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Neuronal 5-HT metabotropic receptors: fine-tuning of their structure, signaling, and roles in synaptic modulation

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Abstract

Serotonin (5-hydroxytryptamine, 5-HT) is, without doubt, the neurotransmitter for which the number of receptors is the highest. Fifteen genes encoding functional 5-HT receptors have been cloned in mammalian brain. 5-HT₃ receptors are ionotropic receptors, whereas all the others are metabotropic G-protein-coupled receptors (GPCRs). 5-HT receptor diversity is further increased by post-genomic modifications, such as alternative splicing (up to 10 splice variants for the 5-HT₄ receptor) or by mRNA editing in the case of 5-HT_{2C} receptors. The cellular and behavioral implications of 5-HT_{2C} receptor editing are of great physiological importance. Signaling of 5-HT receptors involves a great variety of pathways, but only some of these have been demonstrated in neurons. The classical view of neurotransmitter receptors localized within the synaptic cleft cannot be applied to 5-HT receptors, which are mostly (but not exclusively) localized at extra-synaptic locations either pre- or post-synaptically. 5-HT receptors are engaged in pre- or post-synaptic complexes composed of many GPCR-interacting proteins. The functions of these proteins are starting to be revealed. These proteins have been implicated in targeting, trafficking to or from the membrane, desensitization, and fine-tuning of signaling.

Keywords: Serotonin; Serotonin receptors; G-protein-coupled receptors; Receptosome; Signaling

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is certainly one of the neurotransmitters that the general public knows the best but is also the most intriguing for researchers. The name “serotonin” retained in the literature indicates its presence in the serum (released from platelets; Rapport *et al.* 1948); however, 5-HT has also been called “enteramine” because of its early recognized presence, at high concentrations, in enterochromaffin cells (95% of 5-HT is found in platelets and the gastro-intestinal tract; Erspamer and Asero 1952). Serotonin is one of the oldest signaling substances to appear during evolution in coelenterates, arthropods, molluscs, tunicates, and vertebrates (Collier 1958). Unsurprisingly, therefore, serotonergic neurons are also among the earliest neurons to be generated during mammalian development, with 5-HT being released before conventional synapses are established (Gaspar *et al.* 2003). 5-HT is well known by the public, thanks to its roles in normal physiology including sleep, appetite, thermoregulation, and pain perception, and more importantly in human pathologies such as migraine, depression, fear and anxiety, obsessive compulsive disorders, schizophrenia, and addiction. A provocative role for serotonin systems in spiritual experiences has also been proposed in a recent article, published in a respected journal, by Borg *et al.* (2003), although whether this can be taken seriously remains debatable. 5-HT, like a few other neurotransmitters such as acetylcholine, glutamate, and γ -aminobutyric acid (GABA), acts via two categories of receptors: ionotropic and metabotropic receptors. Ionotropic receptors (channel receptors) have a low affinity for their neurotransmitter ligand but a rapid activation constant (a few milliseconds). In contrast, metabotropic receptors (receptors acting through G protein activation and second messenger production) exhibit both a high affinity for their neurotransmitter and a slow activation constant (in seconds or longer). Fifteen genes encoding 5-HT receptors (5-HTR) have been cloned in the mammalian brain (Hoyer and Martin 1997). Recently, three novel 5-HT₃-like genes (5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E}) have been identified (Niesler *et al.* 2003), but to date, no functional analysis has been published. The 5-HT₃ receptors are ionotropic receptors (Van Hooft and Yakel 2003) and will not be discussed in this review. The others are G-protein-coupled receptors (GPCRs) with seven transmembrane domains. They can be categorized into four groups according to their main second messenger system: the 5-HT₁ receptors coupled to $G_{\alpha_i}/G_{\alpha_o}$ proteins (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}); the 5-HT₂ receptors coupled to G_{α_q} proteins (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}); the 5-HT₄, 5-HT₆, and 5-HT₇ receptors coupled to G_s proteins; and the 5-HT₅ receptors (5-HT_{5A} and 5-HT_{5B}) for which the coupling is still uncertain (Raymond *et al.* 2001). The diversity of metabotropic 5-HTR proteins is further increased by post-genomic modifications, such as alternative splicing and mRNA editing. This will be the subject of the first part of this review. Their neuronal localization, including the synaptic localization when known, and their signaling pathways will be discussed in the second section. The third part deals with the nature and functions of a variety of GPCR-interacting proteins (GIPs; Bockaert *et al.* 2003, 2004b) that form, together with 5-HTRs, “receptosomes” implicated in their targeting and trafficking and in the fine tuning of their signaling properties. For lack of space, this review will often take illustrative examples rather than being exhaustive.

2. Genomic and post-genomic complexity of 5-HT metabotropic receptor structure

2.1. Gene structure

Metabotropic 5-HTRs are generated from 12 different genes in the human genome (NCBI, Map Viewer at <http://www.ncbi.nlm.nih.gov/mapview/>). These genes diverged from a common ancestor, and their structure reflects their evolutionary relationship (Barnes and Sharp 1999). 5-HT₁Rs are coded by intronless genes and clearly constitute a subgroup of genes that were derived from a duplicated ancestor (Fig. 1). 5-HT₂R genes are also closely related to two similar introns located at the end of transmembrane domain 2 (TM2) and within TM4-coding sequences. The 5-HT_{2C}R gene presents an additional intron in the extracellular domain of the coding sequences and extends over more than 183kb. Alternative splicing of the 5-HT₂R genes does not generate any functional splice variant (Xie *et al.* 1996). The 5-HT₄R gene is far more complex. The coding sequence extends over more than 185 kb and includes at least 14 exons leading to multiple splicing combinations. To date, ten functional splice variants have been described that can be generated by alternative splicing of the human transcript (Bender *et al.* 2000; Hiroi *et al.* 2001). For nine of these variants, splicing gives rise to alternate C-terminal domains (Fig. 1). As some exons (such as the h or the i exons), in the 5-HT₄R gene, do not present any in-frame stop codons, many additional combinations probably remain to be discovered (Brattelid *et al.* 2004). The relevance and the functional role of such a multiplicity of isoforms also remain to be elucidated. The finding that the less-well represented 5-HT₄R splice variants (e.g., 5-HT_{4(a)}R and 5-HT_{4(e)}R) interact with specific intracellular proteins supports the view that these isoforms are in charge of the fine regulation of signal transduction and are not the result of transcriptional leakage (Joubert *et al.* 2004). Two 5-HT₅R genes have been described in the rodent, but only the 5-HT_{5A}R can be generated in humans (Rees *et al.* 1994). A 5-HT_{5B}R pseudogene had been localized on chromosome 2 in Build 35.1 of the human genome. However, this annotation has been suppressed in the actual build (36.1). The 5-HT₆R gene presents a splicing site that lies in the middle of the intracellular loop 3 and that is similar to the splicing site found in the 5-HT_{5A}R gene. Two separate exons encode TM6 and TM7 of the 5-HT₆R. A non-functional splice variant has been described for this receptor (Olsen *et al.* 1999). The human 5-HT₇R gene is more complex: different acceptor and donor sites can be used to generate three receptors that differ in the composition and length of their C-terminal domains (5-HT_{7(a)}, 5-HT_{7(b)}, and 5-HT_{7(d)}). Because of its altered intron-exon organization, the rat gene generates the 5-HT_{7(c)} but not the 5-HT_{7(d)} isoform (Heidmann *et al.* 1997). This splicing position giving rise to alternate Cterminus is similar to that found in the 5-HT₄R gene. The different C-terminal domains of the 5-HT₇R variants probably recruit specific sets of intracellular proteins that might modulate their function as demonstrated for the 5-HT₄R isoforms.

2.2. Editing

RNA editing is a dynamic mechanism that generates molecular and functional diversity. The most common type of RNA editing involves nucleotide substitution that consists of either cytidine to uridine (C to U) or, most frequently, adenosine to inosine (A to I). Inosine is read as guanosine by the translational machinery of the cell (Higuchi *et al.* 1993). The 5-HT_{2C}R is the only known GPCR to be regulated by editing. Editing takes place at four major positions on exon V (corresponding to the second exon in the coding sequence; Fig. 1), termed sites A, B, C, and D, and one minor site, termed E (Fig. 2a). 5-HT_{2C}R mRNA editing has been identified as A to I discrepancies between genomic and cDNA sequences in the region encoding the second intracellular loop of the receptor. This A to I substitution alters the coding potentials of three codons. Theoretically, 5-HT_{2C}R editing can generate up to 32 different mRNAs that encode 24 receptor isoforms from the non-edited form (INI) to the fully edited form (VGV; Fitzgerald *et al.* 1999; Fig. 2). The editing of 5-HT_{2C} transcripts is tissue-specific. Seven major 5-HT_{2C}R isoforms, encoded by 11 distinct RNA species, are expressed in rat brain compared with 14 receptor isoforms encoded by 23 transcripts in human brain. The most prevalent isoform in human brain is 5-HT_{2C}-VSV, whereas 5-HT_{2C}-VNV is the most abundant in the rat (Fig. 2b).

3. Neuronal and synaptic localizations and signaling functions

The classical view of pre-synaptic 5-HT-containing terminals forming synaptic contacts has been challenged by the team of L. Descarries (Descarries *et al.* 1975). This does not mean that classical 5-HT synapses do not occur, but they are not largely present. Nerve endings often lack the junction membrane specialization that characterizes the synapse. The released 5-HT may diffuse in the extracellular space and reach relatively distant targets. Moreover, the pleiotropic functions of serotonin are not only exerted by the concerted actions of multiple serotonin receptor subtypes but also by the multiple coupling of each receptor subtype. We will focus, in this review, on signaling in native brain tissues.

3.1. 5-HT_{1A}Rs

The highest density of 5-HT_{1A}Rs is found in the limbic areas (Lanfumeey and Hamon 2004). 5-HT_{1A} auto-receptors are localized in cell bodies and dendrites of 5-HT neurons of the dorsal and median raphe nuclei (Sotelo *et al.* 1990; Riad *et al.* 2000; Fig. 3a). They are chiefly expressed along extra-synaptic sites, as previously seen for many metabotropic GPCRs (Riad *et al.* 2000). At the post-synaptic level, such as the hippocampal formation, they are localized on dendrites of pyramidal and granule cells (Fig. 3a; Riad *et al.* 2000). 5-HT_{1A}Rs are negatively coupled to adenylyl cyclase (AC) via pertussis-toxin-sensitive Gα_i and/or Gα_o proteins (De Vivo and Maayani 1986; Weiss *et al.* 1986; Fig. 4). However, 5-HT_{1A}Rs do not seem to be coupled to AC inhibition in dorsal raphe nuclei, in spite of their high density

within these nuclei. Not only 5-HT_{1A} auto-receptors, but also post-synaptic 5-HT_{1A}Rs inhibit neuronal firing. This inhibition is attributable to the activation of G-protein-gated inwardly rectifying potassium (GIRK) currents and the inhibition of Ca²⁺ channels (Andrade *et al.* 1986; Oleskevich 1995; Sodickson and Bean 1998; Fig. 4). Activation of 5-HT_{1A} auto-receptors delays the action of selective serotonin reuptake inhibitors (SSRIs). Indeed, SSRIs are efficient at the post-synaptic level only after desensitization of 5-HT_{1A} auto-receptors (Adell *et al.* 2005). For unknown reasons, 5-HT_{1A} auto-receptors desensitize much faster and to a much greater extent than post-synaptic receptors (Karschin *et al.* 1991). In serotonin transporter (SERT) knock-out mice, 5-HT_{1A} auto-receptors are desensitized and down-regulated in the dorsal raphe nuclei, but not in the hippocampus (Fabre *et al.* 2000). Interestingly, 5-HT_{1A}Rs inhibit N-methyl-D-aspartate (NMDA) function in prefrontal pyramidal neurons by decreasing the transport and expression of NR2B subunits into dendrites (Yuen *et al.* 2005). This effect may explain the inhibition of long-term potentiation (LTP) by 5-HT_{1A}Rs (Edagawa *et al.* 1998). 5-HT_{1A}R activation stimulates neurogenesis in the dentate gyrus and the subventricular zone (Brezun and Daszuta 1999), an effect that seems to be required for antidepressant effects (Santarelli *et al.* 2003). They also reduce the length of dendrites and the number of spines (Yan *et al.* 1997). 5-HT_{1A}R agonists have been developed for anxiety. 5-HT_{1A}R invalidation consistently increases anxiety in mice (Gross and Hen 2004; Lanfumey and Hamon 2004). Positive coupling of 5-HT_{1A}Rs to AC, phospholipase C (PLC) and extracellular signal-regulated kinases (ERK) pathways have only been seen in heterologous systems (Raymond *et al.* 2001) (Fig. 4).

3.2. 5-HT_{1B}Rs and 5-HT_{1D}Rs

5-HT_{1B}Rs and 5-HT_{1D}Rs are not expressed on cell bodies of 5-HT neurons but on terminals of 5-HT neurons and on terminals of other neurons, such as dopaminergic, GABAergic, and glutamatergic neurons, where they inhibit neurotransmitter release (Starke *et al.* 1989; Pauwels 1997; Sari *et al.* 1997; Riad *et al.* 2000). They are highly expressed in the substantia nigra and the globus pallidus (probably on striato-pallidal and striato-nigral GABAergic neurons). 5-HT_{1B}Rs and 5-HT_{1D}Rs are also present in the cortex. 5-HT_{1B/1D} agonists (triptans) are active with respect to migraine, probably by inhibiting trigeminal neurotransmission. 5-HT_{1B}Rs are also present on thalamo-cortical axon terminals carrying sensory afferents from one whisker. These terminals form clusters, called barrels (Gaspar *et al.* 2003), in layer IV (Fig. 3b). 5-HT_{1B}Rs, like the other 5-HT₁Rs, are negatively coupled to AC via Gα_i/Gα_o in native brain tissues, such as the substantia nigra (Bouhelal *et al.* 1988; Fig. 4). In transfected cells, 5-HT_{1B}Rs regulate PLC, PLD, Akt kinase, neuronal nitric oxide synthase (nNOS), and ERK pathways, activate K⁺ channels, and inhibit Ca²⁺ channels (Raymond *et al.* 2001; Fig. 4). Pharmacological and genetic studies suggest a role for 5-HT_{1B}Rs in the pathophysiology of obsessive-compulsive disorders, drug addiction, depression, and sleep. 5-HT_{1B}R knock-out mice are aggressive (Gingrich and Hen 2001). Both in vitro and in vivo experiments indicate that 5-HT_{1B}Rs control the development of axonal harbors

and axon terminal clustering (Gaspar *et al.* 2003). 5-HT_{1E}Rs and 5-HT_{1F}Rs These receptor subtypes are present in the brain, but little data concerning their neuronal localization are available. 5-HT_{1F}R has been found on pre-synaptic trigeminal nerve terminals and mediates some of the effect of a new generation of triptans (rizatriptan, naratriptan, zolmitriptan; Lanfumey and Hamon 2004). Negative coupling to AC has been demonstrated in heterologous cells, but not neurons (Raymond *et al.* 2001; Fig. 4).

3.3.5-HT_{2A}Rs

5-HT innervation of the prefrontal cortex has received extensive attention from neuroanatomists, physiologists, and pharmacologists (Leysen 2004). 5-HT_{2A}Rs are particularly abundant in the pre-frontal cortex (Fig. 3c; Jakab and Goldman-Rakic 1998; Cornea-Hebert *et al.* 1999, 2002; Miner *et al.* 2003) and are certainly the site of action of hallucinogens, such as LSD (Aghajanian and Marek 1999b), and of some atypical anti-psychotics, such as clozapine and risperidone (Adell *et al.* 2005). Alterations in 5-HT_{2A}R function may be a contributing factor to the cognitive impairment in mental disorders such as schizophrenia and depression (Kroeze and Roth 1998). Light- and electron-microscopic immunohistochemistry has been used to determine the cellular and subcellular localization of 5-HT_{2A}Rs in the pre-frontal cortex (Jakab and Goldman-Rakic 1998; Cornea-Hebert *et al.* 1999, 2002; Miner *et al.* 2003; represented in Fig 3c). A dense localization has been found in apical dendrite shafts, proximal to the pyramidal cell soma (Jakab and Goldman-Rakic 1998). Some receptors are present in the soma of pyramidal cells and in the dendrites of local circuited neurons (Jakab and Goldman-Rakic 1998). In one study (Miner *et al.* 2003), but not in another one (Jakab and Goldman-Rakic 1998), a dense localization has been found on the spine heads and necks of pyramidal neurons. A marked intracellular density of 5-HT_{2A}Rs, which may serve as a receptor reserve for trafficking to the plasmamembrane, has also been described (Cornea-Hebert *et al.* 1999, 2002). A minority of 5-HT_{2A}Rs is also found on axon terminals (Jakab and Goldman-Rakic 1998; Miner *et al.* 2003). Whether 5-HT_{2A}Rs are localized on pre-synaptic glutamatergic terminals remains a matter of debate. In the primate cortex, a pre-synaptic localization on glutamatergic terminals has also been reported (Jakab and Goldman-Rakic 1998) although at low levels, whereas in the rat cortex, such a localization remains to be established (Miner *et al.* 2003). Some of these discrepancies may be related to the nature of the antibodies used. In the rat prefrontal cortex, 5-HT_{2A}Rs have also been detected on terminals of aminergic fibers (Miner *et al.* 2003), which may explain the stimulation of this neurotransmitter release following 5-HT_{2A}R activation (Pehek *et al.* 2001). In vitro electrophysiological studies indicate that 5-HT_{2A}Rs enhance spontaneous excitatory post-synaptic currents (EPSCs; Aghajanian and Marek 1999a; Marek and Aghajanian 1999; Zhou and Hablitz 1999). Activation of 5-HT_{2A}Rs also increases late EPSPs evoked by the electrical stimulation of afferent fibers (Adell *et al.* 2005). These effects are probably mediated by a release of glutamate and subsequent activation of α -amino-3-hydroxy- 5-methylisoxazole-4-propionic acid (AMPA) receptors, since they

are inhibited by AMPA antagonists (Aghajanian and Marek 1999a) and by pre-synaptic group II metabotropic glutamate agonists, which are known to inhibit glutamate release (Marek *et al.* 2000). A presynaptic localization of 5-HT_{2A}Rs on glutamatergic terminals may explain these results. However, if 5-HT_{2A}Rs are primarily localized on spine heads of pyramidal cells at asymmetric synapses receiving glutamate innervation, one has to speculate a post-synaptic influence on pre-synaptic glutamatergic release. This could be achieved via the postsynaptic production of a retrograde messenger. An increase in neuronal excitability may also be explained by a 5-HT_{2A}R-mediated inhibition of after-hyperpolarizations (IsAHP) in the prefrontal cortex (Villalobos *et al.* 2005). However, the situation may be more complex because 5-HT inhibits (rather than stimulates) cortical pyramidal neuron activity in vivo (Zhou and Hablitz 1999). This could be the result of several synaptic effects. 5-HT_{2A}Rs induce a massive enhancement of spontaneous inhibitory post-synaptic currents (IPSCs) in pyramidal neurons via the activation of interneurons (Zhou and Hablitz 1999), an inhibition of the Na⁺ current, which reduces dendritic excitability (Carr *et al.* 2002), an inhibition of the L-type Ca²⁺ current (Day *et al.* 2002), and an inhibition of NMDA receptor-mediated transmission (Arvanov *et al.* 1999). Indeed, depending on the kinetics of all these effects and on the kinetics of their desensitization, 5-HT_{2A}Rs may inhibit or excite pyramidal neuron activity. Normal cognitive functions may depend on a fine-tuning of these opposing types of regulation (Williams *et al.* 2002). Interestingly, both the supra-normal level of 5-HT and the depletion of 5-HT result in altered spatial working memory and decision making, respectively (Williams *et al.* 2002). Other electrophysiological studies suggest pre- and postsynaptic localizations of 5-HT_{2A/2C}Rs in the spinal cord. In superficial dorsal horn synapses, pre-synaptic 5-HT_{2A/2C}Rs induce a long-lasting facilitation of miniature EPSC frequency (Hori *et al.* 1996). At the same synapses, postsynaptic 5-HT_{2A/2C}Rs transforms silent glutamatergic synapses into active synapses, a mechanism that requires the interaction of AMPA receptors with PDZ-domain-containing proteins (post-synaptic density/disc large/zona occludens-1 proteins; Li and Zhuo 1998; Li *et al.* 1999). With regard to second messenger production, 5-HT_{2A}Rs activate PLC, PLA2, and the ERK pathway in neurons (Raymond *et al.* 2001). They also activate the Janus kinase (JAK)/transcription (STAT) pathway in skeletal and smooth muscle (Guillet-Deniau *et al.* 1997; Banes *et al.* 2005) and PLD in non-neuronal cells (Raymond *et al.* 2001; Fig. 4). Unexpectedly, some antagonists (mianserin, spiperone, mesulergine) induce desensitization and internalization of 5-HT_{2A}Rs both in vivo and in vitro (Sanders-Bush 1990; Roth and Ciaranello 1991). This process may contribute to the redistribution of 5-HT_{2A}Rs from the membrane of apical dendrites to the cytoplasm of neuronal cell bodies following chronic treatment of rats with atypical antipsychotics (Willins *et al.* 1999).

3.4. 5-HT_{2B}Rs

In the adult brain, 5-HT_{2B}Rs are found only in discrete nuclei of the cerebellum, the lateral septum, the dorsal hypothalamus, and the medial amygdala, contrasting with its wide expression in peripheral

tissues, including the heart and lung. As 5-HT_{2A}Rs, 5-HT_{2B}Rs are coupled to G α_q proteins and activate PLC. However, to date, no study has reported such a coupling within neurons (Wainscott *et al.* 1993). 5-HT_{2B}Rs have also been reported to stimulate Ca²⁺ mobilization in astrocytes derived from various rat cerebral regions (Sanden *et al.* 2000). Additional 5-HT_{2B}R-mediated signaling pathways have been described in non-neuronal cells expressing endogenous 5-HT_{2B}Rs. Stimulation of the 5-HT_{2B}R is associated with an increase in cyclic GMP through the dual activation of constitutive and inducible NOS. G $\alpha_{q/11}$ -coupled 5-HT_{2B}Rs also activate PI3K/Akt and ERK_{1/2} signaling pathways (Raymond *et al.* 2001) (Fig. 4).

3.5. 5-HT_{2C}Rs

A comparison of the distribution of 5-HT_{2C}R mRNA with that of 5-HT_{2C}R protein labeled with ³H-mesulergine in monkey and human brains has revealed both pre- and postsynaptic localizations of 5-HT_{2C}R (Lopez-Gimenez *et al.* 2001; Leysen 2004). These pre- and post-synaptic localizations of 5-HT_{2C}Rs have been further confirmed by electron-microscopic studies performed in the mouse (Becamel *et al.* 2004; Fig. 3d). Data on the localization of 5-HT_{2C}Rs in the ventral tegmental area (VTA) are not consensual. Some suggest that 5-HT_{2C}Rs are primarily localized on non-dopaminergic neurons, probably GABAergic neurons (Di Matteo *et al.* 2001). These receptors may be constitutively active (Berg *et al.* 2005), yielding tonic GABAergic inhibition of dopaminergic neurons. Other data suggest that 5-HT_{2C}Rs are localized on dopaminergic neurons (Ji *et al.* 2006; Fig. 3d). Indeed, all studies agree that the activation of 5-HT_{2C}Rs inhibits dopaminergic neurons (Gobert *et al.* 2000; Ji *et al.* 2006). Atypical anti-psychotics and particularly those with inverse agonism release this inhibition, providing a way to activate dopaminergic systems (Gobert *et al.* 2000; De Deurwaerdere *et al.* 2004; Berg *et al.* 2005). This activation may be relevant for the treatment of depression and the negative symptoms of schizophrenia. In kittens, 5-HT_{2C}Rs have a characteristic columnar expression in layer IV of the visual cortex during the critical period for ocular dominance plasticity. 5-HT_{2C} agonists modulate synaptic plasticity during this period (Gaspar *et al.* 2003). 5-HT_{2C}Rs are also present in hypothalamic pro-opiomelanocortin (POMC)/cocaine amphetamine-regulated transcript (CART) neurons within the arcuate nucleus (Miller 2005). Activation of POMC/CART neurons inhibits feeding. This is certainly the main reason that 5-HT_{2C}^{-/-} mice are obese (Miller 2005). The highest density of 5-HT_{2C}Rs is found in epithelial cells of the choroid plexus, where these receptors control spinal fluid production (Leysen 2004). 5-HT_{2C}R has been reported to activate PLC via G $\alpha_{q/11}$ in many brain regions, including the choroid plexus (Sanders-Bush and Breeding 1988; Chang *et al.* 2000; Fig. 4). These receptors also activate PLD in choroid plexus epithelial cells through a mechanism involving both G α_{13} and G $\beta\gamma$ subunits (McGrew *et al.* 2002). 5-HT_{2C}R-mediated stimulation of PLA2 and AC have been exclusively described in transfected cells (Raymond *et al.* 2001; Fig. 4). As observed for 5-HT_{2A}Rs, 5-HT_{2C}Rs are down-regulated following chronic antagonist exposure (Van Oekelen *et al.* 2003). However, the effects

of such treatments are still controversial. Indeed, Schlag and coworkers (2004) have recently demonstrated that only 5-HT_{2C}R agonists and partial agonists promote receptor desensitization and internalization, whereas neither inverse agonists nor neutral antagonists have these effects (Schlag *et al.* 2004).

Further discussion of 5-HT_{2C}R signaling, in particular with regard to editing, can be found below (*Functions associated with editing*).

3.6. 5-HT₄Rs

5-HT₄Rs are mainly present in limbic structures (hypothalamus, nucleus accumbens, amygdala), islands of Calleja, olfactory tubercle, basal ganglia (striatum, globus pallidus), hippocampus, substantia nigra, and septum. Although the immunolocalization of 5-HT₄Rs is still not possible in brain synapses because of a lack of satisfactory antibodies, indirect evidence suggests that most of 5-HT₄Rs are localized post-synaptically. However, an heterologous presynaptic localization on terminals of cholinergic, GABAergic (dentate gyrus), dopaminergic, and serotonergic neurons is also likely because of the increase in the release of these neurotransmitters by 5-HT₄ agonists. Results from lesion studies and electrophysiological studies are also consistent with a pre-synaptic localization of 5-HT₄Rs (Bockaert *et al.* 1997, 2004a; Bockaert and Dumuis 1998). Two studies have reported the immunolocalization of 5-HT₄Rs. One of them shows the localization of 5-HT₄Rs in respiratory neurons in the pre-Boetzing complex (Manzke *et al.* 2003). Half of these neurons co-express μ -receptors and Substance-P-reactive neurokinin-1 (NK1) receptors. Reverse transcription/polymerase chain reaction experiments have confirmed these findings (Manzke *et al.* 2003). In these neurons, 5-HT₄R activation overcomes fentanyl-induced respiratory depression (by reversing the inhibition of fentanyl-induced cAMP production) and reestablishes stable respiratory rhythms. The second study based on a different antibody has shown the immunostaining of myenteric and submucosal plexuses (Liu *et al.* 2005). Electron-microscopic analysis has indicated an axonal localization, especially in varicosities that contain large dense-cored and small synaptic vesicles. Electrophysiological data have revealed a pre-synaptic action of 5-HT₄Rs on cholinergic neurons leading to the increase in post-synaptic nicotinic EPSCs in mouse myenteric neurons (Bockaert *et al.* 2004a; Liu *et al.* 2005). They are also consistent with observations that 5-HT₄Rs stimulate acetylcholine release in the myenteric plexus (Kilbinger and Wolf 1992; Kilbinger *et al.* 1995). 5-HT₄Rs are positively coupled to AC in mice colliculi neurons and in guinea pig hippocampal membranes (Dumuis *et al.* 1988; Bockaert *et al.* 1990; Fig. 4). In colliculi neurons, the 5-HT₄Rs-mediated increase in cAMP concentration seen after a short application of agonist leads to the inhibition of voltage-gated K⁺ channels, including Ca²⁺-activated K⁺ channels, and their closure (Fagni *et al.* 1992; Ansanay *et al.* 1995). Moreover, the activation of 5-HT₄Rs produces a long-lasting inhibition of K⁺ channels because of the long-lasting blockade of phosphatase (Ansanay *et al.* 1995). In CA1 pyramidal neurons of hippocampus, 5-HT₄Rs reduce the AHP that follows action potentials. Moreover, 5-HT₄R

activation induces a reduction in spike accommodation (Andrade and Chaput 1991; Torres *et al.* 1996). These effects are mediated by cAMP and protein kinase A (PKA) activation. 5-HT_{1A}Rs, 5-HT₄Rs, and 5-HT₇Rs differentially expressed in CA1 neurons allow space-specific modulation of the hyperpolarization-activated current (I_h) current, which is known to adjust synaptic integration and the repetitive discharge behavior of CA1 neurons (Bickmeyer *et al.* 2002). In prefrontal cortex pyramidal neurons, 5-HT₄Rs can activate or inhibit GABAergic synaptic transmission. This regulation of the GABA_A current is also mediated by cAMP- and PKA-dependent signaling pathways (Cai *et al.* 2002). In hippocampal neurons, activation of the endogenous 5-HT₄Rs leads to a decrease in the length and number of neurites through the $G_{\alpha_{13}}$ /RhoA signaling pathway (Fig. 4; Ponimaskin *et al.* 2002). Moreover, of note, 5-HT₄R activation, at least in dorsal root ganglion (DRG) cells, is associated with an increase in the tetrodotoxin-insensitive Na^+ current, but the messenger involved in this effect does not appear to be cAMP (Cardenas *et al.* 1997). 5-HT₄Rs increase, via the production of cAMP, the activated exchange factor, Epac. Epac in turn activates the small G protein Rap1, which increases Ras GTP loading. This Epac-dependent signaling pathway stimulates sAPP α secretion (Lezoualc'h and Robert 2003). In colliculi neurons, the desensitization of 5-HT₄Rs is homologous and results in the activation of G-protein-coupled receptor kinase type 2 (GRK2; Ansanay *et al.* 1992; Barthet *et al.* 2005). In heterologous cells, the GRK2 concentration is too low, and homologous desensitization of 5-HT₄Rs does not occur (Barthet *et al.* 2005). Several reports clearly show the modulation of LTP and long-term depression (LTD) by 5-HT₄ agonists (Kemp and Manahan-Vaughan 2004, 2005). Interestingly, 5-HT₄Rs exert both a tonic and a phasic stimulatory control over dorsal 5-HT raphe neurons (Lucas and Debonnel 2002). This effect might be enhanced by the activation of 5-HT₄Rs expressed in the medial prefrontal cortex (Lucas *et al.* 2005). 5-HT₄Rs also positively modulate learning and memory, reward, sensitivity to novelty, and stress (Bockaert *et al.* 2004a; Compan *et al.* 2004).

3.7. 5-HT₅Rs

Studies on the cellular and subcellular localization of 5-HT₅Rs by using immunohistochemical methods have led to contradictory results. Both astrocytes and neuronal localizations have been reported. One study reports a localization on 5-HT neurons, suggesting a possible role as autoreceptors. However, the quality of the antibodies used and the lack of specific pharmacological tools preclude any definitive conclusion regarding the functional role of these receptors (Nelson 2004). Surprisingly, no study to date has identified a functional coupling of 5-HT₅Rs in native neuronal tissue. Rat 5-HT₅R transfected into C6 glioma cells has been shown to couple to the inhibition of cAMP production. In the same C6 glioma cells, one study has reported that the human 5-HT_{5A}R couples to multiple signal transduction pathways, e.g., the inhibition of cAMP accumulation and of ADP-ribosyl cyclase and the activation of outward currents. This study has indicated that human 5-HT_{5A}R is coupled to $G_{\alpha_i}/G_{\alpha_o}$; it also raises the possibility of a cross-coupling of the receptor to $G_{\alpha_{q/11}}$ (Noda *et al.* 2003; Fig. 4).

3.8. 5-HT₆Rs

5-HT₆Rs are almost exclusively expressed in the central nervous system. They are found at high concentrations in limbic and cortical regions (Woolley *et al.* 2004) but are not expressed in 5-HT neurons. Immunohistochemical studies including electron-microscopic investigations have shown prominent dendritic localization. Some non-motile cilia of the nucleus accumbens, the caudate-putamen, the olfactory tubercles, and the islands of Calleja cells also express 5-HT₆Rs (Brailov *et al.* 2000). In the striatum, 5-HT₆Rs are expressed by medium-sized spiny neurons projecting to the substantia nigra or globus pallidus (Gerard *et al.* 1997; Sebben *et al.* 1994). Preferential GABAergic localization is suggested in most brain localizations. No electrophysiological data are available. Selective antagonists have been synthesized and can now be used to investigate the physiological roles of 5-HT₆Rs. For example, the selective antagonist SB-271046 has been shown to increase glutamate release probably via de-inhibition of GABAergic inhibition (Woolley *et al.* 2004). Many studies have tried to correlate 5-HT₆R polymorphisms with psychiatric disorders (Woolley *et al.* 2004). Contradictory results have been reported. Pharmacological studies and localization studies indicate possible roles in psychotic and affective disorders, anxiety, epilepsy, and memory retention but not the regulation of feeding (Woolley *et al.* 2004). Native 5-HT₆Rs, like 5-HT₄Rs and 5-HT₇Rs, are positively coupled to AC via the G_{α_s} protein in striatal tissue or primary cultures of striatal neurons (Schoeffer and Waeber 1994; Sebben *et al.* 1994). This positive coupling has been confirmed by 5-HT₆Rs being expressed in various heterologous expression models (Ruat *et al.* 1993; Fig. 4).

3.9. 5-HT₇Rs

In the brain, 5-HT₇Rs are mainly found in the thalamus, hypothalamus (suprachiasmatic nucleus), cerebral cortex (both on pyramidal and GABAergic neurons), hippocampus (all CA regions), and amygdala (Hedlund and Sutcliffe 2004; Thomas and Hagan 2004). Most 5-HT₇Rs are localized on both the soma and axon terminals (outside the synapses) of GABAergic neurons. Within the cerebellum, 5-HT₇Rs are exclusively located in Purkinje neurons. 5-HT₇Rs are also found in intra- and extra-cranial blood vessels and meningeal tissues (Hedlund and Sutcliffe 2004). In the guinea pig hippocampus, 5-HT₇Rs have been recognized as being the receptor with a high affinity for 5-carboxamidotryptamine (5-CT) coupled to AC (Bockaert *et al.* 1990, 1997). In heterologous cells, the four 5-HT₇R splice variants activate cAMP production via the G_{α_s} protein (Ruat *et al.* 1993). 5-HT₇Rs also activate the ERK_{1/2} pathway in hippocampal neurons. However, the signaling pathway used by the receptor to activate ERK remains unclear. Most of the 5-HT₇R-mediated activation of ERK has been found to be mediated via G_{α_s}. Both PKA-dependent and -independent pathways might contribute to ERK activation. Until recently, the pathway for ERK activation was shown to require cAMP, PKA, and MAP kinase (MEK) in neurons endogenously expressing 5-HT₇Rs and in 5-HT₇R-transfected cells (including PC12 and

HEK 293 cells; Norum *et al.* 2003). However, another report indicates that 5-HT₇Rs activate ERK in hippocampal neurons and transfected PC12 cells through a pathway independent of PKA but requiring a cAMP-GEF, such as Epac (Lin *et al.* 2003). 5-HT₇Rs inhibit slow AHP in thalamic and trigeminal motor neurons (Goaillard and Vincent 2002). In vivo, SSRIs increase the population spike amplitude in CA1 and CA3 areas. This effect is blocked by SB-269970-A, a specific 5-HT₇R antagonist (Matsumoto *et al.* 2002). In the CA1 region and in thalamic and rat DRG neurons, 5-HT₇Rs increases excitability through a mechanism involving a cAMP-mediated I_h current (Bickmeyer *et al.* 2002). All these effects suggest an important role for 5-HT₇Rs in the control of neuronal excitability and may contribute to greater information transfer within a neuronal network. 5-HT₇Rs endogenously expressed in hippocampal neurons significantly increase neurite length through the Gα₁₂, RhoA, and Cdc42 GTPases-dependent signaling pathway (Kvachnina *et al.* 2005) (Fig 4). Functional studies have also implicated 5-HT₇Rs in thermoregulation and the activation of rapid-eye-movement (REM) sleep via the modulation of suprachiasmatic nucleus neurons (Thomas and Hagan 2004). In this regard, 5-HT₇R antagonists inhibit REM sleep and increase the latency to onset of REM sleep. This effect suggests that 5-HT₇R antagonists are candidates for treating depression, a situation in which the REM sleep is disturbed. A role of 5-HT₇Rs in learning and memory is suggested by the observed decrease in synaptic plasticity in CA1 and contextual fear conditioning in 5-HT₇R^{-/-} mice (Thomas and Hagan 2004). 5-HT₇Rs are recognized for their important role in controlling hormonal release not only at the hypothalamic, but also at the peripheral level. A role in pain and migraine (via their action on blood vessels but also on neurogenic inflammation) is under investigation. Finally, a role in schizophrenia remains to be found in animal models (Hedlund and Sutcliffe 2004; Thomas and Hagan 2004).

4. Fine-tuning of signaling functions

4.1. Constitutive activity

Studies performed mainly in heterologous cells transfected with both native and mutant forms of 5-HT₁Rs (Albert *et al.* 1999; Kiel *et al.* 2000), 5-HT₂Rs (Egan *et al.* 1998), 5-HT₄Rs (Claeysen *et al.* 1999), 5-HT₆Rs, and 5-HT₇Rs have demonstrated that 5-HTRs can exist in a conformation that mimics an active state of the receptor in the absence of agonist, thus revealing that these receptors display constitutive activity. All these studies highlight the importance of i₂ and/or i₃ loops in the regulation of constitutive activity of the majority of 5-HTRs and of the role of the C-terminus in the constitutive activity of 5-HT₄R and 5-HT₇R splice variants, which differ in the length of their C-terminal tail (for a review, see Bockaert *et al.* 2005). However, to date, only 5-HT_{2A}Rs and 5-HT_{2C}Rs have been shown to exhibit constitutive activity in vivo (Berg *et al.* 2005). Harvey (2003) has shown that 5-HT_{2A}R inverse agonists, but not neutral antagonists, retard the learning of the conditioned eye link response in rabbits stimulated by LSD. This effect is not attributable to the blockade of endogenous 5-HT by inverse

agonists, but rather to an inhibition of endogenous constitutive activity, because it occurs after lesions. Evidence of an *in vivo* constitutive activity of 5-HT_{2C}Rs has come from experiments by De Deurwaerdere and co-workers (2004); by using microdialysis, they have demonstrated that SB 206553, an inverse agonist, but not the neutral antagonist SB 242084, enhances basal dopamine release in dopamine-innervated areas, such as the frontal cortex, the accumbens nucleus, and the striatum. The effects of the inverse agonist are insensitive to the lesion of 5-HT neurons and are blocked by the neutral antagonist.

4.2. Functions associated with editing

As seen above, 5-HT_{2C}R exhibits a unique mechanism of generating multiple functional receptor variants through a process called mRNA editing (Burns *et al.* 1997). RNA transcripts encoding the 5-HT_{2C}R undergo A to I RNA editing events at up to five sites (see above). In heterologous cells, RNA editing appears to regulate the pattern of intracellular signaling (Burns *et al.* 1997). The non-edited form (5-HT_{2C-INI}R) is more potent in stimulating PLC and exhibits more constitutive activity than the edited forms (Niswender *et al.* 1999). Moreover, non-edited 5-HT_{2C-INI}R is capable of coupling to the G α_{13} protein (activating the PLD signaling pathway and promoting Rho GTPase activity; McGrew *et al.* 2004), whereas the fully edited isoform 5-HT_{2C-VGV}R fails to activate G α_{13} (Price *et al.* 2001). RNA editing has an important implication for serotonergic signal transduction *in vivo*. Editing is regulated by 5-HT itself. Depletion of 5-HT increases the expression of edited forms having a high affinity for 5-HT, whereas stimulation of the 5-HT_{2A/2C}Rs has the opposite effect (Gurevich *et al.* 2002a). Moreover, altered RNA editing of the 5-HT_{2C}R may play a role in depression. 5-HT_{2C} mRNA isoforms expressed in human prefrontal cortex differ in tissues obtained from depressed suicide victims compared with healthy controls. In suicides, three abundant isoforms, resulting from an increase at the E and C sites and a decrease at the D site and encoding receptors, are expressed. These isoforms exhibit decreased basal activity and decreased agonist affinity and potency (Gurevich *et al.* 2002b). Recently, Kishore and Stamm (2006) have reported that the small nucleolar RNA (snoRNA) HBII-52, which exhibits sequence complementarity to the alternative spliced exon V encoding for a functional 5-HT_{2C}R, regulates receptor mRNA splicing. Notably, Prader-Willy syndrome patients (characterized by neonatal muscular hypotonia, early childhood obesity, hypogonadism, and mental retardation) who do not express HBII-52 snoRNA exhibit abnormally low levels of the non-edited 5-HT_{2C}R isoform, suggesting a role of serotonergic systems in Prader-Willi syndrome (Kishore and Stamm 2006).

5. 5-HTR interacting proteins and modulation of signaling

The first proteins found to have functional interactions with metabotropic 5-HTRs were, of course, G proteins (see above). Although coupling to G proteins remains a central event in 5-HTR-mediated

signaling, an increasing body of evidence indicates that the regulation of their functional status also depends on interactions with a variety of additional protein partners. Like other GPCRs, 5-HTRs associate with β -arrestins in a manner that is dependent on receptor phosphorylation by GRKs. The binding of arrestins to the receptors promotes their internalization. This process requires the recruitment, by arrestins, of binding partners, including clathrin, the central component of the endocytic machinery, AP2, another endocytic protein, and intracellular trafficking proteins such as NSF, ARF6, and ARNO (Lefkowitz and Shenoy 2005). β -Arrestins also serve as scaffolds for signaling enzymes such as c-Src, members of the MAP kinase pathways, and the c-Jun Nterminal kinase 3, which participate in 5-HTR signaling (Lefkowitz and Shenoy 2005). In addition to the binding to β -arrestins, which seems to be a general mechanism, 5- HTR subtypes can recruit specific protein partners. This section will describe our current knowledge concerning these subtype-specific interactions. Metabotropic 5-HTRs are certainly the GPCRs for which the highest number of specific accessory proteins have been identified, thanks to methodologies such as the yeast twohybrid screen and proteomic approaches combining affinity chromatography experiments and the identification of receptor partners by mass spectrometry. However, our present cognizance of 5-HTR protein partners fluctuates from one receptor to another. For some 5-HTRs (e.g., 5-HT_{2A}R, 5-HT_{2C}R, and several 5-HT₄R splice variants), interacting proteins have been extensively characterized by using, in part, proteomic approaches, whereas no binding partner has so far been identified for a number of 5-HTRs (Table 1). As for other GPCRs, most of the hitherto identified 5-HTR interacting proteins associate with their C-terminus, even though some of them have been shown to bind to motifs located on intracellular loops (i₂ and i₃ loops).

5.1. Proteins interacting with the 5-HTR C-terminal domain

5.1.1. PDZ-domain-containing proteins

The acronym PDZ derives from the three first proteins (PSD-95/Disc large/Zonula occludens-1) in which these domains have been described (Nourry *et al.* 2003). To date, PDZ proteins are certainly the most numerous proteins involved in the scaffolding of multiprotein complexes that contribute to the targeting, the trafficking, and the fine tuning of signaling properties of membrane-bound receptors, including GPCRs. Genome sequence analysis predicts the presence of more than 300 PDZ proteins in humans. PDZ proteins generally bind to a conserved consensus sequence (PDZ ligand) located at the extreme C-terminus of target receptors. PDZ domains have been classified according to their specificity for these C-terminal motifs. Class I PDZ domains preferentially bind to S/TX ϕ , class II to ϕ X ϕ and class III to E/DX ϕ motifs, where ϕ represents a hydrophobic residue and X any residue (Nourry *et al.* 2003). Several 5-HTRs express class I or II canonical PDZ ligands, suggesting that they may interact with PDZ-based protein scaffolds. These include the three 5-HT₂R subtypes (SCV, SYV/I, and SSV, for 5-HT_{2A}R, the 5-HT_{2B}R, and 5-HT_{2C}R, respectively), several 5-HT₄R splice variants (SCF-5-HT_{4(a)}R,

VPV-5-HT_{4(e)}R and 5-HT_{4(f)}R), and one 5-HT₇R variant (FVL, 5-HT_{7(b)}R). The first evidence of an interaction between one 5-HTR and one PDZ protein was obtained by Ullmer *et al.* (1998). Screening a human brain cDNA library with the yeast two-hybrid system, this group isolated and cloned a multi-PDZ protein (the protein called MUPP1), which interacts with the C-terminal domain of the human 5-HT_{2C} receptor. MUPP1, which contains thirteen PDZ domains and no obvious catalytic domains, is the PDZ protein that contains the highest number of PDZ domains identified to date. The 5-HT_{2C}R specifically interacts with the 10th PDZ domain of MUPP1, leaving the other PDZ domains free to interact with additional partners within a larger complex (Becamel *et al.* 2001; Parker *et al.* 2003). This interaction is dynamically regulated by agonist-dependent receptor phosphorylation at Ser458, located in the PDZ-binding motif (position-2; Parker *et al.* 2003). The receptor/MUPP1 interaction induces both conformational changes in the MUPP1 protein (Parker *et al.* 2003) and receptor clustering at the cell surface (Becamel *et al.* 2001). MUPP1 also associates with the 5-HT_{2A}R and 5-HT_{2B}R, but the functional significance of these interactions remains to be demonstrated (Becamel *et al.* 2001). A more comprehensive description of PDZ proteins that interact with 5-HT_{2A}R and 5-HT_{2C}R C-termini has been performed by using proteomic approaches comparing proteins “fished out” by using the receptor C-termini as bait and mutant baits in which the C-terminal valine residue, which is critical for interaction with target PDZ domains, has been mutated into an alanine (Becamel *et al.* 2002; Becamel *et al.* 2004). These studies have demonstrated that each 5-HT₂R subtype interacts with specific sets of PDZ proteins, even though they share an identical PDZ ligand (SXV), thereby underpinning the role of residues located outside this canonical sequence in the specificity for PDZ domains (Becamel *et al.* 2004). They have also revealed that 5-HT_{2A}Rs only interact with PDZ proteins located at the post-synapse (e.g., PSD-95), whereas 5-HT_{2C}Rs associate with PDZ proteins exhibiting both presynaptic and post-synaptic localizations (e.g., the Veli3/ CASK/Mint1 complex), consistent with the differential distribution of the receptors at the synaptic junction. The association of 5-HT_{2A}Rs with PSD-95 has been shown to enhance receptor-mediated signal transduction in heterologous cells, a process that can be related to the ability of PSD-95 to inhibit agonist-induced 5-HT_{2A}R internalization (Xia *et al.* 2003a). The functional consequences of the other interactions remain to be demonstrated. However, several studies with receptors mutated at the PDZ ligand indicate that 5-HT₂R/PDZ protein interactions play a critical role in governing receptor targeting in neurons and/or modulating their signal transduction properties. The 5-HT_{2A}R PDZ ligand seems to be essential for preferential receptor targeting to the dendritic compartment of pyramidal cortical neurons (Xia *et al.* 2003b). The deletion of the 5-HT_{2C}R PDZ ligand, which prevents agonist-induced receptor phosphorylation (see above), modulates the desensitization/resensitization of the receptor Ca²⁺ effector pathway in NIH-3T3 fibroblasts (Backstrom *et al.* 2000). A similar approach has indicated the 5-HT_{2B}R PDZ ligand is essential for receptor-mediated activation of constitutive and inducible NOS activities in a murine cell line (Manivet *et al.* 2000). The interaction of 5-HT₂Rs (probably 5-HT_{2A}Rs) with PDZ proteins may also be involved in the persistent facilitation of sensory synaptic transmission in the spinal cord dorsal horn, a process underlying chronic pain,

following tissue injury (Li and Zhuo 1998). This facilitation results from the mobilization of AMPA receptors into the synapses, transforming silent synapses into functional ones (Li and Zhuo 1998). The recruitment of synaptic AMPA receptors induced by 5-HT₂ agonists requires PDZ proteins that physically interact with GluR_{2/3} AMPA receptor subunits (Li *et al.* 1999). Thus, 5-HT₂ and AMPA receptors appear to be functionally linked by a PDZ-based protein scaffold, contributing to the facilitation of nociceptive transmission in the spinal cord. The molecular composition of this PDZ scaffold is still unknown. Specific sets of PDZ proteins interacting with 5-HT_{4(a)}R and 5-HT_{4(e)}R have also been characterized by proteomic screens (Joubert *et al.* 2004). Among the 5-HT_{4(a)}R PDZ-binding partners, NHERF-1 (also designated as Ezrinradixin- moesin-binding phosphoprotein 50, EBP50) and SNX27, a recently cloned sorting nexin, have attracted particular attention. Interaction of the 5-HT_{4(a)}R with NHERF-1 targets the receptor to specialized subcellular microdomains and seems to be involved in receptormediated cytoskeleton remodeling and the modulation of cell shape (Joubert *et al.* 2004). SNX27 belongs to a large protein family involved in the targeting of cell surface receptors to the endocytic compartment. The 5-HT_{4(a)}R/ SNX27 interaction has been consistently found to target part of the receptor pool to early endosomes in heterologous cells (Joubert *et al.* 2004). Two forms of SNX27 that differ in their C-termini (designated as SNX27a and b) have been identified. Both proteins interact with the 5-HT_{4(a)}R C-terminus and are enriched in the brain. SNX27b, but not SNX27a, has been found to be up-regulated in the neocortex of rats following acute exposure to psychostimulants, such as amphetamines and cocaine (Kajii *et al.* 2003). This up-regulation, blocked by D1 receptor antagonists, is reminiscent of stimulant-induced behavioral sensitization and therefore may be involved in the initiation and/or maintenance of long-term neuronal adaptation in response to psychostimulant compounds. As 5-HT₄Rs have been involved in drug sensitization and addiction (Compan *et al.* 2004), the 5-HT₄R/SNX27 interaction may contribute to neuronal adaptation mechanisms underlying these processes.

5.1.2. Other proteins interacting with 5-HTR C-termini

Several 5-HTR subtypes have been shown to interact physically with calmodulin (CaM) via their C-terminus (5-HT_{2A}R and 5-HT_{2C}R; Becamel *et al.* 2002; Turner and Raymond 2005) and/or their intracellular loops (5-HT_{1A}R and 5-HT_{2A}R; Turner *et al.* 2004; Turner and Raymond 2005). Putative CaM-binding sites have also been identified by using a CaM-binding site search engine (http://calcium.uhnres.utoronto.ca/ctdb/pub_pages/search/index.htm) for the intracellular loops of 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2C}, 5-HT₆, and 5-HT₇ receptors and in the C-terminus of 5-HT₄ and 5-HT₇ receptors (Turner and Raymond 2005), but these interactions remain to be established. The 5-HT_{2A}R C-terminal CaM-binding site, located at the juxtamembrane region, overlaps with a putative PKC phosphorylation site. CaM binding and receptor phosphorylation by PKC in vitro have been found to be antagonistic. This suggests that the interaction of CaM with the 5-HT_{2A}R C-terminus can modulate receptor

phosphorylation and desensitization (Turner and Raymond 2005). The 5-HT_{2A}R C-terminus also interacts with ARF1 and, to a lesser extent, ARF6 (Robertson *et al.* 2003). ARF proteins bind to a conserved NPxxY motif that has been found in a number of rhodopsin family GPCRs and that is located at the junction of the transmembrane domain 7 and the C-terminal domain (Johnson *et al.* 2006). This motif is critical in determining selective interaction with isoforms of ARF (Johnson *et al.* 2006). The interaction between 5-HT_{2A}R and ARF proteins appears to be essential for 5-HT_{2A}R-mediated PLD activation, which is independent of G $\alpha_{q/11}$ (Robertson *et al.* 2003). A weaker interaction between ARF1-6 and the i3 loop of the receptor has also been demonstrated. In addition to PDZ proteins and CaM, the 5-HT_{2C}R C-terminal domain recruits two other proteins with unknown functions: PTD4, a putative GTP-binding protein, and PICOT, a novel PKC theta-interacting protein with a thioredoxin homology domain that is involved in the regulation of the thioredoxin system (Becamel *et al.* 2002). The 5-HT_{4(a)}R associates with CRMP2, a member of the collapsin response mediator protein (CRMP) family, essential components of the molecular machinery leading to growth cone collapse in response to semaphorins. CRMP2 induces the outgrowth of multiple axons with a collapse of all dendrites in hippocampal neurons *in vitro*, thereby promoting neuronal polarity (Inagaki *et al.* 2001). 5-HT_{4(a)}Rs have been shown to cause G α_{13} - and RhoA-dependent neurite retraction and cell rounding in neuroblastoma x glioma NIE-115 cells (Ponimaskin *et al.* 2002). Whether the physical interaction between 5-HT_{4(a)}R and CRMP2 contributes to the receptor regulation of neuronal architecture remains to be elucidated. Sec23A, a component of the COPII complex involved in the transport of secretory and plasma membrane proteins from the endoplasmic reticulum to the Golgi apparatus, has been found to associate with the 5-HT_{4(e)}R C-terminus (Joubert *et al.* 2004). This interaction is inhibited by the deletion of the four C-terminal residues of the receptor, indicating a critical role of the extreme Cterminal sequence in the trafficking of short 5-HT₄R variants from the endoplasmic reticulum to the plasma membrane.

5.2. Proteins interacting with the intracellular loops of 5-HTRs

As previously mentioned, CaM-binding sites have been identified in the intracellular loops of several 5-HTR subtypes. CaM has been shown to interact with two distinct motifs located in the N- and C-termini juxtamembrane regions of the 5-HT_{1A}R i₃ loop (Turner *et al.* 2004). Binding of CaM to 5-HT_{1A}R has been found to be antagonistic with PKC-mediated receptor phosphorylation, suggesting a role in receptor desensitization. This interaction may also be critical for the 5-HT_{1A}R-mediated activation of the ERK signaling pathway, a process involving agonist-induced receptor internalization (Della Rocca *et al.* 1999). Interaction of CaM with the i₂ loop of 5-HT_{2A}Rs decreases receptor-mediated [³⁵S]GTP γ S binding to NIH-3T3 cell membranes, suggesting an inhibitory role of CaM in 5-HT_{2A}R-mediated signal transduction by dampening the interaction with the G protein (Turner and Raymond 2005). 5-HT_{2A}Rs interact (via their intracellular loops) with other proteins that influence receptor subcellular distribution within neurons or modulate their signal transduction properties. These include the cytoskeletal

microtubule-associated protein MAP1A, caveolin-1, and the p90 ribosomal S6 kinase 2 (RSK2). The association of 5-HT_{2A}Rs (i₃ loop) with MAP1A may be responsible for the intracellular distribution of 5-HT_{2A}Rs (in an inactive state) in the dendritic compartment of pyramidal neurons of the cerebral cortex (Cornea-Hebert *et al.* 2002). The 5-HT_{2A}R/ caveolin-1 interaction, which has been observed in both C6 glioma cells and rat brain synaptic membrane preparations, appears to be essential for targeting the receptor to lipid rafts. This interaction facilitates 5-HT_{2A}R coupling to Gαq proteins (Bhatnagar *et al.* 2004) and appears to be essential for the serotonin-induced inhibition of voltage-gated K⁺ channels (Kv1.5), which form a ternary complex with 5-HT_{2A}Rs and caveolin-1 upon 5-HT_{2A}R activation in smooth muscle cells of the pulmonary artery (Cogolludo *et al.* 2006). In contrast, the association of 5-HT_{2A}Rs (i₃ loop) with RSK2 seems to be required for the tonic inhibition of receptor signaling exerted by this kinase (Sheffler *et al.* 2006). This tonic inhibition may result from constitutive receptor phosphorylation leading to a constitutively desensitized state. RSK2 expresses a C-terminal class I PDZ ligand (TAL), and RSK2/PDZ protein interactions have been demonstrated to be important for RSK2-mediated modulation of excitatory synaptic transmission. This offers the possibility that 5-HT_{2A}R, RSK2, and PDZ proteins form a unique signaling complex modulating 5-HT_{2A}R function. In fetal myoblasts, serotonin induces a physical association of the 5-HT_{2A} receptor with Jak2, providing a way of recruiting STAT3 by the receptor-kinase complex and the subsequent phosphorylation and nuclear translocation of STAT3 (Guillet-Deniau *et al.* 1997). The binding site of Jak2/STAT3 on the receptor (intracellular loops versus the C-terminus) is still uncertain. Activation of members the JAK/STAT pathway upon 5-HT_{2A}R activation has also been reported in vascular smooth muscle (Banes *et al.* 2005), but whether their physical association with the receptor is required for this activation remains to be demonstrated. A recent study has revealed that 5-HTRs are not only capable of interacting with protein kinases, but that they can also associate with phosphatases, modulating their phosphorylation state. This study has demonstrated a physical interaction between the 5-HT_{2C}R i₃ loop and the tumor suppressor PTEN, an enzyme exhibiting both lipid and protein phosphatase activities (Ji *et al.* 2006). This interaction takes place in dopaminergic neurons of the VTA innervating the accumbens nucleus, these are tonically inhibited by active 5-HT_{2C}Rs. PTEN coupling with 5-HT_{2C}Rs prevents agonist-induced receptor phosphorylation at C-terminal serine residues. Disruption of the 5-HT_{2C}R/PTEN interaction with a membrane-permeant peptidyl mimetic of the PTEN-binding site consistently reproduces the inhibitory effect of 5-HT_{2C} agonists on VTA dopamine neuron firing. Such a peptide also prevents the rewarding effects of cannabinoids, effects that are mediated by the increased activity of VTA dopamine neurons, without the apparent side-effects observed following agonist activation of 5-HT_{2C}R. These experiments indicate that the regulation of 5-HT_{2C}R functional activity mediated by the interaction with PTEN plays an important role in the reinforcing role of drugs (Ji *et al.* 2006). Another recent study has reported the interaction of the third intracellular loop of 5-HT_{1B}Rs with p11 (also called S100A10 or calpactin I light chain or annexin II light chain), a member of the S100 EF-hand protein family (Svenningsson *et al.* 2006). This interaction enhances 5-HT_{1B}R signaling efficacy by promoting its

expression at the cell surface. Several pieces of evidence suggest that the dysfunction of 5-HT_{1B}R regulation by p11 might be involved in depressive states: (1) p11 brain expression is increased by antidepressant treatments, whereas the level of the protein is reduced in an animal model of depression and in the brain of depressed patients; (2) p11 overexpression reproduces some behavior triggered by antidepressants in mice, and (3) p11 knock-out mice exhibit a depression-like syndrome and reduced responsiveness to antidepressant treatments (Svenningsson *et al.* 2006). These last-mentioned examples highlight the essential role of 5-HTR/accessory protein interactions in physiology and pathology and the potential of therapeutic strategies aimed at developing drugs capable of disrupting or reinforcing these interactions and exhibiting fewer side-effects than classical agonist- or antagonist-based treatments.

6. Concluding remarks

Metabotropic 5-HTRs are directly or indirectly the site of action of the most important drugs used to treat widely expressed psychiatric diseases such as depression, schizophrenia, and obsessive-compulsive behaviors. They are also the direct or indirect site of action of several important drugs of abuse such as cocaine and LSD. Finally, they play an essential role in cognition. Therefore, we need increase our knowledge of the 5-HTRs themselves, their signal transduction properties in neuronal cells, their role in synaptic plasticity, and the functional consequences of their interaction with accessory proteins. We have seen in this review that many questions remain to be addressed. With respect to 5-HTR-associated proteins, recent studies have clearly established their important functional and perhaps physiological roles (Bockaert *et al.* 2004b; Ji *et al.* 2006; Svenningsson *et al.* 2006). We are confident that pharmacological agents and perhaps drug targeting will clarify these interactions.

7. References

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Receptor subtype	Intracellular loop	C-terminus	Function of the identified interaction	References
5-HT _{1A}	Calmodulin		Trafficking, signaling	(Turner et al., 2004)
5-HT _{1B}	p11		Increases receptor cell surface expression and function	(Svenningsson et al., 2006)
5-HT _{2A}		PDZ proteins*	Targeting to dendrites, activation of silent synapses in the spinal cord	(Li et al., 1999; Xia et al., 2003b)
		PSD-95	Trafficking, signaling	(Xia et al., 2003a)
		MUPP1		(Becamel et al., 2001)
		SAP97		(Becamel et al., 2004)
		MAGI2		(Becamel et al., 2004)
		MPP3		(Becamel et al., 2004)
		CIPP		(Becamel et al., 2004)
	Caveolin		Internalization, signaling	(Bhatnagar et al., 2004)
	ARF1-6	ARF1-6	Signaling	(Robertson et al., 2003)
	MAP1A		Targeting	(Cornea-Hebert et al., 2002)
	JAK2/STAT3			(Guillet-Deniau et al., 1997)
	Ribosomal S6 kinase		Signaling	(Sheffler et al., 2006)
	Calmodulin	Calmodulin	Receptor phosphorylation / desensitization	(Turner and Raymond, 2005)
5-HT _{2B}		PDZ proteins*	NO signaling	(Manivet et al., 2000)
		MUPP1		(Becamel et al., 2001)
5-HT _{2C}		PDZ proteins*	Desensitization/resensitization	(Backstrom et al., 2000)
		MUPP1	Receptor clustering, phosphorylation ?	(Ullmer et al., 1998) (Becamel et al., 2001) (Parker et al., 2003)
		PSD-95		(Becamel et al., 2002)
		SAP97		(Becamel et al., 2004)
		SAP102		(Becamel et al., 2004)
		MAGI-2		(Becamel et al., 2004)
		MPP3		(Becamel et al., 2002)
		Veli3/CASK/Mint1		(Becamel et al., 2002; Becamel et al., 2004)
		Calmodulin		(Becamel et al., 2002)
		PICOT		(Becamel et al., 2002)
		PTD4		(Becamel et al., 2002)
	PTEN		Receptor dephosphorylation, behavioral responses to drugs	(Ji et al., 2006)
5-HT _{4(a)}		SNX27	Targeting, trafficking	(Joubert et al., 2004)
		NHERF-1(EBP50)	Targeting	(Joubert et al., 2004)
		MAGI2		(Joubert et al., 2004)
		MPP3		(Joubert et al., 2004)
		Veli 1-3		(Joubert et al., 2004)
		CRMP2	Regulation of axon growth ?	(Joubert et al., 2004)
5-HT _{4(e)}		CIPP		(Joubert et al., 2004)
		nNOS		(Joubert et al., 2004)
		Sec23	Trafficking to plasma membrane ?	(Joubert et al., 2004)

Table 1: 5-HTR accessory proteins and their role in receptor functionality.

Only receptor subtypes for which interacting proteins have been unambiguously identified are mentioned. * Refers to experiments in which the receptor PDZ binding motif has been deleted.

Figure legends

Figure 1

Human gene structures of metabotropic 5-HT receptor (HTR) coding sequences. This figure was generated by using released data from the human genome project available on the NCBI site via the map viewer interface: (<http://www.ncbi.nlm.nih.gov/mapview/>). Build 36 version 1 was used to localize the exons, except for 5-HT5BR; which was only annotated on build 35. Column 1 Gene names and cytogenetic localizations. Column 2 Representations of the exon composition of the coding sequences and the part of the receptor encoded by each exon (open boxes exons, bridges splicing events, black boxes transmembrane domains, curved lines loops or N- or C-terminal domains, colors alternative exons and the receptor part encoded in the splice variants). Column 3 Name and size of the gene products and splice variants (aa amino acids). Column 4 Size of the complete coding sequence on the human genome (b bases, kb kilo bases).

Figure 2

RNA editing of 5-HT₂CR transcripts. a Position of the editing sites (A–E) within the human 5-HT₂CR mRNA and the predicted amino acid sequences are shown for the non-edited INI isoform and for the fully edited VGV isoform. b Comparison of 5-HT₂CR editing efficiency at each editing site for the human brain (dark blue bars) and rat brain (pale blue bars).

Figure 3

Examples of well-documented regional and cellular localizations (pink) of 5-HT₁ARs, 5-HT_{1B}1DRs, 5-HT₂ARs, and 5-HT₂CRs. They can be localized on cell bodies (either on 5-HT neurons or other neurotransmitter-containing neurons) or on axon terminals (of 5-HT neurons or other neurotransmitter-containing neurons). 5-HT₂CRs are also localized on the choroid plexus (POMC pro-opiomelanocortin, CART cocaine- and amphetamine-regulated transcript).

Figure 4

5-HTR signaling pathways and effectors (blue 5-HTR signal transduction in neurons, gray signaling linkages only in transfected cell lines, PL phospholipase, ERK extracellular signal-regulated kinase, PK protein kinase, IP₃ inositol triphosphate, DAG diacylglycerol, MAPK mitogen-activated protein kinase, NOS nitric oxide synthase, AHP after-hyperpolarization, JAK Janus kinase, STAT transcription pathway, ih hyperpolarization-activated current, Epa activated exchange factor).

Gene	Gene structure	Gene products	Cds
HTR1A 5q11.2-q13		5-HT _{1A} : 422aa	1269b
HTR1B 6q13		5-HT _{1B} : 390aa	1173b
HTR1D 1p36.3-p34.3		5-HT _{1D} : 377aa	1134b
HTR1E 6q14-q15		5-HT _{1E} : 365aa	1098b
HTR1F 3p12		5-HT _{1F} : 366aa	1101b
HTR2A 5q14-q21		5-HT _{2A} : 471aa	>60kb
HTR2B 2q36.3-q37.1		5-HT _{2B} : 481aa	>15kb
HTR2C Xq24		5-HT _{2C} : 458aa	>183kb
HTR4 5q31-q33		5-HT _{4(a)} : 387aa (b): 388aa, (c): 411aa, (d): 360aa, (e): 371aa, (f): 363aa, (g): 378aa, (hb): 402aa, (i): 428aa, (n): 359aa	>185kb
HTR5A 7q36.1		5-HT _{5A} : 357aa	>14kb
HTR5B 2q14.1		Pseudogene	none
HTR6 1p36-p35		5-HT ₆ : 440aa	>15kb
HTR7 10q21-q24		5-HT _{7(a)} : 445aa 5-HT _{7(b)} : 432aa 5-HT _{7(d)} : 479aa	>116kb

Figure 1

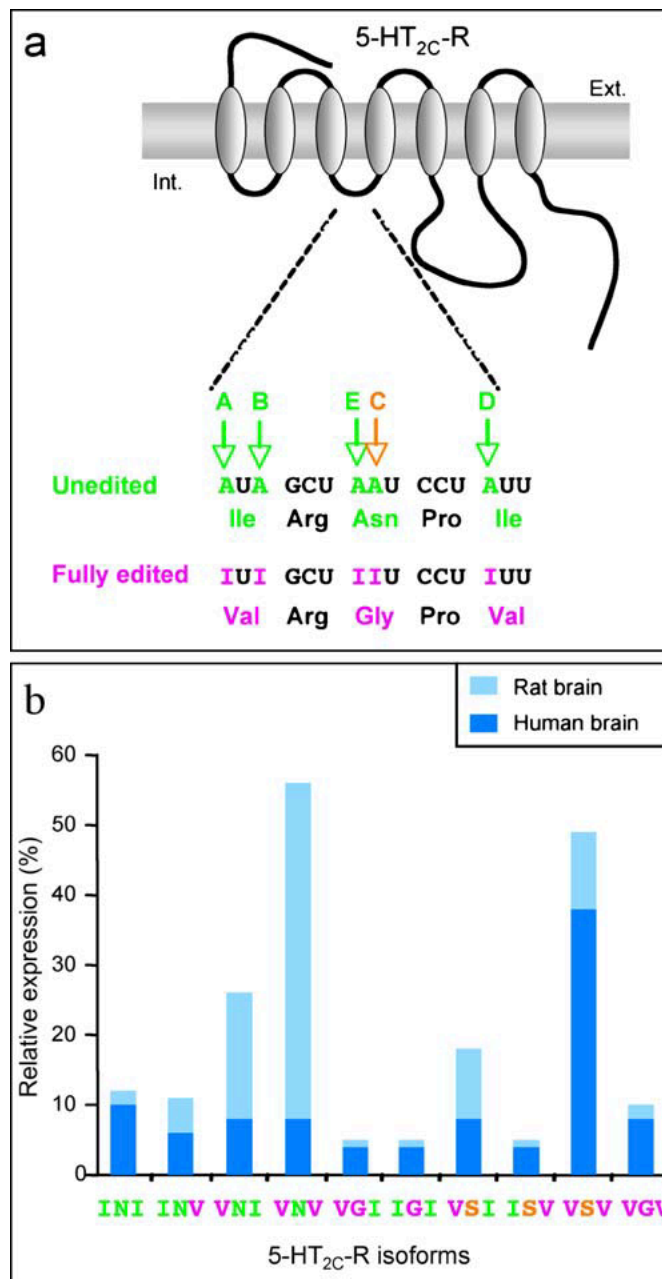


Figure 2

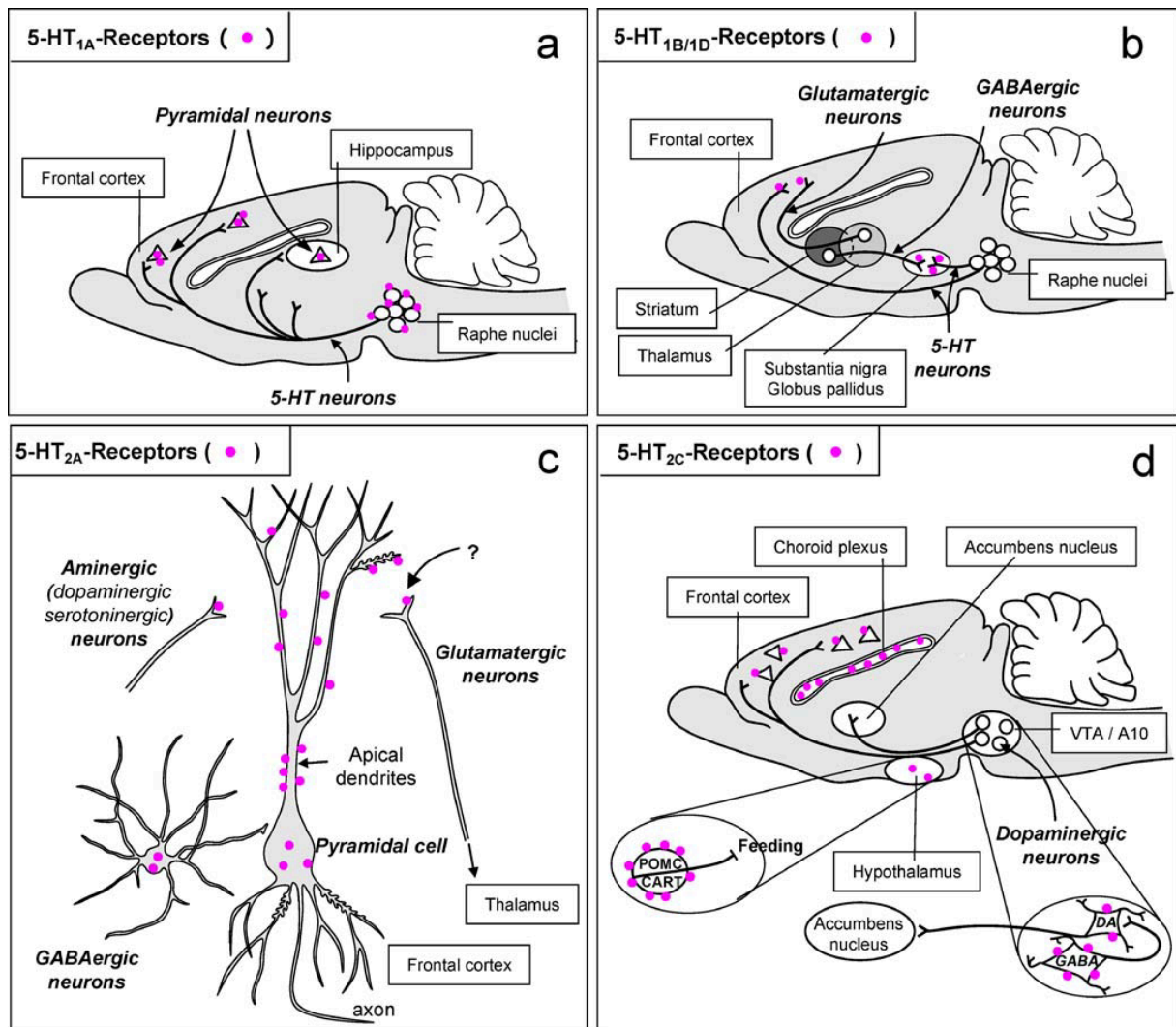


Figure 3

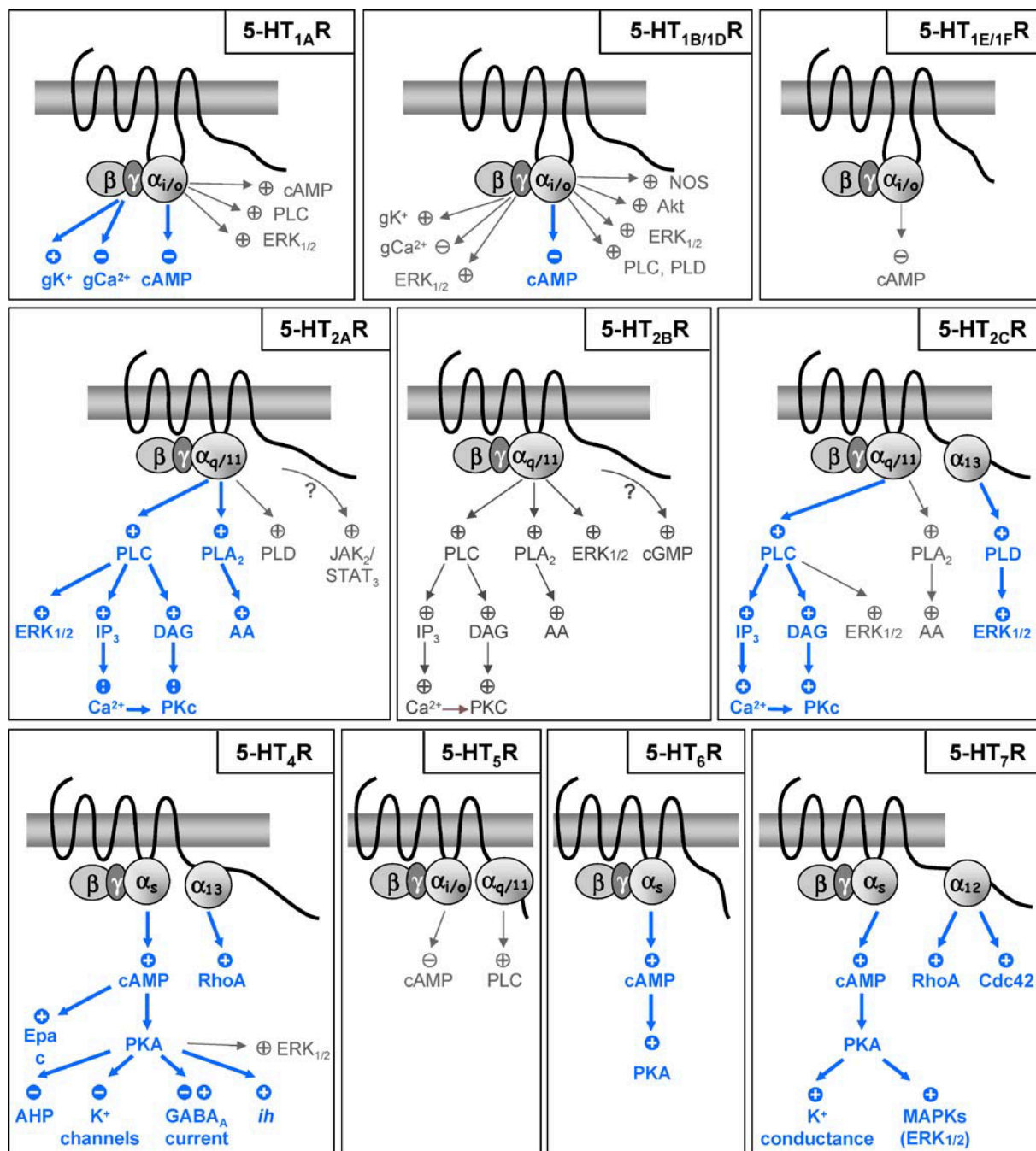


Figure 4