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## 5-HT<sub>4</sub> RECEPTORS

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### Abstract

Serotonin 4 receptors (5-HT<sub>4</sub>Rs) were discovered 15 years ago. They are coded by a very complex gene (700Kb, 38 exons) which generates eight carboxy-terminal variants (a, b, c, d, e, f, g, n). Their sequences differ after position L<sup>358</sup>. Another variant is characterized by a 14 residue insertion within the extracellular loop 2. Highly selective potent 5-HT<sub>4</sub> receptor antagonists and partial agonists which cross the blood-brain barrier have been synthesized, but a specific full agonist for brain studies is still missing.

Based on physiological and behavioral experiments, 5-HT<sub>4</sub>Rs may be targets to treat cognitive deficits, abdominal pain and feeding disorders. One 5-HT<sub>4</sub>R-directed drug (SL65.0155) is already in phase II to treat patients suffering from memory deficits or dementia.

**Key words:** 5-HT<sub>4</sub> receptors; splice variants; G proteins; neurotransmitter release; cognition; pain; anxiety; feeding; CNS drug targets

## I. Historical overview

There are several distinct periods in the history of 5-HT<sub>4</sub> receptors (5-HT<sub>4</sub>Rs): A short **"Naughty but Nice"** period (1988-1989) (see [1]) characterized by a clear scepticism among many serotonergic specialists about the existence of the receptor. Its birth certificate was difficult to obtain [2]. 5-HT<sub>4</sub>R was first described in colliculi and hippocampal neurons as a cAMP stimulatory 5-HT receptor, insensitive to classical 5-HT<sub>1</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptor antagonists [3]. The only exception was tropisetron, a potent 5-HT<sub>3</sub>R antagonist, which is also a weak 5-HT<sub>4</sub>R antagonist (pA<sub>2</sub> = 6.5). Benzamides, such as metoclopramide, renzapride, cisapride and zacopride were rapidly recognized as being potent agonists [4]. This formed a link between these 5-HTRs and an unclassified prokinetic 5-HT receptor in guinea-pig ileum. The latter was insensitive to classical 5-HT<sub>1</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptor antagonists, stimulated by benzamides and blocked by  $\mu$ M concentrations of tropisetron (for a review see [5]).

Then came a **"Flourishing Pharmacological period"** (1990-1997). This was characterized by the description of the distribution of 5-HT<sub>4</sub> receptors in brain and peripheral organs, the analysis of some of their cellular and physiological functions as well as by the synthesis of very specific agonists and antagonists (for reviews see [6, 7]).

An **"Intense Molecular biology period"** (1995-2002) was defined by the cloning of the rodent [8-10] and human [11-14] 5-HT<sub>4</sub>R gene. These studies carried out a structural analysis of ligand binding sites [15] and recognized the existence of multiple allosteric conformations which were stabilized by agonists, antagonists and inverse agonists [10, 16]. The 5-HT<sub>4</sub>R gene is localized on chromosome 5, bands q31-q33 with both dopamine D<sub>1</sub> and  $\alpha$ <sub>2</sub>-adrenergic receptor genes [17] (Fig. 1). There are 38 exons in the 5-HT<sub>4</sub>R gene and nine splice variants have been cloned. They represent an important if not unique discovery amongst genes encoding 5-HT receptors [10, 18, 19] (Fig. 1). A common splicing site produces eight splice variants with an identical DNA sequence up to the codon encoding Leu<sup>358</sup>. On the contrary, the DNA sequence encoding the C-terminus is specific to each splice variant. One of the important issues will be to analyze the functional importance of these splice variants and their potential use as therapeutic targets.

The as yet **"Unwritten period"** needs to be characterized by an extensive analysis of the cellular and physiological functions of 5-HT<sub>4</sub>Rs so that their potential for the treatment of

memory deficit, depression, addiction, epilepsy or eating disorders can be accurately assessed. Some 5-HT<sub>4</sub>R-directed drugs used to be (cisapride), or are on the market (metoclopramide, tegaserod, which is on the US market under the name of Zelnorm™). Tegaserod is used to treat functional gastro-intestinal disorders (FGIDs) including Irritable Bowel Syndrome (IBS). The introduction of 5-HT<sub>4</sub>R drugs for psychiatric and/or neurological diseases seems to be a reasonable possibility.

## II. Cloning and localization

### II-1. Cloning

The cloning of the rat 5-HT<sub>4</sub>R gene was performed in 1995 by Gerald *et al* [8]. They isolated two cDNAs from rats that they called 5-HT<sub>4S</sub> (387 residues) and 5-HT<sub>4L</sub> (406 residues) receptors. According to the present NC-IUPHAR recommendations, they are now called 5-HT<sub>4a</sub> and 5-HT<sub>4b</sub>. Moreover, Van den Wyngaert *et al.* were the first to correct a sequencing error in the 5-HT<sub>4b</sub> (388 residues) receptor which is, in fact, only one residue longer than 5-HT<sub>4a</sub> whatever the species considered, including human [13]. From then on, several laboratories isolated 5-HT<sub>4</sub>R cDNA from rat, mouse and human tissues. Alignment of the putative C-termini amino-acid sequences predicted from all their cDNA (except one, see hereafter), revealed that the divergence in their sequence starts after position L<sup>358</sup>, their last common amino acid. Four C-terminal splice variants have been cloned in mouse, (a, b, e, f) [9, 10], three in rat (a, b, e) [8, 10] and eight C-terminal splice variants in human (a, b, c, d, e, f, g, n) [11, 12, 14, 20-23] (see Fig. **1A**). The 5-HT<sub>4</sub>R isoforms are indeed splice variants generated from the same gene, as revealed by the human genome sequencing. The human 5-HT<sub>4</sub>R gene is one of the longest the G protein-coupled receptor genes (700kb) and consists of at least 38 exons (Fig. **1B**). Its 5' non-translated region is exceptionally long (>500 kb) and complex compared to other 5-HTR genes [19]. The human 5-HT<sub>1</sub>R family has intronless genes [24, 25], the human 5-HT<sub>2A</sub> gene (>20 kb) consists of three exons [26] and the human 5-HT<sub>2C</sub> gene (>230 kb) consists of six exons [27]. It must be noted that the gene of the β<sub>2</sub>-adrenergic receptor, a prototypal GPCR, is located in the longest intron 20 (Fig. **1B** and [19]). The 5-HT<sub>4</sub>R promoter contains potential binding sites for transcription factors (Sp-1, AP-2, AP-4 and GATA) but lacks TATA- and CAAT- boxes.

Variants e, f, and g overlap whereas variants e and f are generated as shorter versions of variant g due to internal 3'-acceptor sites in exon 35 (see Table 1 and insert in Fig. 1B). Note that Bender *et al.* published the correct sequence of 5-HT<sub>4c</sub> [18]. These authors also revealed the existence of a splice variant within the hepta-helical structure of the receptor, the 5-HT<sub>4h</sub> receptor [18]. This variant is a result of the insertion of 14 amino acids (exon 30) into the second extracellular loop of the receptor (Fig. 1). This variant is only found in the 5-HT<sub>4b</sub> isoform and therefore is called the 5-HT<sub>4hb</sub> variant.

The recent description of a 5-HT<sub>4n</sub> isoform containing 359 amino-acids (Fig. 1A) can be explained by two different 5'-donor sites at the end of exon 31 (Table 1) [22]. Thus, exon 31' can be generated and contains an in frame stop codon leading to the shorter C-terminal variant (see insert in Fig. 1B).

## II-2. Localization

The distribution of 5-HT<sub>4R</sub> in the brain has been extensively studied using the labeled antagonists [<sup>3</sup>H]-GR 113808 and [<sup>125</sup>I]-SB 207710 in rat, mouse, guinea-pig, pig, calf, monkey and human (for a review see [28]). 5-HT<sub>4Rs</sub> are expressed in limbic brain structures (hypothalamus, nucleus accumbens, amygdala), islands of Calleja, olfactory tubercle, fundus striati, ventral pallidum, septum, hippocampus, basal ganglia (striatum, globus pallidus) and substantia nigra.

In mice and rats, the [<sup>3</sup>H]-GR 113808 binding site density is higher in the nucleus accumbens shell than in the core [29]. The olfactory tubercles and fundus striati, like the striatum tail in the next posterior level, also exhibit strong labeling in mice. There are a few differences in the rat striatum where the density is identical in the rostral and caudal part, but higher in its lateral than medial part [29]. In the rat cerebral cortex, there is an average concentration of binding sites with a predominant labeling of the cingulate (prefrontal cortex) as compared to the frontoparietal regions. A laminar pattern was observed in the hippocampal formation. The various hypothalamic and thalamic nuclei show moderate density of receptors. The density is weak in the dorsal and medial raphe nuclei in rodents [28, 30].

We know the cellular and subcellular localizations of 5-HT<sub>4Rs</sub> thanks to a diversity of methodological approaches: comparison between 5-HT<sub>4R</sub> mRNA (localized in cell bodies) and 5-HT<sub>4R</sub> binding site distribution, lesion and electrophysiological studies, as well as single

cell PCR studies. The general observation is that 5-HT<sub>4</sub>Rs have a neuronal rather than a glial localization. In the striato-nigral pathways, 5-HT<sub>4</sub>Rs are mainly expressed in somato-dendritic compartments of intrinsic striatal neurons, mainly GABAergic, but their presence on cholinergic neurons is also likely. Some 5-HT<sub>4</sub>Rs are also present on terminals of striatal GABAergic neurons which project to globus pallidus and substantia nigra [29, 31]. In post-mortem putamen of patients suffering from Huntington's disease [32] there is a 50% loss of 5-HT<sub>4</sub>Rs.

In the hippocampus, electrophysiological data indicate an expression in CA1 neurons [33-35] but also in granule cells and other neurons in the dentate gyrus [36]. In the prefrontal cortex, 60% of glutamatergic pyramidal neurons express 5-HT<sub>4</sub>Rs as determined by electrophysiological responses [37] and single cell PCR [38]. Much data indicates an effect of 5-HT<sub>4</sub>Rs activation on acetylcholine release in cortex [39], and also in hippocampus [35]. This suggests a localization on cholinergic neurons, although indirect effects cannot be excluded.

To better understand the putative differential functions of the different 5-HT<sub>4</sub>R splice variants, it will be important to know their differential regional and cellular expression in the brain. Using specific oligonucleotides, but not quantitative PCR, three studies report expression of specific splice variant isoforms. In mouse brain, Claeysen *et al.* found an expression of 5-HT<sub>4a, b, e, f</sub> isoforms. The 5-HT<sub>4e, f</sub> isoforms were not detected in the periphery [10]. Bender *et al.* found a complex pattern of expression of human 5-HT<sub>4</sub>R isoforms, which was dependent not only on the brain region studied, but also on each individual brain. This raises the possibility that there is an individual polymorphism in the 5-HT<sub>4</sub>R variant pattern of expression in man [18]. Using non-quantitative PCR Vilaro *et al.* described a high expression of the 5-HT<sub>4n</sub> isoform in human brain [22]. A quantitative analysis of mRNA levels has been performed by Medhurst *et al.* using human brain [40]. Expression levels observed for the 5-HT<sub>4b</sub> splice variant were virtually identical to those obtained with primers that amplified all 5-HT<sub>4</sub> splice variants. The results indicate that 5-HT<sub>4b</sub> was the dominant variant, followed by the 5-HT<sub>4a, c, g</sub> variants. Interestingly the pattern of expression of the different variants was brain region specific [40].

### III. Multiple structural conformations, pharmacological differences between splice variants, coupling to G proteins, and tool molecules

#### III-1. Multiple structural conformations

Transfections of the different splice variants in several cell lines revealed that 5-HT<sub>4</sub>Rs have a high constitutive activity even when expressed at low and physiological densities (<500 fmol/mg) [10]. At a similar density, they show a much higher constitutive activity than the native and mutated  $\alpha$ 2-adrenergic receptors. Therefore, 5-HT<sub>4</sub>Rs have a high capacity to isomerize from an inactive (R) to an active (R\*) isoform, even in the absence of agonist. We found that 16% of the WT receptors are under the R\* form. Interestingly, the short C-terminal isoforms (5-HT<sub>4e, f</sub> receptors) have a higher constitutive activity than the long C-terminal isoforms (5-HT<sub>4a, b</sub> receptors). Progressive truncation of the C-terminal further increases the constitutive activity [10]. Several cysteines (Cys<sup>328</sup>, Cys<sup>329</sup>, and Cys<sup>386</sup>) of the C-terminal have been shown to be palmitoylated. Cysteine palmitoylation is stimulated by receptor activation [41]. The palmitoylated cysteine 386 is only present in the 5-HT<sub>4a</sub> receptors splice variant, whereas Cys<sup>328</sup> and Cys<sup>329</sup> are present in all splice variants. Mutations of Cys<sup>328</sup>, Cys<sup>329</sup> into Ser, significantly increases the capacity of receptors to convert from the R to the R\* form [41, 42]. When tested on constitutively active mutants some 5-HT<sub>4</sub>R antagonists have been found to be neutral antagonists (for example, RS 100235 and GR 113808), partial agonists (for example SB 204070) or inverse agonists (for example GR 125487, SB 207266 or RS 116-0086) [10, 43]. Inverse agonists have been proposed to stabilize the R form of GPCRs and therefore to reduce their constitutive activity. We found that 5-HT<sub>4</sub>R inverse agonists stabilize a third "silent" form. A specific transmembrane (TM) network, implicated in the stabilization of this "silent" form but not the stabilization of the R\* form, has been described. It includes, the Asp<sup>100</sup>(3.32) in TMIII, Phe<sup>275</sup>(6.51) in TMVI and Trp<sup>272</sup>(6.48) in TMVI [16]. D100 is highly conserved within the biogenic amine receptor family and is indeed necessary for the binding and activity of 5-HT, tryptamines and small indole carbazimidamides. However, D100 is not necessary for the binding of benzamides and other non-indole 5-HT<sub>4</sub> agonists and antagonists. These ligands have affinities in the range of nanomolar concentrations for a mutated 5-HT<sub>4</sub>R D100A [44]. More interestingly, two antagonists of native 5-HT<sub>4</sub>Rs are agonists of this mutated Gs-coupled receptor [44]. The mutated 5-HT<sub>4</sub>R D100A can be added to a class of mutated GPCRs called RASSLs (Receptors Activated Solely by Synthetic Ligands). The idea is to engineer receptors (RASSLs) that would be insensitive to their endogenous ligands but can be fully activated by

synthetic ligands [45]. Heterogeneously expressed in tissues, they can be used to control signal transduction and cellular physiology in a research or therapeutic perspective.

### III-2. Pharmacology and coupling to G-proteins

Several reviews describe in detail the pharmacology of 5-HT<sub>4</sub>Rs [6, 46]. We will only discuss the possible difference in pharmacology of the splice variants and the "non G $\alpha$ s", described signaling pathways (see Table 2).

All the 5-HT<sub>4</sub>R splice variants have the transmembrane domain core and therefore the same primary binding site for 5-HT as well as for most of the synthetic ligands. In binding assays, the pharmacology of the variants (including the 5-HT<sub>4hb</sub>) [18] are not significantly different. However, when functional studies are considered, some differences have been noted in their signaling properties: 1) as already discussed, the constitutive activity of the variants depends on the sequence of their C-terminal [10]; 2) the classical 5-HT<sub>4</sub>R neutral antagonist, GR 113808, is a partial agonist on the 5-HT<sub>4hb</sub> variant [18]; 3) renzapride is a full agonist on the 5-HT<sub>4b</sub> and 5-HT<sub>4d</sub> isoforms and a partial agonist on the 5-HT<sub>4a</sub> and 5-HT<sub>4e/g</sub> isoforms [20]; 4) 5-methoxytryptamine and prucalopride are more potent on the 5-HT<sub>4b</sub> variant, whereas SDZ-HTF 919 and SB 204070 are more potent on the 5-HT<sub>4a</sub> variant [47]; 5) in CHO cells, a selective benzodioxanoxadiazolone (SL 65.0155) is an inverse agonist on 5-HT<sub>4a</sub>R but a partial agonist on 5-HT<sub>4b</sub> and 5-HT<sub>4e</sub> variants [48]; 6) a difference in desensitization of the splice variants is reported in CHO cells, the 5-HT<sub>4d</sub> variant is more rapidly uncoupled to adenylyl cyclase than the 5-HT<sub>4e</sub> variant [49]. In these cells, the desensitization is PKA-dependent. Note that in colliculi neurons, the desensitization of 5-HT<sub>4</sub>Rs is homologous and thus results in an action of G-protein kinase (GRK), independent on PKA activity [50]; 7) an interesting set of data reports that in HEK 293 cells, 5-HT<sub>4b</sub> but not 5-HT<sub>4a</sub> variants are coupled to G $\alpha$ i/G $\alpha$ o [47] (Table 2); 8) the 5-HT<sub>4a</sub> variant is much more potent than 5-HT<sub>4b</sub> in increasing membrane bound Ca<sup>2+</sup> channel activity in HEK 293 cells [47] (Table 2); 8) in COS cells, 5-HT<sub>4b</sub> variant activates the ras and rap1 pathways, via PKA [51] (the other variants have not been tested). The activation of ras but not rap1 led to the downstream activation of ERK1/2 (Table 2); 9) finally, the 5-HT<sub>4a</sub> variant (the other variants have not been tested) has been shown to be coupled to G $\alpha$ 13 in Sf9 cells, but also in mammalian cells, including the neuroblastoma x glioma NIE-115 and fibroblasts NIH 3T3 (Table 2). In NIE-115 cells, 5-HT<sub>4a</sub>Rs provoke a RhoA-dependent neurite retraction and cell rounding [52].



Although interesting, all of the described experiments were performed in heterologous systems and often used overexpression. They indicate that differences in coupling and pharmacology are "possible" but need to be demonstrated in native cells and tissues before their true relevance can be judged.

In a recent series of experiments in brain, we were able to identify GPCR interacting proteins (GIPs) using the different C-termini of 5-HT<sub>4a, b, e</sub> splice variants as bait. The last C-terminus residues of 5-HT<sub>4a</sub>R (SCF) and 5-HT<sub>4e</sub>R (PVPV) correspond to one of the PDZ (type I and type II ligands, respectively [53]). On the contrary, the 5-HT<sub>4b</sub> C-terminus (SQS) is not typical of a PDZ ligand. Interestingly, proteins associated with the different C-termini of different variants suggest that some functional differences mediated via these GIPs may exist (L. Joubert *et al.* in preparation).

#### **IV. 5-HT<sub>4</sub> receptor ligands**

Structure–activity relationship studies (SAR) of 5-HT<sub>4</sub> receptor ligands have been recently reported (for reviews, see [46, 54]). Here, we describe the significant progress made over the last decade in discovering potent and selective 5-HT<sub>4</sub>R agonists, antagonists and inverse agonists and point out those that are commercially available and those whose potential use in CNS disorders has yet to be determined (Table 3).

As previously noted, the 5-HT<sub>4</sub>R ligands are currently related, with some exceptions, to five main chemical classes: the indoles (including analogues of 5-HT, the indole carboxylates and the indole carboxamides), the benzamides, the benzoates, the aryl ketones and the benzimidazolones.

##### **IV-1. Agonists**

The first class of 5-HT<sub>4</sub>R agonists includes the substituted tryptamines or 5-HT analogues, as well as the substituted indole carbazimidamides. 5-Methoxytryptamine is an interesting tool because it lacks affinity for the 5-HT<sub>3</sub>R [55]. From a series of indole carbazimidamide derivatives, HTF 919 (tegaserod) was found to be a potent partial agonist with high affinity at 5-HT<sub>4</sub>R. It is 40- to 80-fold selective over the 5-HT<sub>1A</sub> receptor, 25-fold selective over the 5-HT<sub>2C</sub> and has no appreciable affinity for 5-HT<sub>3</sub> or dopamine receptors [56, 57]. *In vivo* studies

show that tegaserod enhances gastrointestinal motor activity and normalizes impaired motility throughout the gastrointestinal tract. Tegaserod maleate or Zelnorm™ is prescribed for IBS [58]. Further pharmacokinetic investigations in animals revealed a negligible penetration into the brain and so do not represent a useful tool for CNS research. The second class of 5-HT<sub>4</sub>R agonists includes the first generation benzamides bearing the 2-methoxy-4-amino-5-chloro substitution [4]. These benzamides are found to be non-selective and always have antagonistic activity at the 5-HT<sub>3</sub> receptor (zacopride, renzapride and metoclopramide). Structural modification of the basic chain of the benzamides, cisapride SB 205149 and SC 53116 lead to the identification of more potent 5-HT<sub>4</sub>R agonists with lower affinity for 5-HT<sub>3</sub> than the first generation benzamides [59-61]. Nevertheless, cisapride has appreciable affinity for 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenergic receptors.

Interestingly, newer benzamides with large substitutions in their basic chain, such as prucalopride, mosapride and Y-34959, are highly selective but show varying degrees of intrinsic activity depending on the *in vitro* preparations of the 5-HT<sub>4</sub>R used [62-64].

When the amide linkage in benzamide was substituted by an ester, this led to benzoate derivatives with an increased affinity for 5-HT<sub>4</sub>R. The first of this series was ML 10302 with a high affinity for 5-HT<sub>4</sub> receptors and a weak affinity for 5-HT<sub>3</sub> receptors [65]. ML 10302 was also inactive at other 5-HT receptor subtypes. ML 10302 was characterized as being the first potent and selective 5-HT<sub>4</sub>R partial agonist but its susceptibility to hydrolysis limited its utility for *in vivo* investigation. Recently, Moser and coworkers synthesized SL65.0155, a novel compound with high affinity for 5-HT<sub>4</sub>R related to the benzodioxanes[48]. This compound acts as a partial agonist with a remarkable selectivity over other receptor subtypes, except for the  $\sigma$  receptor and  $\alpha_{1D}$ -adrenoceptor.

In the fourth class, the benzimidazolones, BIMU 8 and BIMU 1 are potent and efficacious 5-HT<sub>4</sub>R agonists [66, 67] that enter the CNS. However, their high affinity for the 5-HT<sub>3</sub>R and  $\sigma_2$  recognition site limits their use for CNS applications [68, 69].

The aryl ketones, related to the 5-HT<sub>4</sub>R ester ligands, were prepared in an attempt to overcome their metabolic lability: replacing the ester linkage of the antagonist RS 23597 produced RS 17017 which has a similar affinity for the 5-HT<sub>4</sub>R but has partial agonist activity [70].

Increasing the size of the alkyl group led to a progressive increase in agonist activity with maximal efficacy being achieved with RS 67333 and RS 67506 [71]. They were found to have good selectivity and bioavailability. The incorporation of an n-butyl group on the piperidine ring increases hydrophobicity and, consequently, brain penetration [72, 73]. However, the compounds also bind to  $\sigma_1$  and  $\sigma_2$  receptors [71].

#### IV-2. Antagonists

In the indole class, GR 113808 was the first 5-HT<sub>4</sub>R antagonist with a high affinity for 5-HT<sub>4</sub>R and a low affinity for 5-HT<sub>3</sub>R [74]. This indole ester provided a pharmacophore model that assisted the search for other selective 5-HT<sub>4</sub>R antagonists. Furthermore, [<sup>3</sup>H]GR 113808 was the first commercially available radioligand for 5-HT<sub>4</sub>R-binding studies [75]. The indole ester analogue, GR 125487 is more potent in the CNS and in the periphery, and also has higher bioavailability, presumably because the ester linkage is more stable [76]. SB 203186 is an indole ester with potent and selective 5-HT<sub>4</sub>R antagonist properties in various physiological assays but its short half-life limits its use *in vivo* [77]. In contrast, SB 207266 is a highly potent and selective antagonist with a long duration of action after oral administration [78]. SB 207266 is a very useful tool for research in the CNS [79, 80]. Recently, the indazole carboxamide, LY 353433 was also found to be a potent and selective 5-HT<sub>4</sub>R antagonist with clinically suitable pharmacokinetics [81, 82].

Surprisingly, there are no 5-HT<sub>4</sub> receptor antagonists among the benzamides which only display agonistic activity [4]. However, it is interesting to note that when the amide is substituted by an ester, the compound becomes an antagonist. The first example was the 5-HT<sub>4</sub>R antagonist SDZ 205557 which is the ester analogue of metoclopramide [83]. Increasing the size of the substituent results in an increase in the affinity and selectivity of the drugs. RS 23597, ML 10375 and SB 204070 are good example [86]. The corresponding iodinated compound [<sup>125</sup>I] SB 207710 is an excellent radioligand for binding studies with a remarkable selectivity (>5000-fold) over the other receptor subtypes [87]. The metabolic lability of the ester function leads, however, to limitations with respect to *in vivo* studies. Lack of selectivity of the benzimidazolone derivatives (DAU 6215, DAU 6285) compromised their utility for 5-HT<sub>4</sub>R antagonist studies [88, 89]. New benzimidazole carboxamides and carboxylates have recently been synthesized by Lopez Rodriguez *et al.* [90, 91]. Some of them exhibit high affinity (sub-nanomolar) for the 5-HT<sub>4</sub> binding site and no significant affinity for the 5-HT<sub>3</sub>R.

The aryl ketone class contains molecules that present several advantages; first the replacement of the ester by a ketone provides excellent oral bioavailability. Secondly they enter the CNS following systemic administration. Over the last five years, Roche Bioscience has achieved higher affinity, selectivity and bioavailability as shown by RS 39604 which has subnanomolar affinity and is now commercially available [92] together with RS 100235 and RS 100302 [54, 93].

A new series of tricyclic benzonaphthyridine and azepinoquinoline derivatives has been synthesized (e.g. ATBI 221). They have a very high affinity for the 5-HT<sub>4</sub>R and a high selectivity over other 5-HTR subtypes and non-serotonergic receptors. The connection of the acyl group with the piperidine ring through an iminoether linkage provides high bioavailability and brain penetration [94].

#### **IV-3. Inverse agonists.**

Three specific, high affinity 5-HT<sub>4</sub>R antagonists (GR 125487, SB 207266 and ML 10375) have been reported to exert inverse agonist activity [43, 95]. Recently Roche Bioscience have synthesized three potent 5-HT<sub>4</sub>R inverse agonists, RO 116-2617, RO 116-0086 and RO 116-1148 [16]. RO 116-0086 is the des-amino, des-chloro derivative of the antagonist, SB 204070, and RO 116-1148 is the corresponding amide.

### **V. Control of neuronal ion and receptor channel activity and synaptic transmission**

5-HT<sub>4</sub>Rs have been shown to regulate several ion currents and channels in native neurons (Table 2). In colliculi neurons and in CA1 pyramidal neurons of hippocampus, 5-HT<sub>4</sub>Rs reduce the after-hyperpolarization (AHP) that follows action potentials [96-98]. In colliculi neurons, a concomitant broadening of action potentials and a reduction of spike accommodation were observed [98]. These activities are mediated by cAMP and PKA activation and contribute to increased neuronal excitability. They can be explained by inhibition of K<sup>+</sup> channels including Ca<sup>2+</sup>-activated K<sup>+</sup> channels [33, 98]. In CA1 neurons, a decrease in the calcium-induced calcium release from intracellular stores contributes to the action of cAMP on AHP [33]. In colliculi neurons, a remarkable observation was that short term application of 5-HT<sub>4</sub> receptor agonists or 8-Br-cAMP produces a long lasting inhibition of K<sup>+</sup> channels due to a long lasting blockade of phosphatases [98]. In addition to a reduction

in AHP, 5-HT<sub>4</sub>Rs also increase depolarization in hippocampal CA1 neurons; one group has provided evidence for an activation of the hyperpolarization-activated current I<sub>h</sub> by 5-HT<sub>4</sub>Rs as a mechanism of this depolarization (in mice) [34]. All these electrophysiological effects could influence neuronal excitability and facilitation of neurotransmitter release (see section VI).

Recently, two reports described the effects of 5-HT<sub>4</sub>Rs on GABA<sub>A</sub> receptor activity and GABAergic synaptic transmission. In prefrontal cortex pyramidal neurons, 5-HT<sub>4</sub>Rs have a bi-directional (activation and inhibition) regulation of GABA<sub>A</sub> receptor channels and GABAergic synaptic transmission (measured by the amplitude of the spontaneous GABA<sub>A</sub> receptor mediated inhibitory postsynaptic potentials (IPSPs)) [37]. The authors present evidence for a role of PKA as well as AKAP (A Kinase Anchoring Proteins) in both effects. In guinea-pig dentate gyrus neurons, including granule cells, 5-HT<sub>4</sub>Rs have a different effect on GABAergic transmission. Their activation increases the frequency of GABA<sub>A</sub>-mediated IPSPs [36]. This is a pre-synaptic effect. The latter can be explained by the effects on K<sup>+</sup> channels described above and by data showing a stimulation of GABA release by 5-HT<sub>4</sub>R agonists (see section VI).

A conditional regulation of dorsal raphe nucleus 5-HT neuronal activity has also been observed following intravenous injection of 5-HT<sub>4</sub>R ligands. When the basal firing rate of raphe neurons was high, 5-HT<sub>4</sub>R agonists and antagonists enhanced and reduced the firing rate, respectively [99]. In contrast, when the firing rate of the raphe neurons was low, the same drugs were inactive. These results indicate that 5-HT<sub>4</sub>Rs exert both a tonic and a phasic positive, frequency-related control of 5-HT neurons raising the possibility that 5-HT<sub>4</sub>R may constitute a possible therapeutic target to treat depression.

All these results favor an intriguing role for 5-HT<sub>4</sub>Rs in the fine tuning of neuronal activity. They clearly indicate that their physiological effect may depend on the nature and activity of the neurons in which they are expressed, perhaps explaining why 5-HT<sub>4</sub>R ligands are active in very specific situations (morphine-treated animals, aged animals, scopolamine-induced cognitive deficits, for example).

The long-term potentiation (LTP) of the Schaffer collateral-CA1 synaptic transmission, as well as the population spike amplitude in the CA1 field evoked by Schaffer collateral

stimulation is enhanced by the 5-HT<sub>4</sub>R agonist, SC 53116 [35]. The 5-HT<sub>4</sub>R augmented LTP effect was blocked both by a 5-HT<sub>4</sub>R antagonist as well as by the muscarinic antagonist, scopolamine. These findings suggest functional interactions between the serotonergic system mediated via 5-HT<sub>4</sub>Rs and cholinergic systems associated with cognitive processes.

A very interesting effect of 5-HT<sub>4</sub>Rs on a subpopulation of DRG (dorsal root ganglion) neurons has been described [101]. These DRG are sensitive to capsaicin and express tetrodotoxin (TTX)-insensitive Na<sup>+</sup> channels. They represent subclasses of A $\delta$ - and C-type afferent nociceptors. 5-HT via 5-HT<sub>4</sub>Rs and prostaglandins increases the activity of these particular Na<sup>+</sup> channels. The mechanisms by which 5-HT<sub>4</sub>Rs act are unclear. The 5-HT effect was delayed by 20-30s and was not mimicked by cAMP. This suggests that 5-HT<sub>4</sub>Rs may be involved in the generation of hyperesthesia.

## **VI. Control of neurotransmitter release**

### **VI-1. Acetylcholine release**

In line with the effects of 5-HT<sub>4</sub>Rs on K<sup>+</sup> channels and neuronal excitability, there are numerous reports demonstrating the ability of 5-HT<sub>4</sub>Rs to modulate the release of various neurotransmitters. Because of earlier suggestions that these receptors may be implicated in cognitive functions, and the fact that they facilitate ACh release in gastro-intestinal tract, central ACh release has been particularly studied. Consolo *et al.* were the first to report that i.c.v. injections of the 5-HT<sub>4</sub>R agonist / 5-HT<sub>3</sub>R antagonist, BIMU-1 caused ACh release in the frontal cortex [39]. Yamaguchi *et al.* raised extracellular 5-HT with the 5-HT re-uptake blockers (citalopram or indeloxazine) or a releaser (p-chloroamphetamine) and found that in the frontal cortex, the 5-HT<sub>4</sub>R antagonists, RS 23597 and GR 113808, blocked the associated release in ACh [104]. In the hippocampus, one report found no ACh release following i.c.v. injection of BIMU-1 [39], whereas another group found an increase in ACh release by a local application of the agonist, SC 53116 [35]. As already discussed, the same authors found that the SC 53116-induced enhancement of LTP was mediated by a cholinergic mechanism. In guinea pig brain slices, BIMU-8 increases the electrically-stimulated ACh outflow in cerebral cortex, hippocampus and nucleus basalis magnocellularis slices [105]. In the hippocampus, 5-HT<sub>4</sub>Rs induce ACh release and stimulate and inhibit GABA release via muscarinic M1 and M4 receptors, respectively [106]. 5-HT<sub>4</sub>R antagonists did not decrease ACh release under

basal conditions. This indicates that 5-HT<sub>4</sub>R do not exert a tonic effect on cholinergic neurons.

## **VI-2. Dopamine release**

There are numerous studies that have reported an effect of 5-HT<sub>4</sub>R agonists on DA release. However, a simple picture of the results is difficult to make due to the various experimental conditions used in the studies and also some contradictory results. In general, *in vitro* and *in vivo* studies have reported that when 5-HT or 5-HT<sub>4</sub>R agonists are locally applied to the striatum or substantia nigra, a 5-HT<sub>4</sub>R-mediated increase in DA release is observed (see [25] [99, 107]). Systemic administration of 5-HT<sub>4</sub>R agonists and antagonists is usually ineffective. Striatal DA release induced by haloperidol or morphine but not that induced by cocaine or amphetamine, is reduced by intraperitoneal injection of 5-HT<sub>4</sub>R antagonists and increased by intraperitoneal injection of a 5-HT<sub>4</sub>R agonist [107, 108]. Using identical protocols, mesoaccumbal dopaminergic function was not regulated by 5-HT<sub>4</sub>R ligands. To be able to specifically activate either the nigro-striatal or the mesoaccumbal pathways may be of great therapeutic interest. However, note that systemic injection or local perfusion of the 5-HT<sub>4</sub>R antagonist, SDZ 205557 into the nucleus accumbens shell inhibits cocaine-induced hyperactivity. This result could be explained by an effect of SDZ 205557 on 5-HT<sub>3</sub>R or by an unknown indirect effect [109].

The cellular mechanisms by which the state-dependent facilitatory control of the nigro-striatal pathway occurs are still unclear. The finding that 5-HT<sub>4</sub>R are able to influence DA release in striatal slice preparations indicates that they may control local circuitry within the striatum (cholinergic or GABAergic neurons) (see [25]). Indeed, 5-HT<sub>4</sub>R are present on cell bodies of striatal neurons. However, the finding that 5-HT<sub>4</sub>R modulate the firing rate of nigral DA neurons raises the possibility that striatal 5-HT<sub>4</sub> receptors also affect DA release through an indirect control of DA neurons [107] via modulation of the activity of striatal efferent GABAergic neurons (striato-pallidal or striato-nigral).

## **VI-3. Serotonin release**

The activation of 5-HT<sub>4</sub>R increases 5-HT release in the hippocampus while the 5-HT<sub>4</sub>R antagonists, GR 113808 and GR 125487, reduced basal 5-HT release. This indicates the presence of an endogenous tone on 5-HT<sub>4</sub>R in this preparation [110], perhaps consistent with

the finding that 5-HT<sub>4</sub> binding sites increase in number after the innervation by serotonin neurons has been abolished [29].

## **VII. 5-HT<sub>4</sub>Rs: potential therapeutic CNS drugs targets**

### **VII-1. Cognition**

We proposed several years ago that 5-HT<sub>4</sub>Rs may have pro-cognitive properties [113, 114]. More recent data support this possibility [35, 100]. 5-HT<sub>4</sub>Rs are localized in numerous structures, such as hippocampus and cortex, that are considered important for mnemonic processes. In the hippocampus, localization and functional studies indicate that they play a modulatory role on LTP formation. The latter effect suggests the involvement of a cholinergic relay. 5-HT<sub>4</sub>Rs stimulate ACh release in frontal cortex and hippocampus. There is evidence for a loss of 5-HT<sub>4</sub>Rs in Alzheimer's disease in the cortex (-23%) and, particularly, in the hippocampus (-66%) [31]. Furthermore, in transfected CHO cells, 5-HT<sub>4</sub>Rs were found to stimulate the secretion of non-amyloidogenic soluble amyloid precursor protein (sAPP $\alpha$ ), thought to have neuroprotective and memory enhancing effects [115]. Behavioral experiments have generally confirmed a pro-cognitive effect of 5-HT<sub>4</sub>R ligands with several aspects of memory performance being modulated.

#### **1. Memory acquisition**

- BIMU-1 (5-HT<sub>4</sub> agonist / 5-HT<sub>3</sub> antagonist) increased social olfactory memory in rats when administered before training. This effect was reversed by GR 125487, a specific 5-HT<sub>4</sub>R antagonist [116].
- BIMU-1 and BIMU-8 enhanced acquisition of an auto-shaping task when injected before training. This effect was blocked by GR 125487 [117].
- BIMU-1 and BIMU-8 as well as the specific 5-HT<sub>4</sub>R partial agonist, RS 67333, induce a substantial improvement of acquisition of an olfactory associative memory when injected before training [118, 119] (Fig. 2).
- RS 67333 improved the acquisition of place and object recognition memory in young and old rats. This effect was blocked by GR 125487 [120].
- RS 67333 accelerated learning acquisition in the Morris water maze task when the inter-trial intervals were high (2 h), but not low (30sec). This indicates that



5-HT<sub>4</sub>R agonists can improve memory processes in a high demand memory task [121].

- RS 17017 (a relatively hydrophilic 5-HT<sub>4</sub>R agonist) administered orally and chronically enhanced delayed matching performance in young as well as in old macaques [122].

## 2. Memory deficit

- RS 67333 reversed the performance deficit induced by atropine in the Morris water maze test, an effect eliminated by the 5-HT<sub>4</sub>R antagonist, RS 67532 [73].
- RS 67333 and, to a lesser extent, RS 17017, reversed the deficit in the acquisition of olfactory associative memory induced by the 5-HT<sub>4</sub> antagonist, RS 67532 [119] (Fig. 2).
- The 5-HT<sub>4</sub>R agonist, SC 53116 improved the scopolamine-induced deficit in the passive avoidance test [35].
- BIMU1 and RS 67333 reversed scopolamine-induced impairments in spontaneous working memory, confirming a role for 5-HT<sub>4</sub>R in cholinergically-mediated memory processes.

Taken together, the results suggest that there could be a potential therapeutic role for 5-HT<sub>4</sub> agonists in restoring memory deficits related to short-term working memory disorders [123].

## 3. Memory consolidation

The effect of 5-HT<sub>4</sub>R ligands on memory consolidation is less clear. Meneses and Hong [117] reported that BIMU-1 and -8 have a negative effect on an auto-shaping learning task when injected during the consolidation phase. In contrast, Lamirault and Simon [120] found an increased consolidation of object recognition in old rats with RS 67333.

Sanofi-Synthelabo is currently testing SL 65.0155 (Phase II) in an attempt to treat dementia of the Alzheimer type. The compound does not induce any change in either rat gastrointestinal peristalsis or in rat and dog cardiac rhythm [48]. In particular, SL 65.0155 has been found to alleviate cognitive deficits in aged rats using a linear maze test, as well as scopolamine-induced deficits in Morris water maze performance [48]. Moreover, SL 65.0155 and the cholinesterase inhibitor rivastigmine have a synergic effect in the object recognition test (Fig.

2) and in linear maze performances in aged rats [48]. Similarly, combined treatment with galanthaminium, a new cholinesterase inhibitor and RS 67333, enhanced place and object recognition in young and aged rats [124]. These synergisms are particularly interesting as they suggest a possible co-treatment in Alzheimer's disease and offer the possibility of reducing the therapeutic dose of cholinesterase inhibitors and, consequently, their side effects.

### **VII-2. Pain**

A centrally-mediated antinociceptive effect of both the 5-HT<sub>4</sub>R agonists and the 5-HT<sub>3</sub>R antagonists BIMU-1 and -8 has been reported following noxious thermal (hot-plate test), chemical, (abdominal constriction test) and mechanical (paw pressure test) stimuli [125]. However, the effects of BIMU-1 and -8 may be related to their inactivation of 5-HT<sub>3</sub> receptors; although the 5-HT<sub>3</sub>R / 5-HT<sub>4</sub>R antagonist, SDZ 205557, and a new selective 5-HT<sub>4</sub>R antagonist, ATBI 221, have been found to reduce visceral pain [94, 126]. An increase in 5-HT<sub>4</sub>R mRNA has been found in dorsal root ganglia following hind paw inflammation. The partial 5-HT<sub>4</sub>R agonist, tegaserod (Zelnorm<sup>TM</sup>), which could be acting as an antagonist, is reported to reduce the symptoms of abdominal pain, bloating and constipation [58, 127]. Tegaserod may produce abdominal analgesia *via* a reduction in the activity of extrinsic spinal afferents to the colon. Overall, the results suggest that an inactivation of 5-HT<sub>4</sub>Rs may decrease some types of clinical pain.

### **VII-3. Anxiety and Stress**

The selective 5-HT<sub>4</sub>R antagonists SB 204070, SB 207266 and GR 113808 display anxiolytic-like effects in the elevated plus-maze test, albeit at high doses [128, 129]. In contrast, both SDZ 205-557 and SB 204070 have been reported to attenuate the effects of diazepam and did not modify behavior when administered alone [130]. Similarly, stress-induced anxiety-like behavior, was enhanced in the 5-HT<sub>4</sub>R knockout male mice, again using the elevated plus-maze test ([131] and in preparation). Complex and bi-directional modulation of GABAergic systems by 5-HT<sub>4</sub> receptors, as described by Cai *et al.*, may account for such discrepancies [37].

### **VIII-4. Reward and motor behavior**

The high density of 5-HT<sub>4</sub>Rs in the striatum and nucleus accumbens suggests an involvement in the control of reward and motor behavior. However, the supporting data is not clear and is often contradictory. Under basal conditions, no effect on locomotion has been detected

following the administration of the partial 5-HT<sub>4</sub> receptor agonists, RS 67333 or RS 67506 [73, 109]. Similarly, the 5-HT<sub>4</sub>R antagonist, SB 204070 did not alter amphetamine-induced changes in rat locomotor activity or rotational behavior induced by the unilateral lesion of the nigrostriatal dopaminergic projection [132]. Conversely, it has been shown that hyperlocomotion following 5-MeOT administration was abolished or attenuated by local injection into the hippocampus of the 5-HT<sub>4</sub>R antagonists GR 125487, SB 204070 or RS 23597 [133]. We have further found that 5-HT<sub>4</sub>R knockout mice do not exhibit a change in locomotion in their home cage but show a marked hyposensitivity to novelty-induced locomotion in the open-field, elevated plus-maze and alley tests. Given the high levels of 5-HT<sub>4</sub> receptors in the shell of the nucleus accumbens [29] and taking into account that 5-HT<sub>4</sub>R null mice have been found to be less sensitive to stress-induced anorexia [131], it is hypothesized that 5-HT<sub>4</sub>R null mice will be less sensitive to the motor, rewarding and/or addictive properties of cocaine.

## **Conclusion**

Although 5-HT<sub>4</sub>Rs are the targets for earlier (metoclopramide, cisapride) or recent (tegaserod) therapeutic drugs to treat gastrointestinal diseases, as yet no 5-HT<sub>4</sub>R ligands are used to treat CNS disorders. This is probably due to the lack of availability of specific and stable drugs able to cross the blood brain barrier (Table 3). To our knowledge, SL 65.0155, which is being tested for dementia of the Alzheimer types [48], is the only 5-HT<sub>4</sub> receptor ligand in development (phase II) for a CNS disorder. However, in the light of the emerging profile of the behavioral pharmacology of 5-HT<sub>4</sub> receptor ligands, the possibility that they may also be of therapeutic value in pain, especially of intestinal origin, anxiety and other stress-related disorders cannot be dismissed.

## Legends to Tables

### TABLE 1

#### **Exon-intron boundaries of the human 5-HT<sub>4</sub> receptor gene.**

From [18, 19]. Completed using NCBI Human Genome Resources:

(<http://www.ncbi.nlm.nih.gov/genome/guide/human/>) and Model Maker for HTR4.

This table presents all the identified splicing sites and the corresponding exons in the human 5-HT<sub>4</sub>R gene. Exons 1 to 25 correspond to the 5'-untranslated region (5'UTR) and promoter region of the gene. Exons 26, 27, 28, 29, and 31 are present in all the splice variants cloned and constitute the common part of the receptor. Exon 30 and exons 31' to 38 are alternatively spliced to produce the 8 human splice variants cloned to date.

Splice donor and acceptor sequences between exons (uppercase) and introns (lowercase) are given in bold. The size of exons and introns are indicated.

ND: not determined.

### TABLE 2

#### **5-HT<sub>4</sub>Rs signaling in excitable cells and cell lines. For details see the text**

### TABLE 3

#### **5-HT<sub>4</sub>Rs ligands that could be of interest in CNS research**

A range of affinities taken from the literature (pK<sub>i</sub> and pEC<sub>50</sub>) is indicated for several ligands.

(F): full agonist; (P): partial agonist; (S): specific; (HS): highly specific; (1): commercially available; CNS: useful in CNS research.

## Legend to figures

### FIGURE 1

#### Human 5-HT<sub>4</sub> receptor splice variants and structure of the gene

**A.** C-terminal amino acid sequences and schematic representation of the human splice variants. The cloned 5-HT<sub>4</sub>Rs have identical sequences up to Leu<sup>358</sup> and differ by the length and composition of their C-terminal domain. The 5-HT<sub>4h</sub> variant, which has an insertion of 14 residues in the second extracellular loop, has only been isolated in combination with the b isoform. This variant is called 5-HT<sub>4hb</sub>.

**B.** Organization of the human 5-HT<sub>4</sub>R gene. From [18] and [19]. Completed using NCBI Human Genome Resources: (<http://www.ncbi.nlm.nih.gov/genome/guide/human/>) and Model Maker for HTR4.

**Upper panel:** The locations of exons are indicated as filled bars with numbers denoted over or under the bars. Letters indicate C-terminal splice variant exons. The discontinuous part of the thick line indicates large gaps in intron 17 (>10kb) and 20 (>100kb). ATG indicates the position of the translation start site. The location of the β<sub>2</sub>-adrenergic receptor gene (β<sub>2</sub> AdR) is shown over the solid bar with an arrow indicating the transcriptional direction.

**Middle panel:** Schematic representation of the exons (boxes) present in the coding sequences of 5-HT<sub>4</sub>R variants. The exons in gray are always present and constitute the common part of all the receptor splice variants. Letters indicate which exon is conserved to generate the corresponding isoform.

**Left insert:** Variant n is generated by the use of an alternative 5'-donor site for intron 31. An in-frame stop codon leads to the shorter C-terminal domain. Exons are in uppercase, introns are in lower case.

**Right insert:** The use of different 3'-acceptor sites for intron 34 generates three different exon possibilities (35, 36 and 37) and three corresponding splice variants (g, e and f). Exons are in uppercase, introns are in lower case.

### FIGURE 2

#### 5-HT<sub>4</sub>Rs and cognition

This figure illustrates two experiments showing the positive effects of 5-HT<sub>4</sub>Rs in cognition.

**Olfactory associative task:** In this task, rats, deprived of water for 48 hours, were trained to make two odor-reward associations. Each odor had to be associated with a specific reward, one arbitrary designed as positive (S+) and the other as negative (S-). Rats had to approach

the odor and water ports to interrupt the light beam only when S+ is delivered. Response to odor designated as negative resulted in presentation of non-aversive light, and water was not delivered. Individual trials were presented in a quasi-random fashion. A daily session was made up of 60 trials and five sessions were made with one session per day. The latency to give a response was analyzed and given as mean latencies in seconds for the positive and negative odors. In controls a significant latency difference between S+ and S- was only observed from session 4. After injection of the partial agonist RS 67333 the difference was observed after session 3. After injection of the antagonist RS 67532 the difference was only observed after session 5 whereas a situation similar to the control was observed when both the partial agonist and the antagonist were co- injected (modified from [119]).

**Synergy between subliminal doses of a 5-HT<sub>4</sub> partial agonist (SL 65.0155) and an inhibitor of cholinesterase (rivastigmine) in the object recognition test:** Each experiment consisted of 3 sessions. During the first session the animals were allowed to become familiar with the box. Twenty-four hours later, the animals were again placed in the enclosure in the presence of two identical objects for the amount of time necessary to spend 20s exploring these objects. After another interval of 24 hours, the rats were again placed in the box with a previously presented object and a novel object. Time spent exploring the familiar object and the novel object was measured. Drugs (SL 65.0155: 0.1  $\mu$ g/kg po and rivastigmine: 0.03mg/kg ip) were administered separately or together before each session (modified from [48]).

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Exon number	Positions in cDNA	Exon size (bp)	3'-Acceptor site	5'-Donnor site	Intron size (kpb)
1	1-193	193		GGGCCCAG <b>gt</b> gagttt	10
2	194-292	99	ttctcc <b>ag</b> GTAAAGAA	TAATCCAG <b>gt</b> atccaa	4.3
3	293-420	128	tcttta <b>ag</b> ACCTGACA	TGTTTAAG <b>gt</b> gagcat	3.4
4	421-526	106	ttacgt <b>ag</b> GACCTCTC	TAATTTAG <b>gt</b> atggta	1.7
5	527-670	144	cttctt <b>ag</b> GCTACGTA	ACAGGAAG <b>gt</b> gagtgt	1.1
6	671-872	202	atccac <b>ag</b> GCCACTTC	TTCCATCAG <b>gt</b> gagtag	0.8
7	873-946	74	attggc <b>ag</b> GTGGTTCC	TCAGATTG <b>gt</b> atggct	2.1
8	947-1142	196	ttccac <b>ag</b> GCAGAGGA	TCCCCAAT <b>gt</b> gagtat	6.6
9	1143-1276	134	cgatgc <b>ag</b> GAGCACGA	CCGGCTCAG <b>gt</b> gagtgt	2.9
10	1277-1318	42	ctactt <b>ag</b> GTGGGTTT	TCTGAGT <b>gt</b> gagtag	0.1
11	1319-3013	1695	cettac <b>ag</b> CATCCCAG	TCTAAAG <b>gt</b> aagtat	1.8
12	3014-3194	181	gtgttc <b>ag</b> GTCAGCTT	ACCGCCAG <b>gt</b> gagtca	15
13	3195-3344	150	gtcccc <b>ag</b> GTCCCTCA	GCCTGCAG <b>gt</b> aggcct	2.2
14	3345-3467	123	ccacct <b>ag</b> GCAGCCAT	ACTACCG <b>gt</b> aagtcc	1.3
15	3468-3618	151	gttctt <b>ag</b> GCTGGAGC	AGTCACAG <b>gt</b> cacagg	1.8
16	3619-3819	201	attctt <b>ag</b> GAGATCAG	AGCTGAAG <b>gt</b> aagagc	3.0
17	3820-3938	119	tgagac <b>ag</b> TTCCTCCT	AACTACAG <b>gc</b> aggtgc	>50
18	3939-4151	213	atthgt <b>ag</b> GGCAGTTG	AAGGCAAG <b>gt</b> aagtaa	30.5
19	4152-4351	200	tctgat <b>ag</b> ATGGCTTC	GGTAAAG <b>gt</b> gaatat	0.5
20	4352-4418	67	tcacac <b>ag</b> CAAAAGAC	CCAGAACT <b>gt</b> aagaaa	>240
21	4419-4672	254	tatttc <b>ag</b> TTCCTATG	TAAGTGAG <b>gt</b> gatgag	2.5
22	4673-4716	44	tctcat <b>ag</b> GTCCATAG	CATTCAAG <b>gt</b> aacata	7.5
23	4717-4758	42	aactac <b>ag</b> AGGGAGAG	GATTGAAG <b>gt</b> aagtth	1.8
24	4759-5006	248	tcttca <b>ag</b> GTACTTTA	GCCTGGAG <b>gt</b> gagttg	2.9
25	5007-5088	82	tcctct <b>ag</b> AGACCTCT	TCCAAAAG <b>gt</b> agatct	25
26	5089-5161	73	catttt <b>ag</b> GACCCTGT	AATGTGAG <b>gt</b> atgtta	86
27	5162-5287	126	tctttc <b>ag</b> TTCCTGAGG	CAGCTCAG <b>gt</b> gagcag	1.3
28	5288-5488	201	actcac <b>ag</b> GAAAATAA	CTGGATAG <b>gt</b> aaggac	25
29	5489-5642	154	ctctcc <b>ag</b> GTATTACG	TTGATTT <b>gt</b> gagtat	2.2
30	5643-h	42	tttca <b>ag</b> GAAAGGAG	TTCATGCG <b>gt</b> atgtca	11
31	5643-6211	569	actctc <b>ag</b> ATAGAAAA	GTACTAAG <b>gt</b> gagtat	26
31'	5643-n	708	actctc <b>ag</b> ATAGAAAA	TACTATCT <b>ggt</b> catgg	-
32	6212-b	234	ctctct <b>ag</b> GGATGCAG	TCGCTGGG <b>ct</b> tttctct	1.4
33	6212-c	≥160	tacttt <b>ag</b> TTCCTGGAA	ND	≤4.7
34	6212-d	110	cctacc <b>ag</b> ATTTTGAG	TGGATGT <b>att</b> gtttg	11
35	6212-g	76	actaaa <b>ag</b> TGGCTGTT	AGTGCTCT <b>gt</b> aagtct	-
36	6212-e	55	GTCTCC <b>ag</b> CTTCCTCC	AGTGCTCT <b>gt</b> aagtct	-
37	6212-f	31	TGCAAT <b>ag</b> ACCAGTTC	AGTGCTCT <b>gt</b> aagtct	14
38	6212-a	157	ctttct <b>ag</b> GTACACCG	CCTGGCAT <b>cact</b> ttctg	

Table 1

Receptor variants (when known)	G protein	Second messenger	Ionic current Small G protein	Type of cell
<b>NEURONAL CELLS</b>				
	G <sub>s</sub>	↗ cAMP	⊖ AHP	CA <sub>1</sub> , colliculi neurons
	G <sub>s</sub>	↗ cAMP	⊖ K <sup>+</sup> channels	CA <sub>1</sub> , colliculi neurons
	G <sub>s</sub>	↗ cAMP	⊖ GABA <sub>A</sub> current (PKA-high)	Pyramidal cortical neurons
	G <sub>s</sub>	↗ cAMP	⊕ GABA <sub>A</sub> current (PKA-low)	Pyramidal cortical neurons
	G <sub>s</sub>	↗ cAMP	⊕ I <sub>h</sub> (see text for discussion)	CA <sub>1</sub> neurons
	?	?	⊕ TTX-insensitive Na <sup>+</sup> channels	DRG neurons (Aδ and C types)
<b>CARDIAC CELLS</b>				
	G <sub>s</sub>	↗ cAMP	L-Type Ca <sup>++</sup> channels	Atrial myocytes
<b>TRANSFECTED CELLS</b>				
5-HT <sub>4b</sub>	G <sub>i</sub> / G <sub>o</sub>	↘ cAMP	—	HEK-293
5-HT <sub>4a</sub>	?	?	Ca <sup>++</sup> channels	HEK-293
5-HT <sub>4a</sub>	G <sub>13</sub>	?	rhoA	Sf9, NIE-115, NIH 3T3
5-HT <sub>4b</sub>	G <sub>s</sub>	↗ cAMP	ras (ERK1/2), rap1	COS-cells

**Table 2**

	pK <sub>i</sub> (binding)	pEC <sub>50</sub>	Efficacy	Selectivity	Bio-availability	Status
<b>AGONISTS</b>						
5-HT	7.1 - 7.5	7.4 - 8	(F)	None		(1)
5-MeOT	6.2	7 - 7.7	(F)	5-HT <sub>4</sub> >>5-HT <sub>3</sub>		(1)
HTF919	8	8.8	(P)	S		Zelnorm™ (USA)
Cisapride	7.1	7.25	(F/P)	5-HT <sub>2</sub> , α <sub>1</sub> AR		
SC 53 116	7.5 - 8.1	7.64	(F)	S		
SB 205 149		8	(F/P)	S		
Prucalopride	8 - 8.6	7.5 - 7.8	(P)	HS		
Mosapride	6.8 - 7	7.1	(P)	S		
Y-34959	8.5	8.5	(F)	HS	Long duration	
ML10302	7.9 - 9	7.7 - 8.6	(P)	HS	Short duration	
LS-65 0155	8.78	6.7 - 8.3		HS	Long duration	Phase II, CNS
RS 67333	8.7	8.4	(P)	HS	Long duration	(1), CNS
RS 67506	8.8	8.6	(P)	HS	Long duration	(1), CNS
BIMU 1	6.4 - 7	6.5 - 8.4	(P/F)	LS	Long duration	
BIMU 8	7 - 7.5	7.1 - 8.1	(F)	LS	Long duration	
<b>ANTAGONISTS</b>						
GR 113308	9.2 - 9.8	8.8 to 9.5		S	Short duration	(1) [ <sup>3</sup> H], CNS
GF 125487	10 - 10.4	9.75 - 10		HL	long duration	CNS
SB 203186	9	8.3 to 8.7		HS		(1)
LY 353433				HS	Long duration	CNS
SB 207 266	9.5 - 10	10.6		HS	Long duration	Phase II, CNS
SB 204070	9.2	10.5		HS	Short duration	(1) [ <sup>125</sup> I], CNS
RS 23597	7.8	8.4		LS		(1)
ML 10375	8.5 - 9	9		HS	Short duration	
ATBI 221	8.54	8.3		HS	Long duration	CNS
RS 39606	9.1	9.3		HS	Long duration	(1), CNS
RS 67532	8.7	8.8		HS	Long duration	(1), CNS
RS 100235	10	11.2		HS	Long duration	
RS 100302	10.5	11		HS		
<b>INVERSE AGONISTS</b>						
RO 116-2617	9.6	10.5		HS		
RO 116-0086	9.5	9.5		HS		
RO 116-1148	9.32	9.5		HS		

Table 3

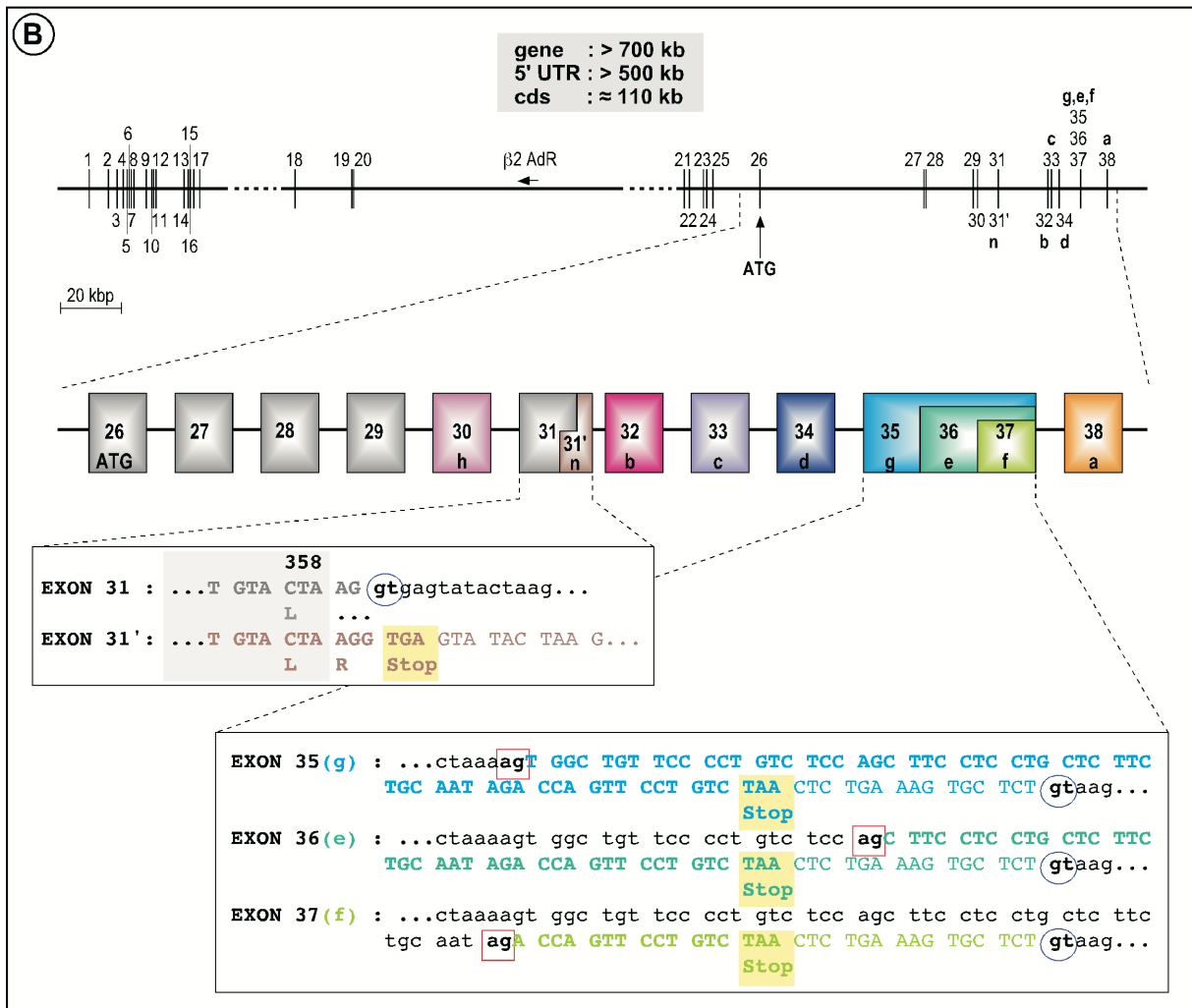
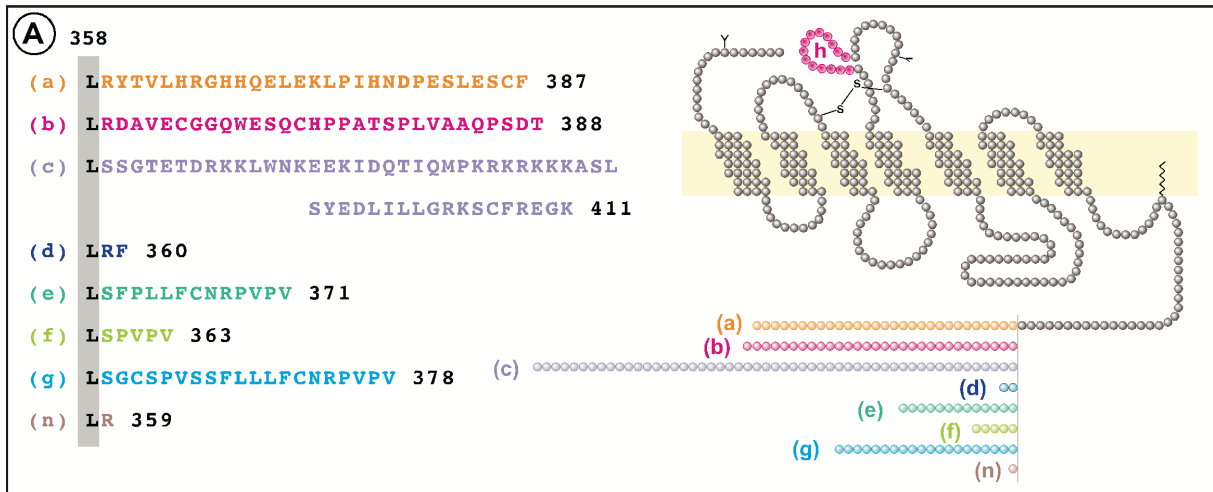


Figure 1

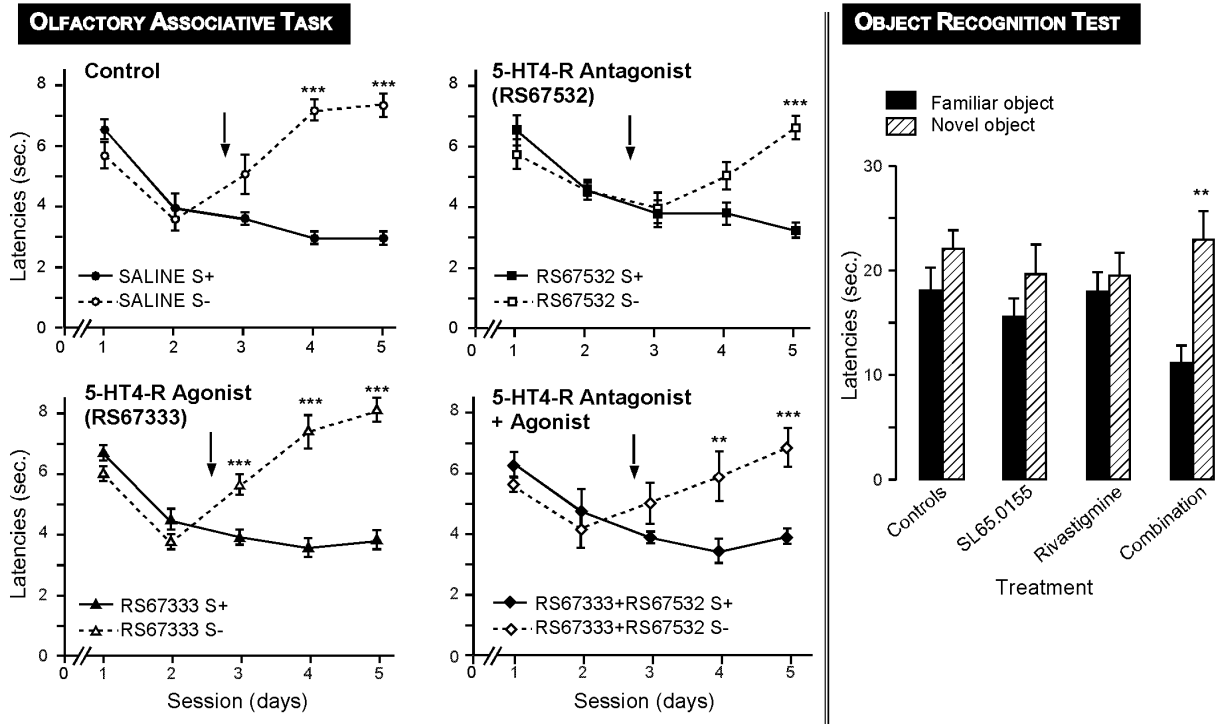


Figure 2