT_{2A/2C} receptor antagonist ritanserin has anxiolytic properties⁶⁵.

5-HT_{2B} receptors

The 5-HT_{2B} receptor mRNA is highly expressed in various peripheral tissues, whereas the level of expression in brain tissue is very low^{52, 245}. The brain distribution of 5-HT_{2B} receptors seems to be restricted to a few regions, including cerebellum^{53, 83}, lateral septum, dorsal hypothalamus and medial amygdala⁸³.

Whereas peripheral effects mediated by this receptor subtype^{30, 96, 148} as well as its effects during embryonic development (for review, see ref. 192) have been described, the function of central 5-HT_{2B} receptors is as yet not clear¹⁵². 5-HT_{2B} receptor selective compounds were initially developed for the treatment of different psychiatric diseases, including anxiety disorders, although these compounds are currently not further characterized in clinical studies. It is possible that peripheral side effects and the influence on embryonic development may limit the clinical utility of 5-HT_{2B} receptor selective compounds¹⁵².

5-HT_{2C} receptors

The distribution of the 5-HT_{2C} receptor has been described in autoradiographic and radioligand binding studies using the radioligand [³H]mesulergine. Very high levels of 5-HT_{2C} receptors are present in the choroid plexus with much lower levels in substantia nigra, globus pallidus, hippocampus and ventromedial hypothalamus^{166, 214}. A similar localization pattern has been described using immunohistochemistry². Expression of mRNA coding for this receptor subtype has been found in several brain regions, including the choroid plexus, cortical regions, hippocampal formation, amygdala, substantia nigra, striatum and ventromedial hypothalamus^{158, 210}. The overlapping patterns of mRNA expression and receptor distribution for several brain regions may indicate that 5-HT_{2C} receptors are localized in the somatodendritic compartment in these brain regions^{158, 166, 210, 214}.

The subcellular localization of 5-HT_{2C} receptors has not been extensively clarified¹⁵². However, it has been demonstrated that in the striatum, 5-HT_{2C} receptor mRNA is co-expressed with enkephalin, and with substance P and dynorphin, indicative of the lo-

calization in both striato-pallidal and striato-nigral neurons, respectively²⁷⁵. Moreover, in the substantia nigra pars compacta and reticulata, 5-HT_{2C} receptors seem to be expressed in GABA-ergic neurons, as mRNAs coding for 5-HT_{2C} receptors are co-expressed with glutamic acid decarboxylase but not with tyrosine hydroxylase mRNA within this region⁸⁶.

5-HT_{2C} receptors have been implicated in feeding behavior as 5-HT_{2C} receptor knock-out mice show disturbed feeding control and are overweight²⁶³ whereas 5-HT_{2C} receptor agonists reduce food intake⁵⁴. 5-HT_{2C} receptor agonists are under clinical evaluation as potential appetite suppressants¹⁵². Other potential indications for 5-HT_{2C} receptor selective compounds include depression and anxiety disorders¹⁵².

5-HT₃ receptors

Central 5-HT₃ receptors are concentrated to lower brainstem nuclei with particularly high levels in the area postrema of the human brain^{18, 142, 167, 206, 271}. Densities are markedly lower in forebrain regions, including amygdala and hippocampus^{142, 167, 206, 271}. The brain expression of 5-HT₃ receptor mRNA is highest in limbic cortical regions, including piriform, cingulate, and entorhinal areas²⁶². Immunocytochemistry studies have demonstrated that in cortical regions, 5-HT₃ receptors are mainly localized in GABA-ergic neurons^{23, 134} and are to a high extent co-localized with cholecystokinin²³.

5-HT₃ receptor antagonists are widely used for the treatment of chemotherapy- and radiotherapy-induced and post-operative emesis²⁸⁴. The anti-emetic effect is suggested partly to be centrally mediated by blockade of 5-HT₃ receptors in the area postrema and nucleus tractus solitarius^{58, 284}. Evidence from animal studies indicate that 5-HT₃ receptor antagonists may have a therapeutic potential in anxiety, drug abuse and withdrawal, schizophrenia and cognitive disorders^{58, 284}. Clinical investigations also indicate efficacy of such drugs in the treatment of anxiety disorders and alcohol abuse (for references, see⁵⁸). However the complex dose-response relationship observed in some of these studies may potentially limit the use of 5-HT₃ receptor antagonists in psychiatric disorders⁵⁸.

5-HT₄ receptors

The brain distribution of 5-HT₄ receptors has been described for several species, including human^{27, 74, 234, 272}. Highest densities of 5-HT₄ receptors are found in regions of the basal ganglia with lower levels in the hippocampal formation and the isocortex^{27, 74, 234, 272}. mRNAs encoding the 5-HT₄ receptor are expressed in the striatum but are, in contrast to the receptor proteins, absent from the globus pallidus and the substantia nigra, indicating that 5-HT₄ receptors are localized in terminals of striatal projections to these regions²⁷. In addition, results from lesion studies provide further support for the localization of 5-HT₄ receptors in terminals of striatal projections to the globus pallidus and substantia nigra^{55, 211}. The density of 5-HT₄ receptors is reduced in the putamen of patients with Huntington's disease²³⁴, consistent with their localization in intrinsic striatal neurons. The cellular localization of 5-HT₄ receptors in cortical regions has not been clearly established, but there is evidence from studies using single-cell

reverse transcription PCR that these receptors are expressed in pyramidal neurons of the prefrontal cortex ⁹⁵.

Behavioral animal studies suggest that centrally active 5-HT₄ receptor agonists may have a therapeutic potential in the treatment of cognitive dysfunction ²⁴. 5-HT₄ receptor agonists have been demonstrated to facilitate acetylcholine release in the frontal cortex of rats ⁵⁶, and to improve memory in different animal models (for review see ref. 24). In support of a possible role of these receptors in cognitive dysfunction associated with neurodegenerative disorders, 5-HT₄ receptor densities are reduced in the hippocampus in subjects with Alzheimer's disease ²³⁴. The preclinical effects observed with 5-HT₄ receptor partial agonists have led to the suggestion that such compounds may have a potential as adjunct treatment strategies for the treatment of cognitive symptoms in schizophrenia ²³⁸.

5-HT₅ receptors

Due to the lack of selective pharmacological tools, the 5-HT₅ receptors are the least thoroughly characterized among the known 5-HT receptor families ¹⁹³. mRNA coding for two different 5-HT₅ receptor subtypes has been identified, denoted 5-HT_{5A} and 5-HT_{5B} ¹⁹³. Whereas both these subtypes have been detected in rodents ^{91, 171, 218}, a functional 5-HT_{5B} receptor is not found in humans, where the coding gene sequence is interrupted by stop codons ¹⁰⁶.

5-HT_{5A} receptor mRNA is expressed in various brain regions including cortex, hippocampus, cerebellum, diencephalon and striatum, whereas the 5-HT_{5B} receptor expression seems to be limited to a few regions of the rodent brain including the CA1 field of the hippocampus, habenula and dorsal raphe nucleus ¹⁹³. The mRNA expression of the 5-HT_{5A} receptor in the human brain has been mapped to the cortex, hippocampus and cerebellum ²⁰⁹.

Immunohistochemistry studies focused on restricted brain regions have provided evidence for the localization of 5-HT_{5A} receptors in the suprachiasmatic nucleus, the intergeniculate leaflet and also in the raphe nuclei, where receptors are co-localized with 5-HT immunoreactivity ⁸². This indicates that 5-HT_{5A} receptors may be involved in the serotonergic regulation of circadian rhythms and have a role as an autoreceptor ⁸².

Non-selective radioligands have been used to compare the binding densities in brain tissue from wild-type and 5-HT_{5A} receptor knock-out mice ¹⁰⁵. Using this strategy, evidence for presence of low levels of 5-HT_{5A} receptors was found in the olfactory bulb, cortex and habenula ¹⁰⁵.

The information on the physiological functions mediated by 5-HT₅ receptors is limited as there are no specific compounds targeting these binding sites ¹⁹³. Studies using 5-HT_{5A} receptor knock-out mice have provided some clues about the possible functional roles of these receptors ¹⁰⁵. These mice display increased exploratory activity when exposed to a novel environment and altered response to LSD ¹⁰⁵.

5-HT₆ receptors

The regional localization of 5-HT₆ receptors has been thoroughly described for the rodent brain^{102, 123} and has recently been characterized in selected regions of the human brain^{85, 123}. These binding sites are concentrated to the striatum with lower densities in the hippocampus and isocortex^{85, 123}. In general, the regional mRNA expression pattern as obtained using *in situ* hybridization histochemistry is in agreement with that of the receptor protein, indicating that in most regions 5-HT₆ receptors are localized in the somatodendritic compartment^{85, 102, 274}. Furthermore, immunohistochemistry studies at the light and electron microscopic levels demonstrated that 5-HT₆ receptors are concentrated in dendrites with no labeling in terminals or cell bodies of the striatum and hippocampus of the rat brain¹⁰². Results from double-label *in situ* hybridization studies give evidence for colocalization of 5-HT₆ receptors with mRNAs for substance P, dynorphin and enkephalin in the rat striatum²⁷⁵. Taken together these results indicate that 5-HT₆ receptors are localized in dendrites of striatal GABA-ergic medium spiny neurons projecting to both the substantia nigra and globus pallidus²⁷⁵.

The physiological functions mediated by the 5-HT₆ receptor have not been clearly established. 5-HT₆ receptor antagonists and antisense oligonucleotides have been shown to enhance learning and memory in rodent behavioral models²⁸⁷, indicating that 5-HT₆ receptor antagonists may be considered for the treatment of pathological states characterized by cognitive dysfunction, such as Alzheimer's disease and schizophrenia^{184, 238}. In addition, such treatment has been shown to reduce food intake and body weight^{286, 287}.

As several atypical antipsychotic agents, including clozapine, show high affinity for this binding site, the 5-HT₆ receptor has attracted interest in the field of psychopharmacology^{143, 237}. The 5-HT₆ receptor 267C/T polymorphism has been associated with response to clozapine in treatment-resistant patients^{129, 291}, although these findings were not replicated in another study¹⁷⁰. Results from animal studies suggest that 5-HT₆ receptor antagonists are not likely to be effective as antipsychotics when given as monotherapy in the clinic²²⁴. However, it cannot be ruled out that interactions with 5-HT₆ receptors may contribute to some of the beneficial effects of atypical antipsychotics, such as improvement in cognitive function²³⁸.

5-HT₇ receptors

Until recently there were no selective radioligands available for the autoradiographic mapping of 5-HT₇ receptors. Therefore, previous autoradiographic studies aimed at mapping 5-HT₇ receptor distribution patterns used non-selective radioligands such as [³H]5-CT^{109, 268} or [³H]mesulergine¹⁶⁹ in the presence of non-labeled compounds to mask other binding sites. The preferential 5-HT_{1A} receptor radioligand [³H]8-OH-DPAT has also been used to examine the distribution of 5-HT₇ receptors in brain tissue from 5-HT_{1A} receptor knockout mice²⁸. From these studies, there is general agreement that 5-HT₇ receptors are concentrated to the hippocampal formation, thalamus, hypothalamus and amygdala. In general, the brain mRNA expression pattern is in agreement with that of the receptor distribution^{109, 195}.

At the cellular level, results from immunocytochemistry studies in the rat brain point to the localization of 5-HT₇ receptors in the somatodendritic compartment¹⁹⁵. Specifically, receptors were found to be localized in layer V pyramidal cells and in small oval cells in layers II-III of the cerebral cortex, and in pyramidal cells of the hippocampus¹⁹⁵. Moreover, a study using single-cell RT-PCR of rat prefrontal cortex provided evidence for the localization of 5-HT₇ receptors in both pyramidal cells and GABA-ergic interneurons²⁸⁸.

The function of central 5-HT₇ receptors has not been clearly established, but based on the localization pattern and functional studies using 5-HT₇ receptor-selective antagonists, the receptor has been implicated both in the regulation of circadian rhythms¹⁶⁰ and body temperature¹¹⁰, and in the mechanisms underlying a number of psychiatric disorders including anxiety, unipolar depression and possibly schizophrenia²⁶⁷.

Studies using animal models point to a role for 5-HT₇ receptor antagonists in the treatment of depression²⁶⁷. Genetic knockout or pharmacological antagonism of 5-HT₇ receptors generated an antidepressant-like profile in the forced swim test¹⁰⁸. Furthermore, 5-HT₇ receptor antagonists reduce the amount of REM sleep and increase REM sleep latency²⁶⁶, an effect often observed with antidepressant treatment⁴¹. In addition, chronic treatment with antidepressants down-regulates 5-HT₇ receptors in the rat hypothalamus²⁵². The 5-HT₇ receptor has been implicated in psychotic disorders, as several antipsychotics show high affinity for this receptor subtype²³⁷, and its mRNA expression is altered in schizophrenia⁸⁴.

SCHIZOPHRENIA

Schizophrenia is a devastating psychotic disorder often affecting patients at early ages. It is characterized by a variety of symptoms, which can be divided into positive symptoms including hallucinations, delusions and disorganization, and negative symptoms including affective flattening, anhedonia and avolition⁶⁰. Cognitive deficits have recently been recognized as major features that severely impair the functional outcome of patients with schizophrenia^{89, 190}. Despite the efficiency of the currently used antipsychotic drugs in providing relief from psychotic symptoms, there are several limitations with these agents including inadequate effect against negative symptoms, treatment resistance among a substantial proportion of the patients and severe side effects.

The underlying pathophysiological mechanisms are largely unclear. There is strong evidence for genetic contribution to the vulnerability of the disorder²⁰⁵. In addition, environmental influences affecting the early development of the nervous system may have an important role in the pathogenesis of schizophrenia²³⁰. Structural brain abnormalities, including enlarged lateral and third ventricles and reduced temporal lobe volume, have been found in a majority of reports, consistent with a neurodevelopmental and possibly a neurodegenerative contribution to the pathophysiology of schizophrenia²⁴⁸.

The suggested neurochemical correlates of the disorder are based on pharmacological mechanisms of compounds that mimic or suppress disease symptoms. The dominating hypothesis, based on the common pharmacological mechanism of antipsychotic

drugs, concerns disturbed dopaminergic function^{4, 48, 202}. All currently available antipsychotic drugs antagonize dopamine D₂ receptors and it was demonstrated in the 1970-ies that the clinical potency of antipsychotics is strongly correlated with the *in vitro* affinity for D₂ receptors²⁴⁷. The findings were later verified in PET studies of D₂ receptor binding in the human brain^{92, 93, 199}. The glutamatergic system has also been implicated in this disorder as the glutamate NMDA receptor antagonist phencyclidine induces schizophrenia-like psychoses in healthy subjects and exacerbates psychotic symptoms in schizophrenic patients^{161, 253}. In addition, evidence from *postmortem* studies give support for a glutamatergic dysfunction, as there are several reports on altered levels of glutamate markers in the frontal and temporal cortex in schizophrenia⁶⁷. The involvement of the serotonin system in schizophrenia has been suggested, as 5-HT_{2A} receptor agonists have hallucinogenic properties¹⁰³, whereas several antipsychotic drugs are 5-HT receptor antagonists³⁶. Moreover, *postmortem* studies have found differences in brain densities of 5-HT receptors in schizophrenic patients compared to controls^{16, 71}.

INVOLVEMENT OF THE SEROTONIN SYSTEM IN PSYCHOSIS

Serotonergic effects of hallucinogens

The early hypothesis associating the 5-HT system with psychosis was based on the interaction of the hallucinogenic agent LSD with the serotonergic system and structural similarities of LSD and 5-HT²⁸⁵. It was proposed that the formation of psychotogenic N-methylated indoleamines during 5-HT metabolism could underlie schizophrenia. However, this theory was not supported by subsequent studies, which could not consistently find an association of schizophrenia with endogenous N-methylated indoleamines (for review, see ref. 162).

Later, it became clear that 5-HT₂ agonism is an important mechanism of the hallucinogenic effects of LSD and the phenethylamines, since the 5-HT₂ receptor affinities of these agents are correlated with their hallucinogenic potencies¹⁰³. However, in some aspects, the psychosis induced by LSD is different from characteristic symptoms in schizophrenia and consequently the validity of this model has been questioned³⁶.

Serotonergic abnormalities in schizophrenia

Early studies examined the levels of 5-HT and 5-HIAA in brain tissue or CSF of schizophrenic patients. There have been some reports on lower CSF levels of 5-HIAA in schizophrenia^{7, 33, 100}, although this has not been replicated in several other investigations^{203, 217, 220, 221, 235}. Other studies measuring CSF concentrations of 5-HIAA in patients with schizophrenia have associated 5-HIAA levels with enlarged cerebral ventricles^{203, 221} and to a family history of schizophrenia²⁴⁶. There have been reports on higher levels of 5-HT in regions of the basal ganglia in brain tissue from patients with schizophrenia^{59, 94, 144}. On the other hand, lower 5-HT levels have been indicated in the hypothalamus, hippocampus and brainstem, and lower levels of 5-HIAA were found in the cingulate and frontal cortices²⁸¹. However, most of the findings on the level of 5-

HT or 5-HIAA in brain tissue from patients with schizophrenia compared to controls have not been confirmed in subsequent studies.

More recently, *postmortem* investigations have been performed to compare the densities of 5-HT binding sites in brain tissue from patients with schizophrenia and controls. Among these, the 5-HT_{1A} and 5-HT₂ receptors have been the most thoroughly studied subtypes. Higher density of 5-HT_{1A} receptors has been found in prefrontal and temporal cortical regions^{45, 46, 107, 116, 249, 257} and in the cerebellar vermis²⁵¹ in brain tissue from schizophrenic patients. These findings were not supported by two recent PET studies^{17, 289}, although another investigation found a higher 5-HT_{1A} receptor binding potential in the medial temporal cortex in schizophrenic patients compared to controls²⁶¹. Concerning the 5-HT_{2A} receptor subtype, a majority of studies indicate lower levels in different cortical regions in schizophrenia^{20, 45, 68, 107, 147, 189, 225}. However, other investigations found either higher levels^{138, 277} or no difference in the densities^{70, 233} of 5-HT_{2A} receptors in the cerebral cortex of schizophrenic patients compared to controls. A limitation of these studies was the use of the relatively non-selective radioligands [³H]ketanserin and [¹²⁵I]LSD. [³H]Ketanserin has been shown to label other binding sites including the adrenergic α_1 receptor¹⁴⁵ and tetrabenazine-sensitive binding sites associated with a dihydroxy-phenylacetic acid release system¹⁵⁰, and [¹²⁵I]LSD shows nanomolar affinity for several 5-HT receptors in addition to the 5-HT_{2A} subtype, including 5-HT_{1A}, 5-HT_{2C}, 5-HT₅, 5-HT₆ and 5-HT₇ receptors¹⁹⁷.

Other 5-HT receptors and the 5-HT transporter have been less extensively studied in schizophrenia and data concerning these receptors are limited to a few reports from selected brain regions. There are reports on fewer 5-HT transporter binding sites in the prefrontal cortex^{138, 147} and one study demonstrating higher levels of 5-HT transporters in the striatum¹³⁸. One study gave indications for a trend towards higher 5-HT_{1B} receptor mRNA expression in the hippocampal formation in schizophrenia¹⁵⁶. In contrast, a radioligand autoradiography study using [³H]sumatriptan binding in hippocampal brain sections found no differences for the methiothepin-sensitive component (5-HT_{1B/1D}), whereas the level of methiothepin-insensitive [³H]sumatriptan binding sites (consistent with 5-HT_{1F}) was lower in the hippocampus of schizophrenic patients compared to controls²⁴⁴. Lower 5-HT_{2C} receptor mRNA expression has been found in brain tissue from schizophrenic patients⁴⁹. The few studies comparing the levels of 5-HT₃ and 5-HT₄ receptors in selected brain regions from schizophrenic patients and controls found no evidence for differences between the groups^{1, 69, 244}. There is evidence for reduced levels of 5-HT₆ and 5-HT₇ receptor mRNA expression in the hippocampus and prefrontal cortex, respectively, in schizophrenic patients compared to controls⁸⁴.

Serotonergic effects of antipsychotic drugs

The recent interest for the 5-HT system in the pharmacological mechanism of antipsychotic drugs is mainly due to the particularly high affinity of the second-generation antipsychotics for 5-HT receptors¹⁷⁹. The second-generation antipsychotic drugs are commonly referred to as atypical due to a low frequency of extrapyramidal side effects (EPS) in doses that produce antipsychotic effect, and it has been argued that these agents have other beneficial properties as compared to the classical antipsychotics. In

particular, the prototype atypical antipsychotic clozapine has been considered superior to other antipsychotic drugs as it shows efficacy in treatment resistant patients^{139, 177} and may be more efficacious compared to classical antipsychotics in improving cognitive function^{178, 222}. In addition, a recent meta-analysis demonstrated superior efficacy of some atypical (e.g. clozapine, olanzapine, risperidone) as compared to classical antipsychotics⁶³.

It has been proposed that the *in vitro* affinity ratios of 5-HT_{2A} to D₂ receptors may predict the atypical characteristic of an antipsychotic¹⁷⁶. It was subsequently confirmed in PET studies that clozapine shows high 5-HT_{2A} receptor occupancy and lower D₂ receptor occupancy than previously observed for classical antipsychotics in clinically relevant doses²⁰⁰. However, interaction with the 5-HT_{2A} receptor may not be a prerequisite for atypicality as the substituted benzamides (e.g. remoxipride and sulpiride), which are selective D₂ receptor antagonists²⁹⁸, have also been designated atypical due to their low incidence of extrapyramidal side effects.

Nevertheless, the 5-HT_{2A} receptor has been considered a potential target for the treatment of psychosis and 5-HT_{2A} receptor antagonists have been evaluated as possible antipsychotics in clinical trials. Two open clinical studies indicated beneficial effects of the 5-HT₂ receptor antagonists amperozide¹¹ and ritanserin²⁷⁸ in the treatment of patients with schizophrenia. The selective 5-HT_{2A} receptor antagonist M100907 was developed as a potential antipsychotic drug and reached phase III clinical trials for the treatment of acute schizophrenia⁶⁴. However, the studies were discontinued due to poor effects compared to haloperidol, although significant efficacy was observed as compared to placebo^{64, 168}. 5-HT_{2A} receptor inverse agonists are currently under clinical evaluation for the treatment of schizophrenia¹⁵².

Several antipsychotics are partial agonists for 5-HT_{1A}^{8, 47, 136, 196} and inverse agonists for 5-HT_{1B}, 5-HT_{1D}¹⁰ and 5-HT_{2C} receptor subtypes^{120, 232}. Moreover, some of these compounds interact with 5-HT₃²²⁹, 5-HT₆ and 5-HT₇ receptor subtypes²³⁷, although it is not clear whether binding to these receptor subtypes contribute to the favorable properties of these compounds. The 5-HT₃ receptor antagonist ondansetron have been evaluated for its possible efficacy in schizophrenia, but no conclusive evidence has as yet been provided for an antipsychotic potential of 5-HT₃ receptor antagonists⁵⁸. Antagonists for 5-HT₆ and 5-HT₇ receptor subtypes are not expected to have antipsychotic effects based on results from animal studies^{223, 224}. However, it cannot be ruled out that interactions with these receptors may contribute to some of the other beneficial effects of atypical antipsychotics, such as improvement in cognitive and depressive symptoms. For instance, 5-HT₆ receptor antagonists are under evaluation as potential cognition enhancers in schizophrenia^{184, 238} and 5-HT₇ receptor selective compounds may have antidepressant-like effects^{108, 267}.

AIMS OF THE STUDY

The general aims of this thesis were:

- to characterize the distribution of 5-HT transporters and G-protein-coupled 5-HT receptor subtypes in human brain whole hemisphere sections
- to examine possible differences in the density of brain 5-HT binding sites in psychotic patients compared to controls

Specific aims of the investigations were:

- to analyze the regional mRNA expression and distribution patterns of 5-HT_{1B} and 5-HT_{1D} receptors in the human brain
- to visualize the distribution of 5-HT₄ receptors by means of the high-sensitivity radioligand [¹²⁵I]SB-207710 and to provide high-resolution anatomical correlates for *in vivo* neuroimaging studies using the ¹²³I-labeled analog
- to analyze the distribution of 5-HT₆ and 5-HT₇ receptors in human brain whole hemisphere sections
- to compare the distribution patterns and densities of 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT₄, 5-HT₆ and 5-HT₇) and transporters in brain tissue from patients with schizophrenia-like psychosis and control subjects by the use of whole hemisphere autoradiography.

EXPERIMENTAL PROCEDURES

BRAIN TISSUE

Human brains were obtained *postmortem* at clinical autopsy at the National Institute of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden (Papers I-VI), the Department of Forensic Medicine, University of Oulu, Finland (Papers IV and VI) and the Department of Forensic Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary (Papers IV and VI). The study was approved by the Ethics Committee at Karolinska Institutet and the Swedish Board of Social Welfare. None of the brains exhibited damages or neurological abnormalities. Brains were divided at the mid-sagittal plane and frozen ($-70\text{ }^{\circ}\text{C}$).

For the analysis of receptor densities in schizophrenia-like psychosis, brain tissue from six cases and six control subjects were examined (see Paper VI for a detailed description of controls and cases, including antipsychotic medication). Four of the patients were diagnosed with schizophrenia, one with schizoaffective disorder, and one with psychosis not otherwise specified (NOS) and depression. The groups were matched with respect to hemisphere, sex and origin of tissue, and were not significantly different with respect to age ($p = 0.94$) or *postmortem* interval ($p = 0.43$).

CRYOSECTIONING

The frozen hemispheres were placed into a specially designed rubber box with a specimen holder (see Fig. 3). A semi-liquid gel of carboxymethylcellulose was added into the rubber box and the block was frozen. The rubber box was removed from the frozen block and whole hemispheres were cryosectioned as described^{112, 114} using a heavy-duty cryomicrotome (Leica cryomacrocut CM3600). Briefly, a thin tissue paper and a transparent tape (3M) were placed onto the tissue block, which was subsequently cut into $100\text{ }\mu\text{m}$ horizontal or coronal sections. The tissue cryosections adhered to the paper and were transferred to gelatinized or poly-L-lysine-treated glass plates, dried at room temperature, and then stored with dehydrating agents ($-25\text{ }^{\circ}\text{C}$) until use.

In addition to the above-mentioned whole hemisphere brain tissue, other human brain specimens were obtained at autopsy under approved ethical guidelines. The brains were immediately cut into 1.5 cm -thick coronal blocks, frozen in dry-ice-cooled isopentane, and stored at $-70\text{ }^{\circ}\text{C}$. The tissue was subsequently cut into coronal blocks containing specific brain regions (brainstem, cerebellum, frontal cortex). Tissue blocks were subsequently cut into $20\text{ }\mu\text{m}$ -thick cryosections by using a Jung-Frigocut 2800E cryostat (Leica, Heidelberg, Germany), dried onto Superfrost or poly-L-lysine-treated glass plates, and stored at $-25\text{ }^{\circ}\text{C}$.

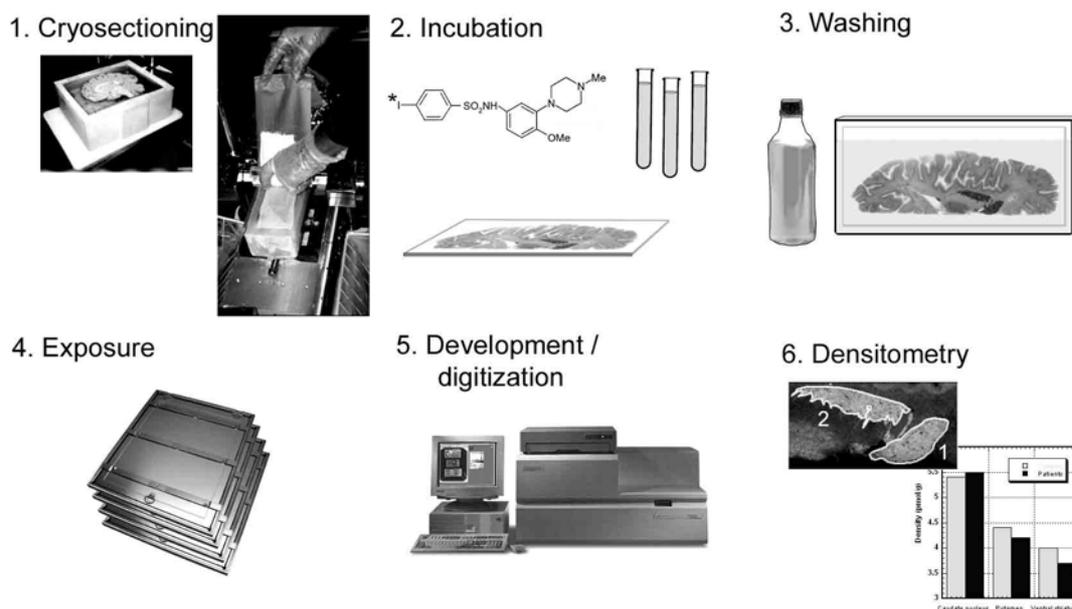


Figure 3. Whole hemisphere autoradiography.

For comparative purposes, male Sprague-Dawley rats (ALAB Sweden) were used for rat brain autoradiography. The experiments were approved by the ethical committee at Karolinska Institutet. Rat brains were removed, frozen in dry ice and cryosectioned into coronal or horizontal sections by using a Jung-Frigocut 2800E cryostat. Sections were dried onto Superfrost-treated glass plates, and stored at -25°C .

RADIOLIGAND AUTORADIOGRAPHY

The autoradiographic experiments were performed essentially as described by Hall et al. ^{112, 114}, see Fig. 3. In Table 2, detailed assay protocols are described for the different binding sites. Sections were pre-incubated to eliminate the endogenous transmitter and remaining drugs in the tissue. Incubations with radioligands were performed at room temperature in Tris-HCl or phosphate buffers (pH 7.4). Incubation volumes were approximately 14 ml for whole hemisphere sections and 2 - 4 ml for the 20 μm -thick sections. The radioligand concentrations were selected based on reported K_i values (see Tables 2 and 3). Non-specific binding was defined in anatomically adjacent sections in the presence of saturating concentrations of a non-labeled structurally unrelated compound with high affinity for the target binding site (Table 2). The concentrations of the competitive agents were selected to occupy more than 100 times the IC_{50} values as calculated by the equation below for competition at a single binding site ⁵¹:

$$K_i = \frac{\text{IC}_{50}}{1 + \frac{[L]}{K_D}}$$

where K_i is the equilibrium dissociation constant of the competitive ligand, IC_{50} is the inhibitory concentration 50%, $[L]$ is the radioligand concentration, and K_D the equilibrium dissociation constant of the radioligand.

Table 2. Assay protocols for autoradiographic studies of 5-HT transporters and receptors

Receptor/ Transporter	Radioligand	Conc.	Preincubation time (min)	Incubation time (min)	Washing time (min)	Definition of non- specific binding
5-HT transporter	[³ H]Citalopram	2 nM	15	90	3 x 10	10 μM fluoxetine
5-HT _{1A}	[³ H]WAY-100635	2 nM	2 x 15	60	2 x 10	10 μM 8-OH-DPAT
5-HT _{1B/1D}	[³ H]GR 125743	2 nM	2 x 15	90	3 x 10	10 μM 5-HT
5-HT _{2A}	[³ H]M100907	2 nM	3 x 10 ^a	60	3 x 5	10 μM ketanserin
5-HT ₄	[¹²⁵ I]SB-207710	17 pM	3 x 5 ^a	60	3 x 10	10 μM 5-HT
5-HT ₆	[¹²⁵ I]SB-258585	50 pM	3 x 5	60	3 x 60	10 μM methiothepin
5-HT ₇	[³ H]SB-269970	4 nM	3 x 10	120	3 x 10	10 μM 5-HT

Incubation buffers: **5-HT transporter** 10.14 mM Na₂HPO₄, 137 mM NaCl, 2.7 mM KCl, 1.76 mM KH₂PO₄; **5-HT_{1A}** 50 mM Tris, 2 mM CaCl₂; **5-HT_{1B/1D}** 170 mM Tris, 4 mM CaCl₂, 0.1 % ascorbic acid, 10 μM pargyline; **5-HT_{2A}** 50 mM Tris, 0.1 % ascorbic acid; 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂; **5-HT₄** 50 mM Tris, 5 mM MgCl₂, 1 mM EGTA, 10 μM pargyline; **5-HT₆** 50 mM Tris, 5 mM MgCl₂, 0.1% ascorbic acid, 0.5 mM EDTA, 10 μM pargyline; **5-HT₇** 50 mM Tris, 4 mM CaCl₂, 1 mM ascorbic acid, 0.1 mM pargyline. Preincubation solutions contained the same salts as incubation buffers, but pargyline was omitted. (^a Preincubation was not performed for distribution studies of 5-HT_{2A} and 5-HT₄ receptors, Papers III and V).

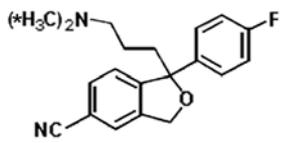
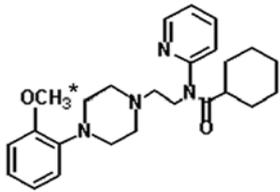
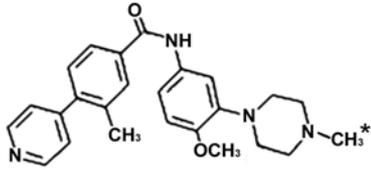
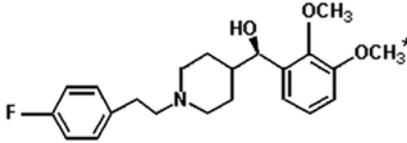
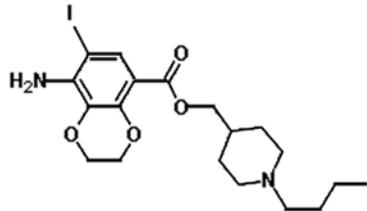
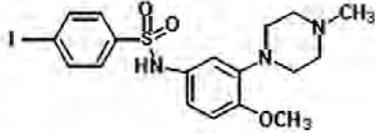
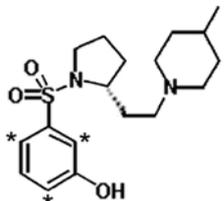
In order to distinguish 5-HT_{1B} and 5-HT_{1D} receptor binding sites, series of consecutive sections were incubated in parallel. In addition to sections defining total and non-specific [³H]GR 125743 binding (Table 2), two adjacent sections were used for the selective labeling of 5-HT_{1B} and 5-HT_{1D} receptors. The separate binding to 5-HT_{1B} and 5-HT_{1D} receptors was defined in the presence of ketanserin (300 nM) and SB-224289 (1 μM), respectively.

To separate bound radioligand from free, several subsequent washes were performed with cold (4 °C) buffer, followed by a brief cold wash by dipping the sections into distilled water to remove excess buffer salts. Sections were rapidly dried to avoid dissociation and diffusion of the bound radioligand before exposure. Detection was performed using autoradiographic films (Kodak BioMax MR, Eastman Kodak Company, Rochester, New York, USA and ³H-Hyperfilm, Amersham; developer Kodak D19) or phosphor imager analysis (Fuji BAS-1500 scanner; imaging plates: BAS IP-TR 2040, Fuji Photo Film, Co., LTD, Japan). Film autoradiograms were digitized using a Scan-Maker high-resolution scanner and Adobe Photoshop.

COMPOUNDS

[³H]Citalopram was obtained from NEN Life Science Products, Inc., Boston. [³H]GR 125743 was obtained from Amersham Pharmacia Biotech, Uppsala. Radiosynthesis of [³H]WAY-100635, [³H]M100907 and [¹²⁵I]SB-207710 is described in Paper V. [¹²⁵I]SB-258585 and [³H]SB-269970 were kindly provided by GlaxoSmithKline, Harlow, UK. See Table 3 for radioligand characteristics. Other compounds and chemicals were obtained from commercially available sources and were of analytical grade wherever possible.

Table 3. Characteristics of radioligands used for whole hemisphere autoradiography studies

Radioligand	Binding site	K _D or K _i	Selectivity	Chemical structure	Ref.
[³ H]Citalopram (Paper V)	5-HT transporter	1.0 nM	> 100-fold		137, 185
[³ H]WAY-100635 (Paper V)	5-HT _{1A}	2.5 nM	> 100-fold		97, 111
[³ H]GR 125743 (Papers I, II, and V)	5-HT _{1B} 5-HT _{1D}	1.02 nM 6.2 nM	100-fold		9, 75
[³ H]M100907 (Paper V)	5-HT _{2A}	0.85 nM	>100-fold		140
[¹²⁵ I]SB 207710 (Papers III, V and VI)	5-HT ₄	79 pM ^a 50 pM ^a	1000-fold		13, 37
[¹²⁵ I]SB-258585 (Paper VI)	5-HT ₆	1.2 nM	>100-fold		85, 122
[³ H]SB-269970 (Papers IV and VI)	5-HT ₇	2.3 nM	100-fold except for 5-HT _{5A} (50-fold)		159, 265

K_D and K_i values are given for human receptors and transporters, as determined using radioligand binding or autoradiography. ^aFor the cloned human 5-HT₄ receptor splice variants 5-HT_{4(a)} and 5-HT_{4(b)}, respectively. Asterisks indicate position of radiolabel for tritiated ligands.

TISSUE THICKNESS AND OPTICAL DENSITY

For tritium autoradiography, tissue sections thicker than 5 μm are considered infinitely thick with regard to the low energy β -radiation emitted¹⁰¹ and the resulting optical density signal is independent on the thickness of the brain section. On the other hand, due to the high-energy radiation of ^{125}I , surface emission will depend on the thickness of the tissue. As the commercially available ^{125}I -standards are calibrated for sections of 20 μm thickness, calibration will result in higher apparent binding densities if values are not adjusted for the section thickness. Experiments were carried out to elucidate the relationship between section thickness and radiation at the surface of tissue. Autoradiography was performed using [^{125}I]SB-207710 (see Table 2 for assay protocol) and rat brain sections of different thickness (10, 20, 50, 100, 200 and 300 μm). Autoradiographic films (Kodak BioMax MR) were applied to the sections for 5 days before development. Regions of interest were taken from the cortex and caudate-putamen, respectively. Quantitative determinations of [^{125}I]SB-207710 binding were achieved by transforming the measured pixel gray levels into apparent binding densities (pmol/g tissue) using ^{125}I -calibrating scales (Microscales, Amersham, U.K.).

There was a linear relationship between the apparent binding density and section thickness for thickness up to 100 μm (see Fig. 4). At higher section thickness, the curve diverged from linearity. The direct linear relationship between the radiation at tissue surface and section thickness enables the quantification of ^{125}I autoradiograms from sections of thickness up to 100 μm . Consequently, linear transformation of the apparent binding density can be applied to adjust for differences in optical density obtained when section thickness (up to 100 μm) is different from that of the standard.

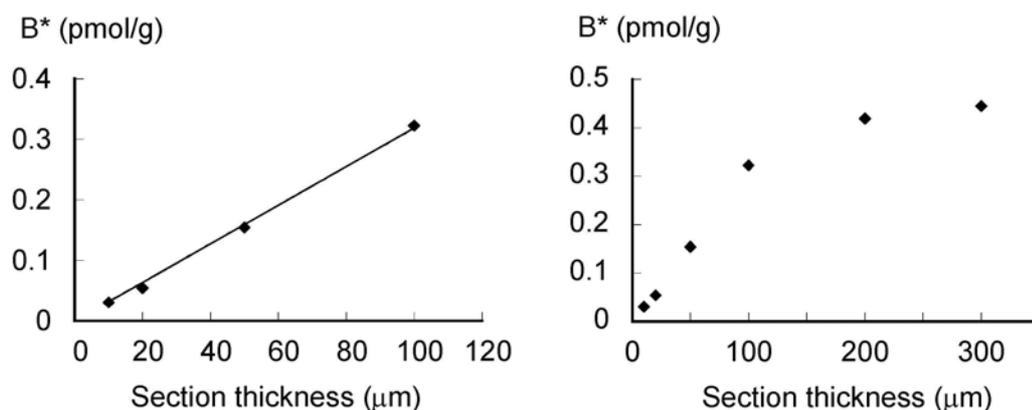


Figure 4. Relationship between section thickness and radiation at tissue surface for autoradiography using ^{125}I -labeled ligands. Measurements are from the cortex of rat brain tissue of different thickness. B^* denotes the apparent binding density, defined as the density extrapolated from values for 20 μm thickness. Representative graphs illustrating a linear relationship ($y = 0.00319x$, $r = 0.999$) between section thickness and apparent binding density for thickness up to 100 μm (left panel). The linear relationship was lost at a higher thickness (right panel).

IN SITU HYBRIDIZATION HISTOCHEMISTRY (PAPER II)

The brain sections were prefixed as previously described¹³¹ in 4% formaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 5 min, rinsed twice in PBS, treated with 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl, for 10 min. Sections were dehydrated in 70%, 80%, 95% and 100% ethanol, delipidated in chloroform for 5 min, rinsed in ethanol (100%, 95%) and air-dried. Solutions were made from autoclaved 0.1% diethylpyrocarbonate-treated water.

Templates for synthesis of 5-HT_{1B} receptor riboprobes were obtained by PCR amplification of cDNA reverse transcribed from human brain mRNA. PCR primers selected from the published sequence (Gene Bank accession number: NM_000863) corresponded to the human 5-HT_{1B} cDNA sequence 603-622 and 765-783 for the upper and lower strand, respectively. Sense and antisense primers were flanked at the 5' ends with T7 and SP6 RNA polymerase promoter sequences, respectively (sense primer: ctgtaatacgactcactataggggaacaccgaccacatcctct, antisense primer: gggatttaggtgacactatagaagggggagtcggttatcagc). ³³P-Antisense and -sense probes were generated by transcription with SP6 and T7 polymerase, respectively. *In vitro* transcription was performed using [α -³³P]uridine triphosphate (New England Nuclear, Boston, MA, USA or Amersham, U.K.) to radiolabel the 5-HT_{1B} receptor riboprobe.

The hybridization was carried out with 2000 cpm labeled probe per mm². Sections were covered with heat-denatured hybridization solution (4X standard sodium citrate (SSC; 1X= 3M sodium chloride, 0.3M sodium citrate, pH 7.0), 10% (w/v) dextran sulfate, 1X Denhardt's (0.02% Ficoll, 0.2% polyvinylpyrrolidone, 0.2 mg/ml bovine serum albumin), 0.5 mg/ml sheared, single-stranded salmon sperm DNA, 250 µg/ml yeast tRNA, 200 mM DTT and 50% formamide). The tissue sections were hybridized overnight at 65°C with 4X SSC/50% formamide humidifying environment. Subsequently, the coverslips were removed and the tissue sections were placed in 2X SSC/1 mM DTT solution and were carried through the following washes: 10 min in RNase A buffer (0.5 M NaCl, 0.04 M Tris (pH 8.0), 1 mM EDTA (pH 8.0)) at 37 °C, 30 min in RNase A (40 µg/ml) plus RNase A buffer at 37 °C, several 5–10 min series of washes in decreasing concentrations of SSC in 1 mM DTT at room temperature, 60 min in 0.5X SSC/1 mM DTT/50% formamide at 48 °C and 60 min in 0.1X SSC/1 mM DTT at 53 °C. Tissue sections were rinsed for 1 min in 0.1X SSC/1 mM DTT at room temperature and dehydrated by ascending 1 min ethanol rinses, 100% ethanol, and air-dried.

The specificity of the hybridization signal was assessed in control experiments with the sense probe or RNase A treatment (100 µg/ml, 30 min at 37 °C in 0.1 M triethanolamine/0.9% NaCl, pH 8.0) prior to the hybridization with the probe. The slides were covered with autoradiography films (Kodak BioMax MR, Eastman Kodak Company, Rochester, New York, USA) in X-ray cassettes for 6 weeks, and developed (D19, Kodak).

IMAGE ANALYSIS

Software for image processing and densitometry included Adobe Photoshop and Image Gauge 3.12 (Fuji Photo Film, Co., LTD). For radioligand autoradiograms regions of interest were taken from images representing total and non-specific binding, respectively, for each brain region. Mean pixel gray levels obtained were transformed into radioactivity values and to binding density (pmol/g tissue, original wet weight) using calibrating scales (Microscales, Amersham, U.K and American Radiolabeled Chemicals Inc., St. Louis, Mo, USA). Specific binding densities were calculated by subtracting the level of non-specific binding from the total binding for each brain region. In Paper VI, binding densities were transformed to B_{\max} values to adjust for concentration differences between experiments according to the equation:

$$B = \frac{B_{\max} \times [L]}{K_D + [L]}$$

where B is the specific binding, [L] the radioligand concentration and K_D the equilibrium dissociation constant of the radioligand. For *in situ* hybridization autoradiograms, mean pixel gray levels were transformed to dpm/mg values by means of ^{14}C -calibrating scales. Background labeling in the surrounding white matter was subtracted from dpm/mg values obtained in gray matter structures. Adjacent sections stained with Cresyl violet were used as anatomical correlates.

STATISTICAL ANALYSIS

The Mann-Whitney U test was used to analyze differences in receptor density between the groups. Correlations of the densities with age and *postmortem* interval were analyzed with Spearman's rank correlation coefficient. No correction for multiple comparisons was performed in this preliminary study. Significance levels presented are given as Mann-Whitney exact p-values. A p-value below 0.050 was considered significant and a p-value less than 0.10 was considered as a trend towards a difference between the groups.

RESULTS AND COMMENTS

5-HT TRANSPORTERS

As previously described^{57, 165}, high levels of [³H]citalopram binding to the 5-HT transporter was found in the raphe nuclei, midline thalamic nuclei, the entorhinal cortex and cingulate gyrus. In addition, this study extended the previous analyses to additional structures of the forebrain (Paper V).

At the cortical level, binding was concentrated to the cingulate gyrus, insular and entorhinal cortices, uncus, the temporal pole and orbitofrontal gyrus (Fig. 5), regions referred to as the greater limbic lobe, according to the definition of Heimer¹¹⁸. Within the cingulate gyrus, the highest levels were seen in the subcallosal area followed by the anterior cingulate, and the binding was lowest in the posterior cingulate region.

The binding was heterogeneous in the striatum, with higher levels in restricted zones in the medial caudate nucleus and the lateral putamen. In the ventral striatum, higher levels of binding were found in medial as compared to lateral parts (Fig. 5A). Dense binding was observed in parts of the olfactory tract (Fig. 5B).

There was a trend towards lower levels of 5-HT transporters in the caudate nucleus and putamen ($p < 0.070$) and in the superior temporal gyrus and the basolateral amygdala ($p < 0.10$) in patients with schizophrenia-like psychosis compared to controls. No trends or significant differences in the densities were found for other regions examined, including frontal and cingulate cortices, thalamus and pallidum.

Our results are contradictory to previous findings of lower levels of 5-HT transporters in the frontal cortex^{138, 147} and higher levels in the striatum¹³⁸ in schizophrenic patients compared to controls. These discrepancies could reflect different characteristics of the subject populations analyzed.

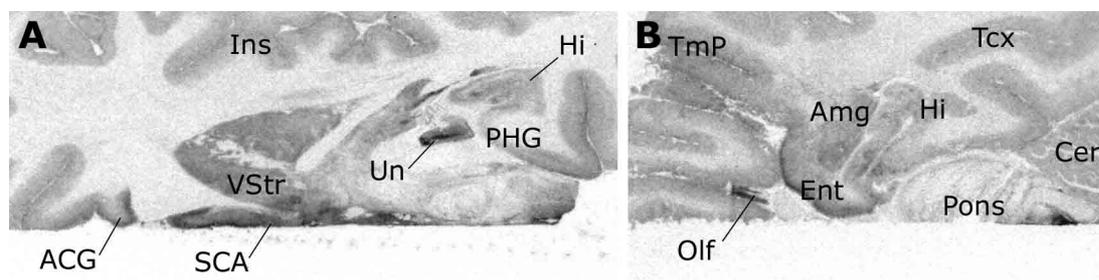


Figure 5. Details of whole hemisphere autoradiograms showing [³H]citalopram binding to the 5-HT transporter at the level of the ventral striatum (A) and pons (B); horizontal sections, 86.7 mm and 109.1 mm, respectively, from the vertex. ACG, anterior cingulate gyrus; Amg, amygdala; Cer, cerebellum; Ent, entorhinal cortex; Hi, hippocampus; Ins, insular cortex; Olf, olfactory tract; PHG, parahippocampal gyrus; SCA, subcallosal area; TmP, temporal pole; Tcx, temporal cortex; Un, uncus; VStr, ventral striatum (A, from Paper V; B, unpublished).

5-HT_{1A} RECEPTORS

Studies examining the distribution of 5-HT_{1A} receptors were performed using the selective radioligand [³H]WAY-100635 (Paper V). In general, our results on the distribution of 5-HT_{1A} receptors are in agreement with earlier studies¹¹¹. Similar to the distribution of the 5-HT transporter, higher densities of 5-HT_{1A} receptor binding sites were found in limbic as compared to isocortical regions. Thus, higher levels were detected in the subcallosal area and the anterior cingulate gyrus compared to the lateral frontal cortex and in the temporal pole as compared to the temporal isocortex (Fig. 6). This is in contrast to previous findings obtained with the radioligand [³H]8-OH-DPAT, which shows equally high binding in the perigenual cingulate as in other regions of the frontal cortex²¹⁴. In agreement with our findings, a study comparing the levels of binding with these two radioligands found that the cingulate gyrus display exceptionally high [³H]WAY-100635 to [³H]8-OH-DPAT binding ratio⁴⁶. The observed discrepancy may result from the different binding characteristics of the radioligands. The antagonist [³H]WAY-100635 does not discriminate between high- and low-affinity states, in contrast to the agonist [³H]8-OH-DPAT, which preferentially labels the high-affinity state, i.e. receptors functionally coupled to G-proteins^{97, 141}. From these results it could be

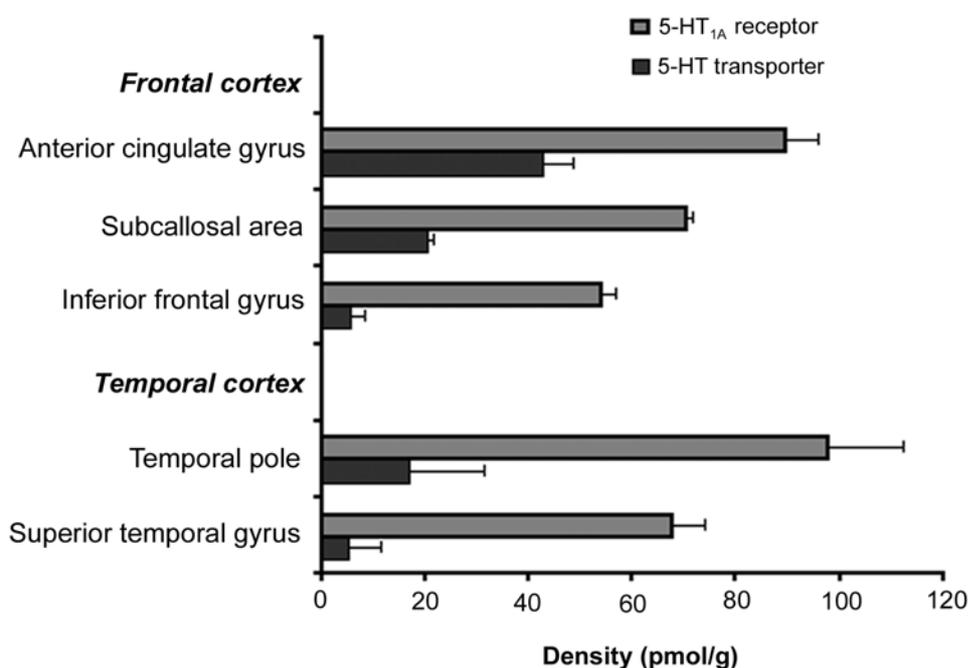


Figure 6. Quantitative analysis of 5-HT_{1A} receptors and 5-HT transporters in external layers of frontal and temporal cortical regions of the human brain. The densities of 5-HT_{1A} receptors (light gray) and 5-HT transporters (dark gray) were defined using the radioligands [³H]WAY-100635 and [³H]citalopram, respectively, and adjacent whole hemisphere sections. Values are given as specific binding, expressed in pmol/g tissue, and are presented as averages and standard errors of the means. Higher levels of 5-HT transporters and 5-HT_{1A} receptors were found in limbic cortices (subcallosal area, anterior cingulate, temporal pole) as compared to isocortical regions (inferior frontal gyrus, superior temporal gyrus) (Data from Paper V).

suggested that 5-HT_{1A} receptors in a low-affinity state, not functionally coupled to G-proteins, are more abundant in the limbic cortices as compared to the isocortex.

High levels of both 5-HT_{1A} receptors and 5-HT transporters were found in anterior and ventral regions of the cingulate gyrus, with the highest levels in the subcallosal area (Fig. 6). Abnormal activity and reduced cortical volume of the subcallosal area has been reported in depressed patients⁷⁷. In addition, this region is activated during sadness in control subjects and its activity seems to be reduced with recovery from depression¹⁷⁴. The high levels of 5-HT transporters and 5-HT_{1A} receptors in the subcallosal area may indicate that these sites are main targets in the pharmacological treatment of depression.

The densities of 5-HT_{1A} receptors were compared in frontal, temporal, insular and cingulate cortices, and in the basolateral amygdala and hippocampal formation of psychotic patients and controls. No significant differences in the densities were found in patients compared to controls, although there was a trend towards lower levels of 5-HT_{1A} receptors in the dentate gyrus ($p = 0.093$) for the patient group.

This study did thus not replicate the previous *postmortem* findings of increased cortical 5-HT_{1A} receptor densities in schizophrenia^{46, 107, 116, 249}. Two recent PET studies using [¹¹C]WAY-100635 found no increases in the levels of 5-HT_{1A} receptors in patients with schizophrenia^{17, 289}, in agreement with our *postmortem* results. As most previous autoradiography studies used the radioligand [³H]8-OH-DPAT, the divergent results could possibly be due to the different binding properties of the radioligands. Alternatively, the contradictory results may reflect the heterogeneous symptom profile of the disorder. For instance, in a recent investigation, the *in vivo* 5-HT_{1A} receptor binding potential was inversely correlated with negative symptoms and depression/anxiety scores in patients with schizophrenia²⁸⁹.

5-HT_{1B} RECEPTORS

Prior to the publication of Papers I and II, the separate distribution of 5-HT_{1B} and 5-HT_{1D} receptor subtypes in the human brain had not been examined in detail due to the lack of selective pharmacological tools to distinguish between human 5-HT_{1B} and 5-HT_{1D} receptors. In addition, the mRNA expression pattern of these receptors in the human brain had not been thoroughly characterized. The aims of this work were therefore to analyze the separate distribution of 5-HT_{1B} and 5-HT_{1D} receptor subtypes (Paper I) and their regional mRNA expression patterns (Paper II) in the human brain. Receptor autoradiography was performed using the non-selective 5-HT_{1B/1D} receptor radioligand [³H]GR 125743 and unlabeled compounds that preferentially occupy either 5-HT_{1B} or 5-HT_{1D} receptor subtypes. The mRNA expression pattern was examined by means of *in situ* hybridization histochemistry using ³³P-labeled riboprobes. In addition, the distribution of the 5-HT_{1B} receptors was compared for the human and rat brain to analyze potential species differences in the distribution of this receptor subtype.

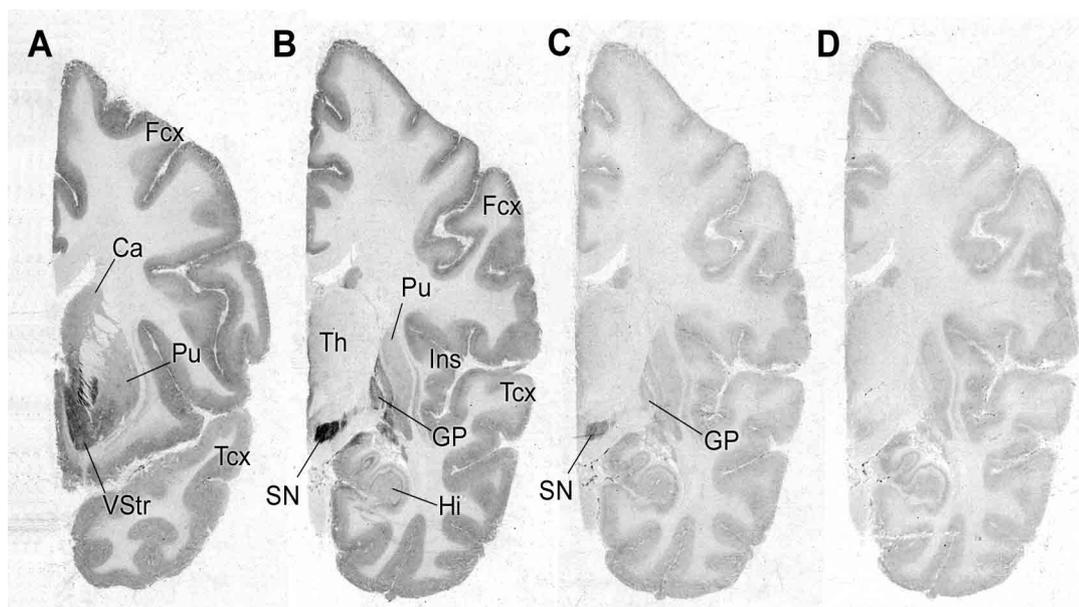


Figure 7. Whole hemisphere autoradiograms illustrating distribution of [^3H]GR 125743 (2 nM) binding to 5-HT $_{1B}$ and 5-HT $_{1D}$ receptors in the human brain. Coronal sections at the level of the striatum (A, 72.6 mm from the frontal pole) and globus pallidus (B, C and D; 93.0 - 93.2 mm from the frontal pole). Binding to the 5-HT $_{1B}$ receptor in the presence of 300 nM ketanserin (A and B), binding to the 5-HT $_{1D}$ receptor in the presence of 1 μM SB 224289 (C), and non-specific binding in the presence of 10 μM 5-HT (D). Ca, caudate nucleus; Fcx, frontal cortex; GP, globus pallidus; Hi, hippocampal formation; Ins, insular cortex; Pu, putamen; SN, substantia nigra; Tcx, temporal cortex; Th, thalamus; VStr, ventral striatum (from Paper I).

General distribution

Results from the autoradiographic studies provide evidence for high levels of 5-HT $_{1B}$ receptors in the pallidum and substantia nigra, with lower levels in the striatum (Fig. 7A and B). The highest level of 5-HT $_{1B}$ receptors was found in the ventral pallidum. Higher levels were found in ventromedial as compared to dorsolateral striatal regions (Fig. 7A). Markedly higher levels of 5-HT $_{1B}$ receptor binding sites were found in the medial occipital cortex (corresponding to the striate area) as compared to other cortical regions (Paper I). Evidence for high levels of 5-HT $_{1B}$ receptor mRNA expression was found in the isocortex, striatum, thalamus and raphe nuclei (Paper II).

Basal ganglia

There was a mismatch for the mRNA expression and the receptor localization in regions of the basal ganglia (Paper II). Whereas both mRNAs and receptors were detected in the striatum, no mRNA expression was found in the substantia nigra and pallidum where very high levels of receptor binding sites were detected (compare Fig. 8A to 8B and Fig. 8C to 8D). However, similar to the receptor distribution pattern, mRNA expression was higher in ventral as compared to dorsal striatal regions. The mismatch for the regional mRNA expression and receptor distribution in the basal ganglia is consistent with the localization of 5-HT $_{1B}$ receptors in terminals of striatal projections to the substantia nigra and pallidum in the human brain. In particular, the

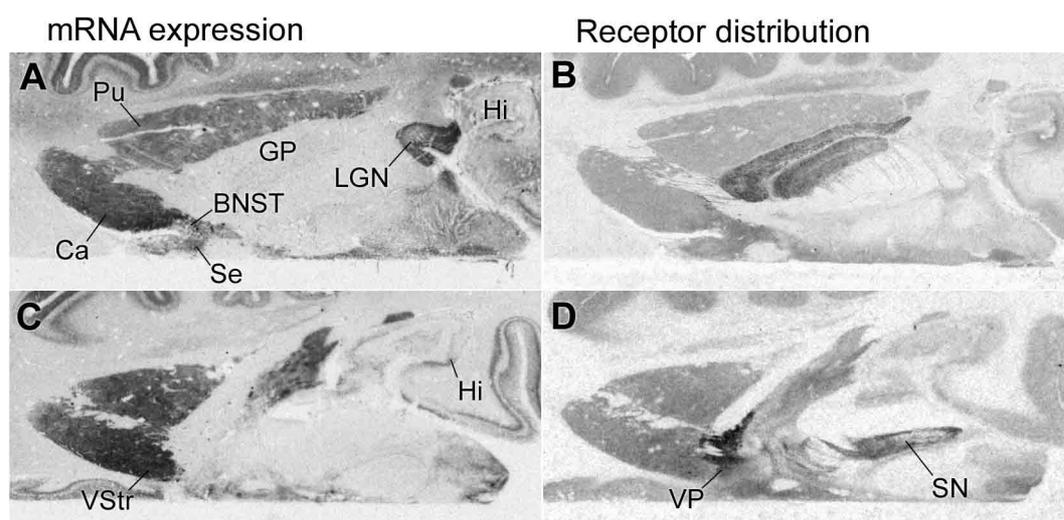


Figure 8. Details of whole hemisphere sections showing regional expression of 5-HT_{1B} receptor mRNA and distribution of the 5-HT_{1B} receptor in adjacent horizontal sections at the level of the globus pallidus (A and B, 80.7 mm and 80.6 mm, respectively, from the vertex) and substantia nigra (C and D, 87.7 and 87.2 mm, respectively from the vertex). (A and C) Sections hybridized with a 5-HT_{1B} receptor antisense mRNA probe. (B and D) [³H]GR 125743 binding in the presence of the 5-HT_{1D} receptor selective compound PNU-142633, 800 nM. BNST, bed nucleus of stria terminalis; Ca, caudate nucleus; GP, globus pallidus; Hi, hippocampus; LGN, lateral geniculate nucleus; Pu, putamen; Se, septal nuclei; SN, substantia nigra; VP, ventral pallidum; VStr, ventral striatum (from Paper II).

results illustrate that the highest level of mRNA expression is found in the ventral striatum. This region projects predominantly to the ventral pallidum¹¹⁷, where the highest levels of receptors were detected.

Thalamus

The distribution patterns also indicated a mismatch for the mRNA expression and receptor distribution in the thalamus. Thus mRNA expression was found in several thalamic nuclei, including lateral geniculate (Fig. 8A), mediodorsal and pulvinar, whereas no or very low levels of receptors were found in these nuclei. The results suggest that 5-HT_{1B} receptors are localized in cortical projections from these thalamic nuclei. The high levels of mRNA expression, but very low levels of receptor binding sites in the lateral geniculate nucleus is consistent with the very high levels of 5-HT_{1B} receptors detected in the visual cortex (Paper I), the main cortical projection of this thalamic nucleus.

Basal forebrain

The bed nucleus of stria terminalis and septal nuclei displayed intermediately high levels of both mRNA expression and receptor densities (Fig. 8A and B). This indicates that 5-HT_{1B} receptors may be localized in intrinsic neurons in these regions.

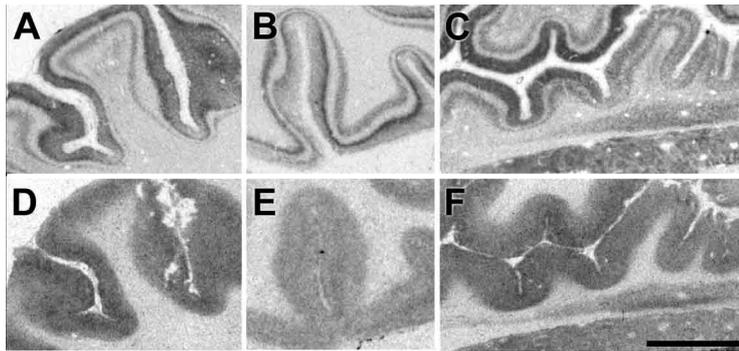


Figure 9. Distribution of 5-HT_{1B} receptor mRNAs (A-C) and 5-HT_{1B} receptors (D-F) in the human cortex. Frontal (A and D), temporal (B and E) and insular cortex (C and F). Distance from the vertex: (A, C, D and F) ca. 81 mm, (B and E) ca. 87.5 mm. Scale bar 10 mm. (B) from Paper II, (A and C-F) unpublished.

Cortex

A distinct, layer-specific mRNA expression pattern was observed in cortical regions with most intense labeling in layer IV, lower levels in layers II-III and VI and no or very low expression in layer V (Fig. 9). In the frontal cortex, higher levels of mRNA expression were also found in external as compared to deep cortical layers (Fig. 9A). A similar tendency was observed for the receptor localization in this region, although the distribution pattern was more homogeneous as compared to the cortical mRNA expression. When comparing densities in external cortical layers, higher levels of both mRNA expression (Fig. 9A and B) and binding sites (Fig. 9D and E) were found in the frontal as compared to the temporal cortex. This rostro-caudal gradient was also evident for the insular cortex, where expression in external layers was higher in anterior as compared to posterior parts (Fig. 9C). The overlapping patterns of mRNA expression and receptor distribution in the isocortex may indicate that 5-HT_{1B} receptors are localized in cortical interneurons.

Species differences

Similar to the distribution pattern in the human brain, high levels of 5-HT_{1B} receptors were found in the rat substantia nigra and pallidum (results not shown). Furthermore, the general localization of 5-HT_{1B} receptor mRNA and binding sites in the basal ganglia is in agreement with the distribution pattern of 5-HT_{1B} receptors in the mouse³¹ and guinea-pig brain²⁶. On the other hand, the localization pattern in cortical regions of the human brain differed from the distribution pattern in the other species. Thus, very high densities of 5-HT_{1B} receptors were found in the rat subiculum, whereas the densities detected in the human hippocampal formation were markedly lower (Fig. 10). In contrast, higher levels of 5-HT_{1B} receptors were found in the isocortex of the human as compared to the rat brain (Fig. 10).

In the guinea-pig cerebral cortex, 5-HT_{1B} receptor mRNA expression is concentrated to deep layers²⁶, while in the human brain intense hybridization signals were also detected in external and middle cortical layers. In addition, moderately high levels of 5-HT_{1B} receptor binding sites were found in the human cortex, in contrast to the guinea-pig brain where levels of 5-HT_{1B} receptors are very low or not detectable²⁶. The 5-HT_{1B} receptor is suggested to be involved in spatial learning and memory, based on

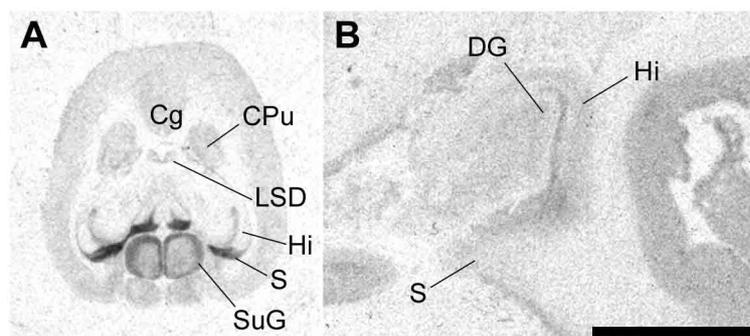


Figure 10. Comparison of the anatomical distribution of [^3H]GR 125743 binding to the 5-HT_{1B} receptor in the rat and human hippocampus. (A) Horizontal rat brain section at the level of the caudate-putamen. (B) Detail from a horizontal human brain whole hemisphere section showing distribution of the 5-HT_{1B} receptor in the hippocampus (91.4 mm from the vertex). Cg, cingulate cortex; CPu, caudate-putamen; DG, dentate gyrus; Hi, hippocampus; LSD, lateral septal nucleus, dorsal; S, subiculum; SuG, superficial gray layer of superior colliculus; scale bar 10 mm (unpublished).

results obtained in rodent models and has been proposed as a novel target for the treatment of conditions characterized by memory dysfunction^{42, 295}. The different distribution patterns of 5-HT_{1B} receptors in the rat as compared to the human cortex as demonstrated in this study may be useful to consider in the future development of 5-HT_{1B} receptor antagonists for the treatment of memory disorders.

Implications for psychiatric disorders

The 5-HT_{1B} receptor is a new potential target for the development of antidepressant drugs¹⁷². Also, the modulation of prepulse inhibition by 5-HT_{1B} receptor agonists⁸⁰ and the inverse agonist activity of some antipsychotics¹⁰ indicate that 5-HT_{1B} receptor antagonists may have antipsychotic properties. The combined pattern of mRNA expression and receptor distribution illustrates that 5-HT_{1B} heteroreceptors are localized in ventral striato-pallidal regions, projections from the mediodorsal thalamus and the bed nucleus of stria terminalis, circuits implicated in the pathophysiology of various neuropsychiatric disorders, including depression, schizophrenia and obsessive compulsive disorder^{79, 183, 258}.

In brain tissue from psychotic patients compared to controls, there were no significant differences in the density of 5-HT_{1B} receptors in the striatum, globus pallidus, substantia nigra, bed nucleus of stria terminalis, dorsal prefrontal cortex, basolateral amygdala or hippocampus. However, there was a trend towards lower levels of 5-HT_{1B} receptors in the lateral orbitofrontal cortex ($p = 0.067$) and the ventral pallidum ($p = 0.082$) of psychotic patients compared to controls. Previous studies of the 5-HT_{1B} receptor in psychosis have been limited to the hippocampal formation^{156, 244}. Our results are in agreement with a recent study, which also failed to show differences in the density of hippocampal 5-HT_{1B} receptors in patients with schizophrenia as compared to controls²⁴⁴. However, another study indicated a trend towards higher 5-HT_{1B} receptor mRNA expression in the hippocampus in schizophrenia¹⁵⁶. Further studies are required to confirm the findings from the present and previous investigations.

5-HT_{1D} RECEPTORS

The combined 5-HT_{1B/1D} receptor radioligand [³H]GR 125743 was used to study the distribution of 5-HT_{1B} and 5-HT_{1D} receptors in the human brain. The density of 5-HT_{1D} receptors was defined by two separate calculation methods: either as the [³H]GR 125743 binding remaining in the presence of the 5-HT_{1B} receptor ligand SB 224289 or as the binding occupied by the 5-HT_{1D} receptor-selective compound ketanserin.

The results demonstrate that 5-HT_{1D} receptors are present in markedly lower levels than 5-HT_{1B} receptors in the human brain (Fig. 7C). Low levels of 5-HT_{1D} receptors were found in the pallidum and substantia nigra and binding was virtually absent in other brain regions examined.

As the selectivities of these compounds are only about 60-70 fold, it is not possible to completely occlude the binding to the one of the receptor subtypes without affecting binding to the other subtype. Therefore the recently developed compound PNU-142633 (800 nM) which shows over 3000-fold selectivity for 5-HT_{1D} versus 5-HT_{1B} receptors was also applied in later studies of the 5-HT_{1B} and 5-HT_{1D} receptor subtypes. Results from these studies are in agreement with the earlier findings with the less specific ligands described above. Thus, by using this method, only very low levels of 5-HT_{1D} receptors were found in the substantia nigra and the pallidum.

Results from preliminary *in situ* hybridization studies indicated that 5-HT_{1D} receptor mRNA is expressed in low levels in a few regions including the raphe nuclei, striatum and external layers of the temporal cortex and the cerebellar cortex (Fig. 11). However, future studies are required to verify the distribution of the 5-HT_{1D} receptor mRNA in the human brain.

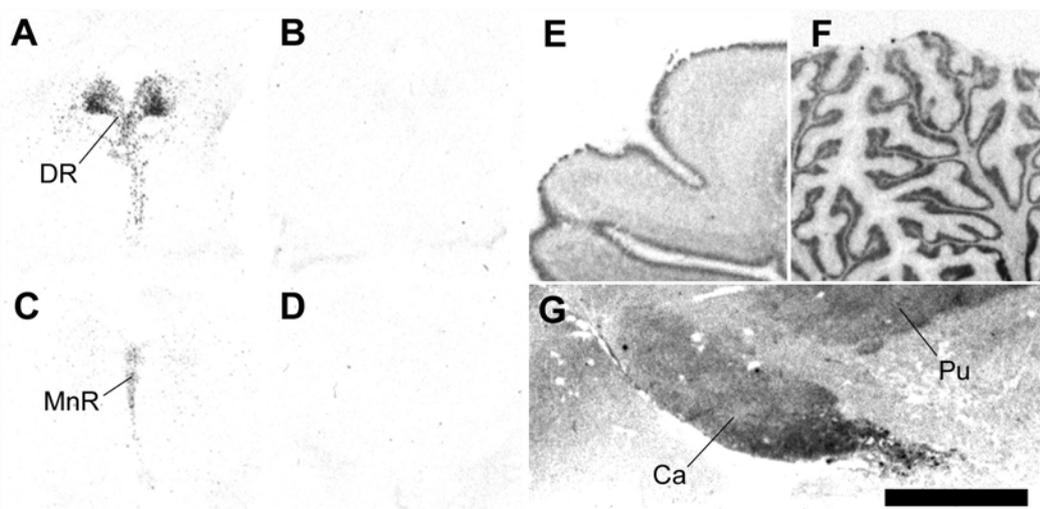


Figure 11. Autoradiograms showing hybridization of 5-HT_{1D} receptor antisense (A, C, E-G) and sense (B, D) mRNA probes to human brain tissue. (A and B) Dorsal raphe nucleus. (C and D) Median raphe nucleus. (E) Temporal cortex. (F) Cerebellar cortex. (G) Striatum. (A-D), 20 μ m and (E-G), 100 μ m sections, 100.1 mm (E and F) and 80.9 mm (G), respectively, from the vertex. Ca, caudate nucleus; DR, dorsal raphe nucleus; MnR, median raphe nucleus; Pu, putamen; scale bar 10 mm (unpublished).

The expression of 5-HT_{1D} receptor mRNA in the striatum and presence of binding sites in the substantia nigra and pallidum may indicate that these receptors are localized in terminals of striato-nigral and -pallidal projections of the human brain. As no evidence for 5-HT_{1D} receptor binding sites were found in the temporal cortex and cerebellum, where mRNAs coding for this receptor were detected, it is possible that some 5-HT_{1D} receptors remain undetectable by the current methodology. A selective 5-HT_{1D} receptor radioligand is therefore required to confirm the localization of 5-HT_{1D} receptors in the human brain.

5-HT_{2A} RECEPTORS

As has been described previously ¹¹³, high levels of 5-HT_{2A} receptors were found in isocortical regions, with lower densities in subcortical structures. Densities were markedly lower in the entorhinal cortex as compared to the isocortex (Paper V).

Most previous autoradiographic studies have indicated reduced levels of 5-HT_{2A} receptors in schizophrenia ^{20, 45, 68, 107, 147, 189, 225}. Some other investigators have found either increased ^{138, 277}, or unchanged ^{70, 233} 5-HT_{2A} receptor densities in the cerebral cortex of schizophrenic patients compared to controls. However, as previous studies used relatively non-selective radioligands ^{145, 150, 197}, the observed differences could reflect changes in densities of other receptor subtypes. To clarify this, we used the selective 5-HT_{2A} receptor radioligand [³H]M100907 to investigate the densities of 5-HT_{2A} receptors in brain tissue from psychotic patients and controls.

Lower levels of 5-HT_{2A} receptors were found in cortical regions of psychotic patients compared to controls (Figs. 12 and 13), although the differences were not statistically significant ($p > 0.10$ for all regions). However, the exclusion of values for the outlier

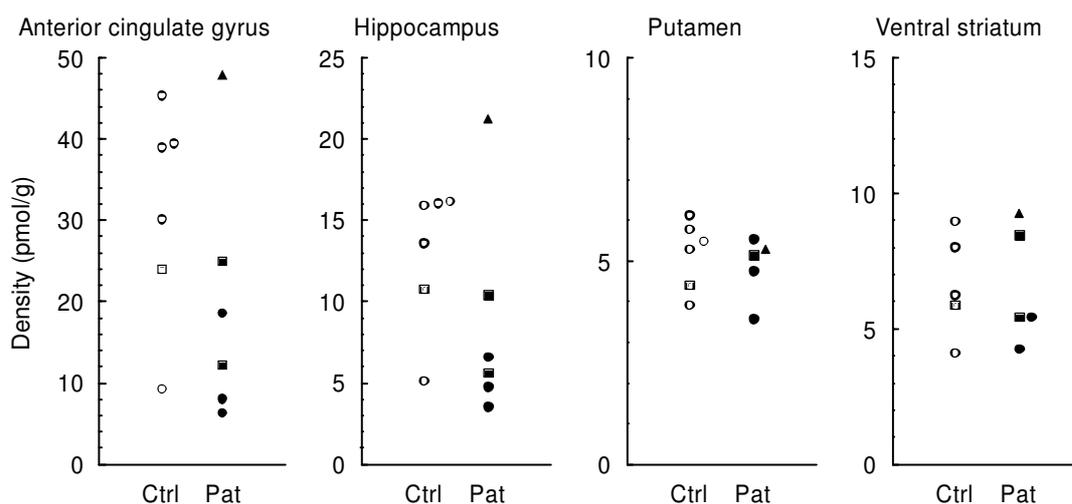


Figure 12. 5-HT_{2A} receptor densities in brain tissue from psychotic patients compared to controls. Open symbols, controls; black symbols, psychotic patients; □ = control, suicide; • = schizophrenia; ■ = schizophrenia or schizoaffective disorder, suicide; ▲ = psychosis NOS. Mann-Whitney exact p -values, all data: anterior cingulate gyrus, 0.24; hippocampus, 0.18; putamen, 0.66; ventral striatum, 1.0; exclusion of outlier: anterior cingulate gyrus, 0.052; hippocampus, 0.030 (unpublished data).

patient (diagnosed with psychosis NOS), gave significantly lower levels in the hippocampus for patients as compared to controls ($p = 0.030$, see Fig. 12 and 13). Differences approached significance for the anterior cingulate ($p = 0.052$) and insular cortex ($p = 0.052$) and a trend towards lower levels was found for lateral frontal and temporal cortices ($p < 0.10$). No differences in the densities of 5-HT_{2A} receptors were found in the striatum (Fig. 12) or the occipital cortex ($p = 0.66$).

The results obtained using a selective 5-HT_{2A} receptor radioligand is in agreement with most previous studies, where non-selective radioligands were used to compare densities of 5-HT_{2A} receptors in patients with schizophrenia and control subjects^{20, 45, 68, 107, 147, 189, 225}. In the present study, differences were most marked in the hippocampus, anterior cingulate gyrus and insular cortex. These regions are part of the greater limbic lobe, and have been implicated in psychiatric disorders¹¹⁸. The observed differences are unlikely to result from residual antipsychotic drugs in the tissue, as this would probably reduce the level of binding to a similar extent throughout the different brain regions. On the other hand, it is possible that adaptive mechanisms due to antipsychotic medication may have affected the level of 5-HT_{2A} receptors, as chronic treatment with antipsychotics may downregulate 5-HT_{2A} receptors⁸⁸.

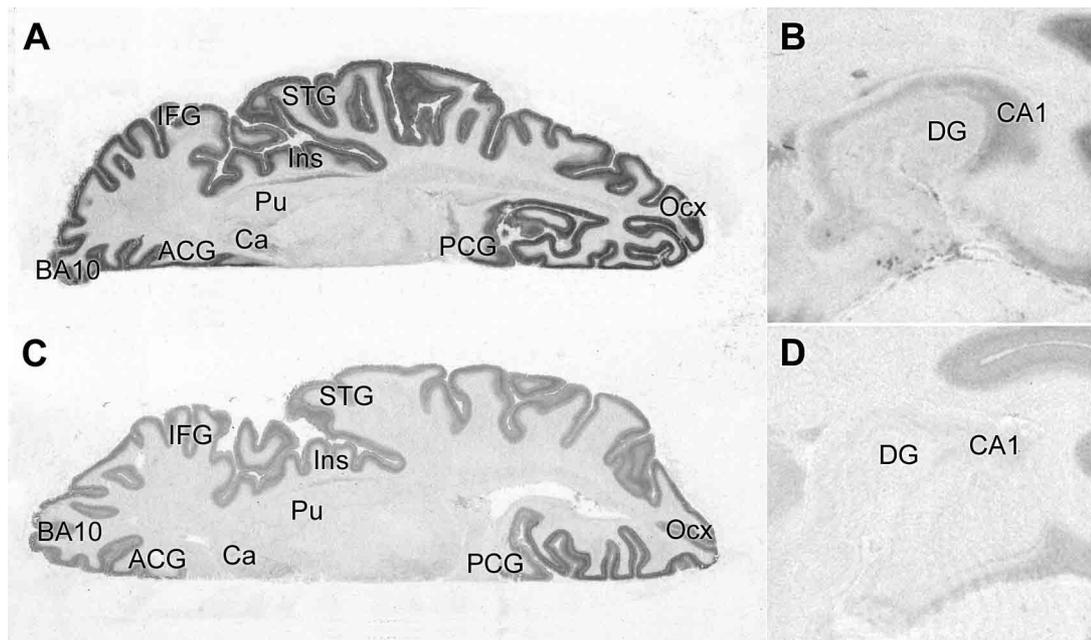


Figure 13. Autoradiograms of horizontal sections comparing 5-HT_{2A} receptor densities in a control subject (A and B) and a psychotic patient (C and D). (A and C) whole hemisphere sections at the level of the dorsal striatum, 76.7 mm and 74.3 mm, respectively, from the vertex. (B and D) Magnification of details from whole hemisphere sections at the level of the hippocampus, 92.5 mm and 97.9 mm, respectively, from the vertex. Autoradiograms are from a control and a patient with densities closest to the median value for the highest density region (frontal cortex). ACG, anterior cingulate gyrus; BA10, frontal pole; Ca, caudate nucleus; DG, dentate gyrus; IFG, inferior frontal gyrus; Ins, insular cortex; Ocx, occipital cortex; PCG, posterior cingulate gyrus; Pu, putamen, STG, superior temporal gyrus (unpublished).

5-HT₄ RECEPTORS

The distribution of 5-HT₄ receptors in the human brain was examined using the radioligand [¹²⁵I]SB-207710. As [¹²³I]-labeled SB-207710 has recently been developed as a SPET radioligand for the study of 5-HT₄ receptors *in vivo*, one of the aims of the investigation was to provide anatomical correlates for the lower resolution *in vivo* studies. Furthermore, we wished to take advantage of the higher detection sensitivity of [¹²⁵I]- as compared to [³H]-labeled ligands.

In general, the results are in agreement with previous studies on the 5-HT₄ receptor distribution in the human brain^{27, 74, 234, 272}. The highest level of [¹²⁵I]SB-207710 binding to 5-HT₄ receptors was found in the basal ganglia and the hippocampus. In contrast to a previous study using a similar autoradiographic methodology and tritiated radioligands²⁷, we observed low but non-negligible specific binding in regions such as the raphe nuclei and the thalamus (anterior and pulvinar nuclei, see Fig. 14A, Paper III), possibly due to the lower detection limit of [¹²⁵I]- compared to [³H]- labeled compounds. Moreover, by using this high sensitivity ligand, extended information of the binding pattern in the cerebral cortex was obtained (Fig. 14B). The binding was higher in temporal as compared to frontal cortical regions, and lower in the occipital cortex. Binding was highest in external cortical layers, lower levels were found in deep layers and the lowest intensities were seen in middle cortical layers. In the frontal cortex, binding in deep layers was concentrated to a distinct band consistent with layer V (Fig. 14B).

A comparison in adjacent sections revealed that the cortical distribution pattern of 5-HT₄ receptors show some qualitative similarities to that of the 5-HT_{1A} receptor (Paper V). Both receptors are more abundant in the temporal as compared to frontal cortical regions, and both display markedly lower levels in the occipital cortex. Also, the cortical lamination pattern is similar, with highest levels in external layers and a band of diffuse labeling in deep layers. It is possible that these receptors are colocalized in pyramidal neurons and may mediate opposite effect on neuronal excitability as has been demonstrated for the hippocampus²³⁹. The results show that [¹²⁵I]SB-207710 is suitable for human brain whole hemisphere autoradiography and could provide an important complement to the lower resolution SPET studies.

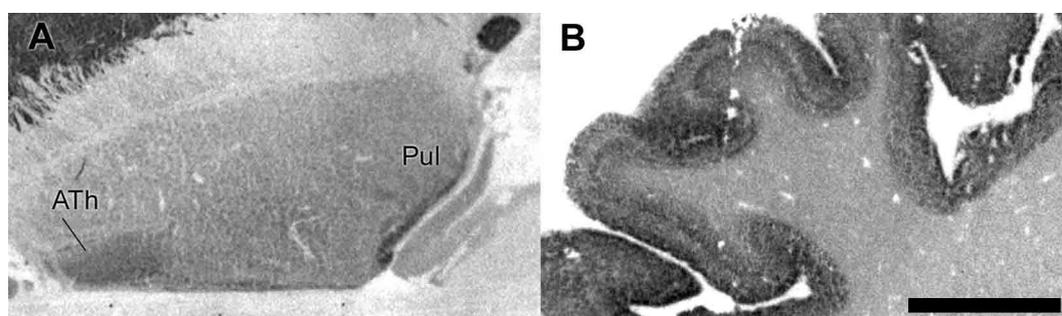


Figure 14. Details from horizontal whole hemisphere autoradiograms showing [¹²⁵I]SB-207710 binding to 5-HT₄ receptors in the thalamus (A) and frontal cortex (B); 65.3 mm and 70.5 mm, respectively, from the vertex. ATh, anterior thalamus; Pul, pulvinar; scale bar 10 mm (unpublished).

Descriptive statistics indicated lower levels of 5-HT₄ receptors in cortical regions of psychotic patients compared to controls (Paper VI). However, these differences did not reach statistical significance. Our results of 5-HT₄ receptors are in agreement with previous studies, which failed to demonstrate significant differences in the densities of 5-HT₄ receptors in the frontal cortex⁶⁹ and hippocampus²⁴⁴ of patients with schizophrenia compared to controls.

5-HT₆ RECEPTORS

Results from whole hemisphere autoradiography studies demonstrated that 5-HT₆ receptors are concentrated in the striatum with higher levels in dorsal as compared to ventral striatal regions (Fig. 15A), as has been previously demonstrated^{85, 123}. In addition, we found evidence for the presence of 5-HT₆ receptors in the substantia nigra (Fig. 15C). Other regions including the amygdala and the hippocampal formation (dentate gyrus and CA3) displayed markedly lower levels of [¹²⁵I]SB-258585 binding. The specific binding was low or very low in thalamus, hypothalamus, isocortex, the CA1 region of the hippocampus, and in the cerebellum (Paper VI).

For comparison of brain tissue from psychotic patients vs. controls, densities of 5-HT₆ receptor binding sites were analyzed in the striatum (see Table 4), as the high level of non-specific binding in other brain regions precluded accurate quantification. There were no significant differences between the groups in the densities of 5-HT₆ receptors in either the caudate nucleus ($p = 0.59$), the putamen ($p = 0.31$) or the ventral striatum ($p = 0.69$; Paper VI).

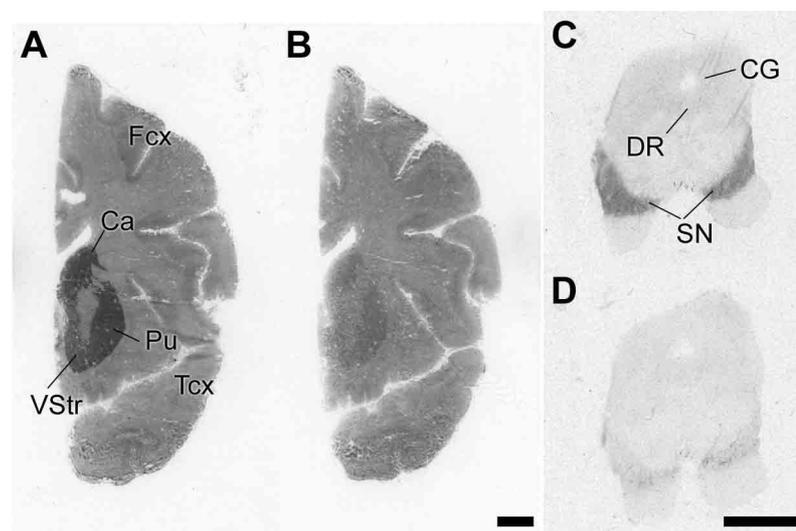


Figure 15. [¹²⁵I]SB-258585 binding to 5-HT₆ receptors in coronal whole hemisphere sections at the level of the striatum (100 μm; A and B; 71.2 mm and 70.8 mm, respectively, from the frontal pole) and brain-stem sections at the level of substantia nigra (20 μm; C and D) of the human brain. Total binding (A and C) and non-specific binding, in the presence of 10 μM methiothepin (B and D). Ca, caudate nucleus; CG, central gray; DR, dorsal raphe nucleus; Fcx, frontal cortex; Pu, putamen; SN, substantia nigra; Tcx, temporal cortex; VStr, ventral striatum; scale bar 10 mm (from Paper VI).

Table 4. 5-HT₆ receptor densities in striatal regions of controls and psychotic patients

Brain region	Controls			Patients		
	<i>n</i>	Median	Inter-quartile range	<i>n</i>	Median	Inter-quartile range
Caudate nucleus	6	5.5	4.9 – 5.9	6	5.0	4.2 – 5.8
Putamen	6	4.4	4.0 – 4.7	6	3.8	3.6 – 4.4
Ventral striatum	5	4.4	3.1 – 4.8	5	3.6	3.5 – 4.0

Densities are presented as B_{max} values, calculated based on the K_D value reported by East et al.⁸⁵ and are expressed in pmol/g tissue.

Previous studies have found evidence for reduced levels of 5-HT₆ receptors in the hippocampus⁸⁴ with no alterations in receptor densities or mRNA expression in the dorsolateral prefrontal cortex^{84, 85}. Thus, alterations in the levels of 5-HT₆ receptors in schizophrenia may be concentrated to the hippocampus, a region that was not investigated in the present study.

5-HT₇ RECEPTORS

The novel selective 5-HT₇ receptor antagonist radioligand [³H]SB-269970 was used for the autoradiographic analysis of 5-HT₇ receptors in human brain whole hemisphere sections. [³H]SB-269970 binding to the 5-HT₇ receptor was mainly concentrated to the thalamus and the hippocampal formation. The highest levels were detected in the anterior thalamus. Higher levels of binding were found in the anterior cingulate gyrus as compared to isocortical regions (Fig. 16). Intermediate levels of 5-HT₇ receptor binding sites were found in the hypothalamus, hippocampus, amygdala and certain brainstem nuclei (substantia nigra, ventral tegmental area and dorsal raphe nucleus; Paper IV).

The anterior cingulate gyrus, where high densities of 5-HT₇ receptors were found, has been associated with affect regulation²⁸². This distribution pattern gives further support for the involvement of 5-HT₇ receptors in affective behaviors^{267, 273}. The concentration of 5-HT₇ receptors to the anterior thalamus and the hippocampal formation is in agreement with rodent studies implicating this receptor subtype in learning and memory¹⁸². The 5-HT₇ receptor may therefore be considered a putative target for the development of antidepressants and cognition enhancers. Moreover, it is possible that interaction with this receptor subtype may contribute to the beneficial effects on cognitive and depressive symptoms of some atypical antipsychotics.

Mean values of 5-HT₇ receptor densities were lower for anterior cortical regions and thalamus of psychotic patients compared to controls. There was a trend towards lower levels of 5-HT₇ receptors in the inferior gyrus of the lateral prefrontal cortex (Figs. 16 and 17; *p* = 0.065) and pulvinar thalamic nucleus (*p* = 0.082) of patients versus controls. No differences were found for the caudate nucleus (*p* = 0.59), the putamen (*p* = 1.0) or the posterior cingulate gyrus (*p* = 0.54; Paper VI). Although the effect of medication cannot be ruled out, the observed differences are unlikely to result from residual antipsychotics in the tissue, as this would probably reduce the level of binding to a similar extent throughout the different brain regions. Our results on the 5-HT₇ receptor in schizophrenia-like psychosis are in agreement with a previous study demonstrating

lower levels of 5-HT₇ receptor mRNA expression in the dorsolateral prefrontal cortex in schizophrenia⁸⁴. However, as the number of subjects was small, these results need to be replicated in larger samples to allow a conclusion about the 5-HT₇ receptor densities in psychosis.

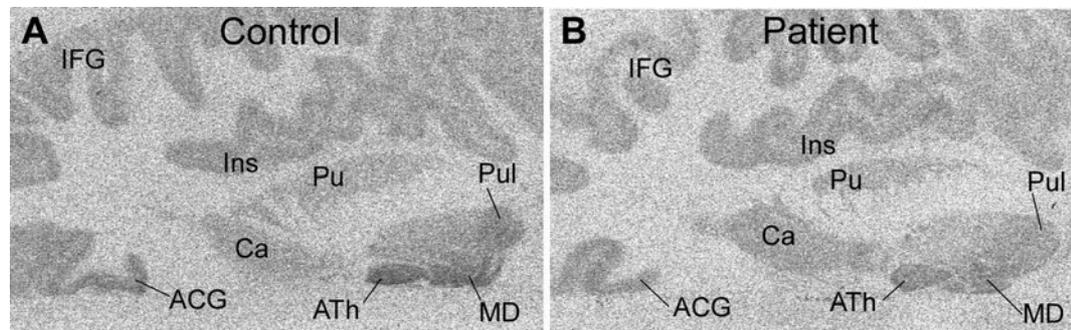


Figure 16. Autoradiographic distribution of 5-HT₇ receptors in brain tissue from a control subject (A) and a psychotic patient (B) at the level of the dorsal striatum (horizontal sections; 66.9 mm and 65.9 mm, respectively, from the vertex). Images are from subjects with densities closest to the median value for the region with highest density (anterior thalamus) for controls and patients, respectively. ACG, anterior cingulate gyrus; ATh, anterior thalamus; Ca, caudate nucleus; IFG, inferior frontal gyrus; Ins, insular cortex; MD, mediodorsal thalamus; Pu, putamen; Pul, pulvinar (from Paper VI).

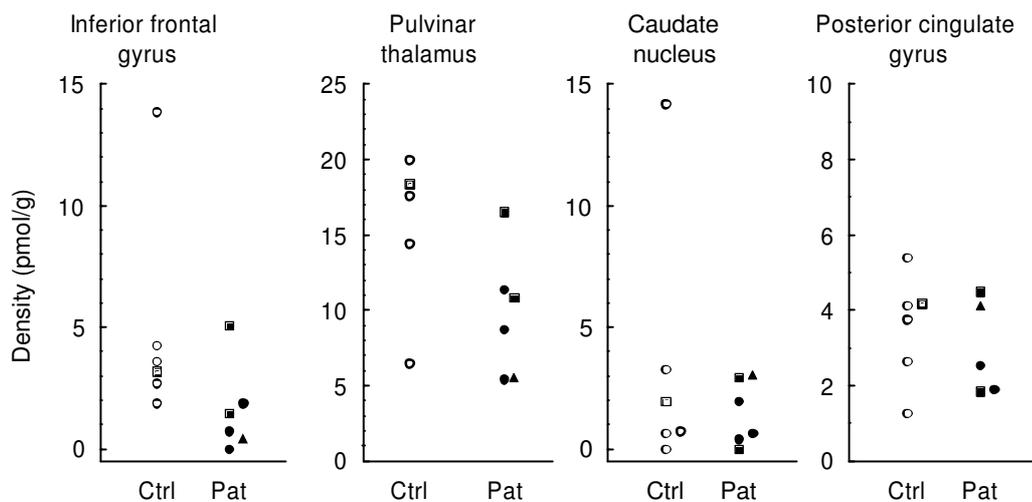


Figure 17. 5-HT₇ receptor densities in brain tissue from psychotic patients compared to controls. Open symbols, controls; black symbols, psychotic patients. □ = control, suicide; ● = schizophrenia; ■ = schizophrenia or schizoaffective disorder, suicide; ▲ = psychosis NOS. Mann-Whitney exact *p*-values: Inferior frontal gyrus, 0.065; pulvinar thalamus, 0.082; caudate nucleus, 0.59; posterior cingulate gyrus, *p* = 0.54 (data from Paper VI).

SUMMARY OF FINDINGS

- In cortical regions, 5-HT transporters are concentrated to several regions of the greater limbic lobe (anterior cingulate, subcallosal area, insular and entorhinal cortices, uncus, temporal pole and orbitofrontal gyrus). Higher levels of [³H]WAY-100635 binding to the 5-HT_{1A} receptor were also found in the limbic cortices (subcallosal area, anterior cingulate gyrus, temporal pole) as compared to the isocortex.
- 5-HT_{1B} receptors are localized in the basal ganglia with higher densities in ventral compared to dorsal striato-pallidal regions of the human brain. Very high levels of 5-HT_{1B} receptors were detected in the occipital cortex. High levels of 5-HT_{1B} receptor mRNA expression were found in the isocortex, ventral striatum, thalamus (lateral geniculate, mediodorsal and pulvinar nuclei) and raphe nuclei.
- There was a mismatch between the regional 5-HT_{1B} mRNA expression pattern and the receptor distribution in the human basal ganglia and thalamus, in support of the localization in terminals of striatal and thalamic projections. On the other hand, overlapping distribution patterns of mRNA and binding sites were found in the isocortex.
- We found evidence for species differences in the cortical distribution of 5-HT_{1B} receptors between the rat and human brain. Markedly higher levels of 5-HT_{1B} receptors were detected in the rat than in the human hippocampal formation, whereas densities were higher in the isocortex of the human as compared to the rat brain.
- 5-HT_{1D} receptors are present in markedly lower levels as compared to 5-HT_{1B} receptors and appear to be confined to the substantia nigra and the pallidum. The results indicate expression of 5-HT_{1D} receptor mRNA in the raphe nuclei, striatum, external layers of the temporal cortex and in the cerebellum.
- [¹²⁵I]SB-207710 is a sensitive tool for the autoradiographic mapping of 5-HT₄ receptors in the human *postmortem* brain. The high sensitivity of this radioligand enables the visualization of 5-HT₄ receptors in low-density regions including the dorsal raphe nucleus and the thalamus.
- 5-HT₇ receptors are concentrated in regions implicated in memory, affect regulation and emotional processing including the anterior thalamus, the hippocampal formation, the anterior cingulate gyrus and the amygdala of the human brain.

- There was a trend towards lower levels of 5-HT transporters in the striatum and the temporal cortex, as well as lower levels of 5-HT_{2A} receptors in cortical regions and 5-HT₇ receptors in the lateral frontal cortex and pulvinar thalamus of psychotic patients compared to controls. Data also indicated lower levels of 5-HT_{1A} and 5-HT_{1B} receptors in the hippocampal formation, and in the ventral pallidum and orbitofrontal cortex, respectively. No trends or significant differences were observed between the groups in the densities of 5-HT₄ and 5-HT₆ receptors (see Table 5 for summary).

Table 5. 5-HT binding sites in schizophrenia-like psychosis. Summary of findings from whole hemisphere autoradiography studies (Paper VI and unpublished results)

Brain region	5-HT transporter	5-HT _{1A}	5-HT _{1B}	5-HT _{2A}	5-HT ₄	5-HT ₆	5-HT ₇
<i>Basal ganglia</i>							
Caudate nucleus	↓ ²	-	↔	↔	↔	↔	↔
Putamen	↓ ²	-	↔	↔	↔	↔	↔
Ventral striatum	↔	-	↔	↔	↔	↔	N.A.
Globus pallidus	↔	-	↔	-	↔	-	N.A.
Ventral pallidum	↔	-	↓ ¹	-	↔	-	N.A.
<i>Limbic cortices</i>							
Basolateral amygdala	↓ ¹	↔	↔	N.A.	↔	N.A.	N.A.
Hippocampal formation	N.A.	↓ ¹	↔	↓ ^{3, a}	↔	N.A.	N.A.
Anterior cingulate gyrus	↔	↔	↔	↓ ^{2, a}	↔	-	↔
Posterior cingulate gyrus	↔	↔	↔	↔	↔	-	↔
Orbitofrontal cortex	↔	N.A.	↓ ²	N.A.	N.A.	-	N.A.
<i>Isocortex</i>							
Inferior frontal gyrus	↔	↔	↔	↓ ^{1, a}	↔	-	↓ ²
Superior temporal gyrus	↓ ¹	↔	↔	↓ ^{1, a}	↔	-	↔
<i>Thalamus</i>							
Anterior	↔	-	-	-	↔	-	↔
Mediodorsal	↔	-	-	↔	N.A.	-	↔
Pulvinar	↔	-	-	-	N.A.	-	↓ ¹

↓ = Indications for lower densities for psychotic patients compared to controls (¹ p < 0.10; ² p < 0.070; ³ p < 0.050); ↔ = no group difference, N.A. = not analyzed; - = not detectable by the current methodology, ^a schizophrenia or schizoaffective disorder, patient diagnosed with psychosis NOS excluded.

CONCLUDING REMARKS

This study illustrates that the different 5-HT receptors have unique distribution patterns in the human brain, reflecting their different physiological effects. It can be concluded that the examined binding sites are localized in specific structures of the greater limbic lobe (5-HT transporter, 5-HT_{1A}, 5-HT₇) and major output channels from limbic cortices, such as the ventral striato-pallidal system and the bed nucleus of the stria terminalis (5-HT_{1B}, 5-HT transporter). These localization patterns are consistent with the documented role for 5-HT in the modulation of mood and emotional state, and the suggested involvement of this system in the pathophysiology of a variety of psychiatric disorders.

While previous autoradiographic investigations of the localization of 5-HT receptors have been limited to a few selected brain regions, the whole hemisphere autoradiography methodology, as applied in this study, enabled the mapping of the distribution of 5-HT receptor subtypes throughout several regions of the human forebrain. The rationale for choosing this experimental setup was to collect information on a number of receptors from several regions in a common brain material. The possibility to compare the detailed regional distribution of 5-HT receptor subtypes in adjacent sections of the same individual may demonstrate important aspects of the subregional co-localization of different binding sites. Additionally, the information obtained in this study may provide high-resolution anatomical correlates for *in vivo* imaging studies of 5-HT binding sites, in particular given that several of the compounds used in this study are available as PET or SPET radioligands for the mapping of 5-HT receptors *in vivo*.

Previous studies of 5-HT receptor densities in psychosis have concentrated on specific brain regions and most of the studies have been focused on the frontal cortex. In contrast, this pilot investigation examined the densities of the 5-HT receptor subtypes 5-HT_{1B}, 5-HT_{2A}, 5-HT₄, 5-HT₆ and 5-HT₇ in whole hemisphere brain tissue from psychotic patients compared to control subjects. The data indicated lower levels of 5-HT transporters in the striatum and temporal cortex, lower levels of 5-HT_{2A} receptors in prefrontal and temporal cortices and 5-HT₇ receptors in the frontal cortex and thalamus. As most of the patients in the study had been chronically treated with antipsychotic drugs, which interact with 5-HT receptors, it is possible that some of the differences may be the result of drug treatment rather than disease-related alterations. Moreover the cause of death was suicide for two of the patients and previous autoradiographic studies have found altered levels of 5-HT binding sites in suicide victims⁵. As human whole hemisphere brain tissue is not easily available, the number of subjects was small, and the results need to be interpreted with caution. Nevertheless, these findings may give directions for large-scale studies examining specific brain regions of psychotic patients as compared to controls.

Future studies are thus required to verify our results on 5-HT receptor densities in psychosis. Moreover, further investigations of brain 5-HT_{1B/1D} receptors in depression and anxiety disorders would be of interest as these receptors have been hypothesized to be hypersensitive in these conditions^{87, 191, 194, 294}. The recent findings that 5-HT₇ receptor knock-out mice have fewer signs of depression¹⁰⁸ may encourage studies of this

receptor subtype in depressed patients compared to controls. The brain distribution of 5-HT_{5A} receptor binding sites is currently not known due to the lack of selective detection methods. The development of specific radioligands for the 5-HT_{5A} receptor will enable the future autoradiographic mapping of this less well-characterized receptor subtype in the human brain. Moreover, as LSD shows high affinity for the 5-HT_{5A} receptor and may mediate some of its psychoactive effects through this receptor subtype¹⁰⁵, studies of 5-HT_{5A} receptors in psychosis may be relevant. The application of *post-mortem* techniques for the analysis of neurochemical abnormalities in psychiatric disorders is associated with several obstacles, including the limited availability of brain tissue, difficulties related to the *postmortem* assignment of *antemortem* diagnosis¹²¹, and variability due to differences in *postmortem* interval and agonal state. Moreover as described above, suicide as cause of death and chronic treatment with psychoactive drugs may be important confounders when analyzing the 5-HT system in psychiatric disorders and these factors are not easily controlled for in *postmortem* studies. Therefore, it is likely that future studies will utilize *in vivo* imaging technologies for comparison of brain receptor densities in different subject groups.

In addition to the comparison between patients and controls, it is likely that future neuroimaging studies of the 5-HT system will address the issue of interindividual variability in receptor density. As has been described for 5-HT receptors *in vivo*^{29, 226}, our data showed a marked variability in receptor density between different subjects (see e.g. Figs. 12 and 17). Although differences due to *postmortem* effects cannot be ruled out in this study, it is possible that these observations reflect interindividual differences in brain 5-HT receptor densities that could manifest as differences in personality traits among individuals as has been described for the 5-HT_{1A} receptor²⁹.

Drugs interacting with the serotonin system are prescribed for several psychiatric disorders. However, with few exceptions, the currently used drugs show a low degree of selectivity at the receptor level. Given the number of 5-HT receptor selective compounds recently developed (see Table 1), it is likely that future psychoactive drugs will specifically target 5-HT receptor subtypes. The regional localization of 5-HT binding sites in the human brain may provide clues about the function of the different 5-HT receptors, which may be useful to consider in the clinical development of receptor-selective drugs. In this respect, the distribution patterns of 5-HT_{1B} (enriched in the ventral striato-pallidal system) and 5-HT₇ receptors (concentrated to the anterior cingulate gyrus) suggest that these may be potential targets for the development of drugs for the treatment of psychiatric disorders.

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